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Increased and Indiscriminate Use of Germicides during the Covid Pandemic: Possible Impacts on Our Health and the Environment

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Abstract

Sudden outbreak of the Covid pandemic a couple of years ago called for widespread use of disinfectants to prevent dissemination of infection. While the importance of the preventive measure is obvious, the adverse effects of the disinfectants also cannot be ignored. **Impacts of the biocides on human health and the environment are discussed with relevant examples in this article and possible link between indiscriminate use of the germicides and the global crisis of antibiotic- resistance of bacteria is also highlighted.**

Keywords: Covid pandemic, germicides, environmental impact, antimicrobial agents, antibiotic resistance

Introduction

The term "biocide" represents a broad range of products including disinfectants, preservatives, antiseptics, herbicides, fungicides, and insecticides. They are commercial products intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means. The wide range of products denoted by the term biocide also includes wood preservatives, rodenticides, antifouling agents (on boats), and in-can preservatives used in homes and public places such ashospitals and industries. However, **the term biocide is commonly used as synonym of antimicrobial agents or disinfectants / sanitizers.**

Increased use of disinfectants during the pandemic

Sharp increase in the use of germicides was noticed all over the world during the Covid-19 pandemic. According to a report, at least 2000 tons of disinfectants was dispensed at the Wuhan city of China alone**.** A questionnaire-based survey in China revealed that use of disinfectants for household purpose was increased from 19.9% (before the pandemic) to 48.7% (after the outbreak of Covid-19). **Enveloped viruses (e.g SARS-CoV-2) are known to be susceptible to inactivation by chlorine-based disinfectants (CBDs). Hence, during the pandemic a number of countries undertook programs for large-scale disinfection of public places using the CBDs.**The most common CBDs used for this purpose included sodium hypochlorite (bleach), calcium hypochlorite (bleaching powder), sodium dichloroisocyanurate (NaDCC), chloramine, and chlorine dioxide [1]. The mode of action of various types of disinfectants was reviewed earlier [2].

Harmful effects on the users

Adverse effects of disinfectants on the users are well-documented. **Use of CBDs, widely applied for the prevention of dissemination of infection, is associated with the possibility of the release of a number of chemical substances harmful for health and environment.** Common people are not supposed to be exposed to high concentration of chlorine and other disinfectants during their day to day activities. But health workers, especially those actively engaged in spraying of the disinfectants, are regularly exposed to harmful amounts of these biocides. The application of CBDs is known to induce direct health problems such as respiratory issues, irritation of the skin and eyes, and psychological discomfort. Several cases of poisoning, caused by accidental inhalation of chlorine gas, were reported by the U.S Department of Health and Human Services during the Covid-19 pandemic. Accidental inhalation of chlorine may give rise to sudden onset of the attack of asthma known as reactive airways dysfunction syndrome (RADS) the symptoms of which could last for months or years. Moreover, **free chlorine, released from most of the CBDs in presence of water, reacts with various organic substances to form different types of organochlorine compounds called disinfection byproducts (DBPs)** such as-trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), haloketones (HKs), and trihalophenols (THPs). **Many of these DBPs are carcinogenic and also mutagenic. Besides being associated with rectal cancer and colon cancer, they are also known to be associated with reproductive and developmental disorders** [1]. In addition to these problems, the DBPs can seep through the soil and contaminate the table of under-ground water, leading to far-reaching consequences. So far, more than 600 DBPs are identified from different aqueous environments. Only some of them are regulated in drinking water. WHO has provided guidelines values for some of the DBPs. **Some inorganic substances present in wastewater (e.g ammonia, nitrate, bromide and iodide) induce the formation of nitrogenous (N-DBPs), brominated (Br-DBPs), and iodinated (I-DBPs) compounds, which are potentially more toxic and also carcinogenic compared to their chlorinated counterparts. Hospital wastewater contains a number of anthropogenic substances that also contribute to the formation of DBPs.** Diagnosis, treatment, medication, surgeries, and activities related to research and development, engender these substances. Excreta of patients also add various types of pharmaceuticals to the sewage. For example, use of the antiviral drug umifenovir was significantly enhanced following the outbreak of the pandemic. Subsequently, the drug was detected in municipal wastewater. It reacts with sodium hypochlorite to form a wide range of DBPs (predominantly Br-DBPs). Iodinated contrast media are widely used in X-ray based imaging systems (e.g computer tomography scans, angiography). It is not surprising therefore that these compounds are found in hospital waste water and iodine present in these compounds can contribute to the formation of iodinated DBPs [1]. Besides causing allergic reactions to skin, accidental inhalation of the dilute solution of sodium hypochlorite is known to be associated with acute cardiopulmonary arrest, nausea, vomiting, diarrhea, and also renal problems [2].

Adverse effects on the environment

Disinfectants released during the use, exert a number of detrimental effects also on the environment, and even on the flora and fauna they come in contact with. Chlorine used as disinfectant, is converted into chloride ion which is stable in soil environments. It is absorbed by plants and accumulated especially in leaves. That may ultimately lead to the death of the whole

plant. Some plants harbor various types of microorganisms in their rhizosphere (zone of soil surrounding the root of a plant). These microorganisms, essential for the healthy growth of the plant, are destroyed by the CBDs. **Spraying of concentrated solution of the disinfectants during the pandemic might be associated with the transport of chloride ion to the agricultural field through drainage or rainwater. Needless to say, that is damaging to the crops.** Chloride ion is known to affect even the regeneration of plant tissues. **Chlorine gas that goes to the higher atmospheric level, plays a significant role in the destruction of the stratospheric ozone layer that protects us from the harmful ultraviolet-ray coming from the sun** [1]. Chlorine gas is also produced by various types of anthropogenic activities e.g, burning of agricultural wastes, municipal wastes, biofuels and industrial activities. The consequent **increase in the atmospheric concentration of chlorine is associated with a rise in the concentration of particulate matters having diameter less than 2.5 micron (P 2.5). These pollutants are known to have a number of adverse effects on health (aggravated asthma, decreased lungs function, premature death of people with lungs or heart-problem)** [1]**.**

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants predominantly generated by human activities (viz, residential heating, coal gasification and liquefying plants, carbon black coal-tar pitch and asphalt production, coke and aluminum production, catalytic cracking towers and related activities in petroleum refineries) and also present in motor vehicle exhaust**. Many of them are toxic, mutagenic and carcinogenic.** Because of their lipophilic properties, they are readily absorbed from the gastrointestinal tract of animals and rapidly distributed in various types of tissues. They also have a marked tendency for localization in the body fat. **When municipal wastes containing chlorine are destroyed by burning, chlorinated polycyclic aromatic hydrocarbons are produced. During the pandemic, because of sudden increase in the quantity of the clinical wastes, it was necessary in some places to burn clinical wastes with municipal wastes. But the clinical wastes had to be disinfected with CBDs before carrying them for incineration.** Consequently, the concentration of hydrogen chloride gas emitted from the incinerator was substantially increased. **That also led to the formation of chlorinated PAHs which are more toxic** [1]**.**

DBPs, that reach water bodies, adversely affect the life of aquatic animals in various ways. Retarded growth rate, increased mortality rate, morphological deformities such as severe curvature in body axis and pericardial oedema and decreased locomotor behavior of the embryo (which stimulates the hatching process) were observed in Zebra fishes following exposure to several types of DBPs. Accumulation of the chlorination byproducts in fishes and planktons may result in chronic adverse effects on the ecosystems. **Disinfectants, that enter the food chain, may have long-term damaging effects on human and animal health.** However, the adverse effects of DBPs on different forms of aquatic life need further in-depth investigations [3]. The damaging effect of the overuse of disinfectants on the biodiversity was evident when a total of 135 animal bodies belonging to 17 species (blackbirds, wild boars and weasels) were found in Chongqing, which borders the Hubei Province in China. According to the experts some of the animals died after being poisoned by disinfectant sprayed by workers to prevent the spread of the coronavirus [4].

Extensive use other germicides (e.g, Quaternary ammonium compounds or QACs, Hydrogen peroxide, alcohols) also, is associated with a number of adverse effects on health and environment.QACs (Benzalkonium chloride, Cetrimide, Chlorhexidine) are widely used in

community and healthcare settings for disinfection. These germicides are found to be linked with aggravation of asthma. Excess exposure to alcohol-based disinfectants or hydrogen peroxide can lead to the damage in the respiratory tract [5]. Presence of QACs in human blood is known to be associated with increase in inflammatory cytokines, decreased mitochondrial function, and disruption of cholesterol homeostasis in a dose-dependent manner [4]. **Widespread use of disinfectants on porous surfaces, including fabrics / textiles and consumer plastics, may lead to degradation of plastic materials and release of microplastics in the environment. Less than 5mm in length and almost ubiquitous in nature, these environmental pollutants take nearly 1000 years to degrade. They are suspected to cause several inflammatory ailments, genotoxicity (damages that cause mutations) chronic diseases and autoimmune diseases** [6]. For persons suffering from chronic obstructive pulmonary diseases, exposure to microplastics appears to be dangerous since they can enter the respiratory tract and damage lung tissues.

Bacterial resistance to disinfectants

Extensive use of disinfectants has raised concern about development of resistance in bacteria to the germicides. Bacteria possess some efflux pumps (special transporter proteins) that push out a number of structurally unrelated compounds from the interior of the bacterial cell to the external environment and thus do not allow them to accumulate in sufficient concentration required for activity. Changes in the membrane permeability also make the bacteria cell impervious to various growth-inhibitory substances. These mechanisms appear to play an important role in bacterial resistance to germicides. Target-site mutations (one of the causes of antibiotic-resistance) leading to germicide resistance is known in some rare cases. Use of germicides contaminated with bacteria is known to cause outbreaks of healthcare-associated infections. Interested readers are referred to an informative article cited in the bibliography [7].

Correlation between germicide-tolerance and antibiotic-resistance

Correlation between bacterial resistance to germicides and clinically useful antibiotics has been found in a number of cases. However, a definite relationship between germicide-resistance and antibiotic-resistance, is yet to be established. But, scientists are not dismissing the possibility of getting the problem of antibiotic-resistance aggravated by widespread use of germicides. **Bacteria may develop resistance against germicides and simultaneously cross-resistance to antibiotics. The possibility assumes a significant dimension, when there is an overlap between the mechanisms of resistance to germicides and antibiotics.** For example, an efflux pump present in the bacterium *Staphylococcus aureus*, was reported to push out a wide variety of chemical substances viz, β-lactams, fluoroquinolones, tetracyclines, fungicides, dyes, quaternary ammonium compounds and preservatives from the bacterial cell and render the cell resistant to all of these inhibitory substances [8]. The non-specific nature of the efflux pumps enables them to confer resistance to a number of structurally-unrelated growth-inhibitory substances at a time. Hence **the possibility, that overuse of germicide may worsen the global crisis of antibiotic-resistance, cannot be ruled out. While the necessity of using germicides to control sudden outbreak of an infectious disease in a large scale cannot be denied, we cannot wink at the possible impact of the germicides on the worldwide crisis of antibiotic-resistance.** The habit of many people to clean their palms before eating anything using a few drops of a germicide instead

of washing the hand with soap water, also appears to be questionable since it appears to foster emergence of germicide-resistant bacteria that are also antibiotic-resistant.

The Triclosan Controversy

In this context, it appears relevant to recall the episode of **triclosan**. It **is an antimicrobial compound developed in 1966.In the beginning its use was confined into hospitals and health care settings.** It was also used in surgical scrubs and hand-washes. **Subsequently it was incorporated into a number of consumer products e.g soap, toothpaste, hand -sanitizer and mouth- wash.During 2014, triclosan was added to more than 2000 consumer products in the U.S.The widespread use of triclosan facilitated its entry in the environment.** Its antibacterial potential, based on its ability to inhibit bacterial fatty acid synthesis, is resistant to anaerobic degradation, thus contributing to its ability to persist in the environment. It was detected in 57.6% of the water samples, collected from the streams and rivers, in a program conducted by the US Geological Survey during 1999 to 2000. The possibility of association between triclosan-tolerance and antibiotic-resistance was studied in several laboratories. An investigation, performed at the University of Tennessee Health Science Center, Memphis (TN, US), revealed increased resistance to tetracycline and norfloxacin in triclosan-resistant strains of *Pseudomonas aeruginosa* and *Stenotrophomonasmaltophilia*, when they were further exposed to triclosan [9]. Existence of a multidrug efflux system, that plays an important role in the antibiotic-resistance and triclosan-resistance in the opportunistic pathogen *Pseudomonas aeruginosa*, was also demonstrated. Hence **the apprehension, that random use of triclosan may select triclosan-resistant bacteria that are resistant also to various therapeutically useful antibiotics, was not unfounded. The Food and Drug Administration USA conducted detailed investigation and in September 2016, banned the incorporation of triclosan and 18 other antimicrobial chemicals into household soap products** [9]**.** However, the use of triclosan in toothpaste, hand sanitizer, and mouthwash was not prohibited. **Dr. Stuart B. Levy, the Emeritus Professor of molecular biology and microbiology at the Tufts University, was one of those who challenged the utility of adding triclosan to the consumer products.** Besides making significant contribution to the basic understanding of the mechanisms involved in bacterial resistance to several antibiotics, he untiringly delivered lectures and published articles throughout his career, to promote prudent and judicious use of antibiotics. **His reservation against the widespread use of triclosan in the consumer products earned him the wrath of the manufacturers. But he did not retract from the controversy. The measure taken by FDA ultimately vindicated his stance** [10].

Concluding remarks

Thus it is obvious from the foregoing discussion that widespread application of germicides on the environment may engender highly toxic disinfection byproducts and we have to strike a balance between use and overuse of germicides. **Judicious and rational use of germicides will save us from disaster. On the other hand, improper and indiscriminate use, besides causing health damage and environmental pollution, may contribute to the already existing crisis of antibiotic-resistance of bacteria.** Evaluation of CBD-alternatives (e.g, ozone, UV radiation, or sunlight/solar disinfection) is recommended in order to reduce the amount of DBPs formed and also to avoid exposure to the harmful effects of CBDs**. Pharmacists can assume an important**

role in formulating a policy in the use of germicides under a given situation and also in creating awareness in the society on the importance of prudent use of germicides.

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Pharmacological and Medicinal Properties of *Areca catechu* L.: A Comprehensive Review

Liza Das[1], Sneha Biswas[1], Hira Dey[1], Manisha Nath[1], Setu Mallick[1], Pranomi Chakroborty[1], Pritesh Ranjan Dash[2], Emdad Hossain[3], Abul Kalam Lutful Kabir[3,4*]

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Abstract

Under the family Arecaceae, *Areca catechu* L. brought its importance for various traditional uses by different tribal people round the Globe. It isgenerally known to as Betel nut palm. It is naturally grown as well cultivated in the tropical belts of Southeast Asia to India and extended to prominent islands of Pacific. The nut of this plant is an important component in the cultural practices of many Pacific Islanders, and when chewed either with betel leaves alone or with lime as a stimulant to produce 'betel quid'. This comprehensive review backs up current data regarding its traditional uses, phytochemical and pharmacological activities. The bioactive constituents such as alkaloids, tannins and polyphenols which are responsible for its various pharmacological actions like anti-inflammatory and antioxidant in the plant were also explained. Recent research has supported its anti-migraine, anti-venom, hypoglycemic and even anti-HIV activity. Arecoline, a potential substancein the plant, inhibited the proliferation of cancer cells. The nut also showedanti-aging properties which is suitable for cosmetic preparations. Potential hazards of the nut explained to avoid the serious side effects like mouth cancer. The review emphasizedfurther information which can be utilized for isolation of phyto-constituents, can be suitable for future medicine.

Keywords: *Areca catechu,* Arecaceae, Pharmacological activities,Medicinal Properties, Supari

Introduction

Since Archaicages, our existence has been dependent on plants as a source of food, medicine, predominantly used as a source of sanative components. Plants possess remarkable source for the unboltingofinitiate products of medicinal value. Many of the drugs used today are minor synthetic modification or copies of the natural occurring substances [1].In the current scenario, due to severe undesirable side effects of synthetic medicines, people are returning to nature's lifestyle by using plants. Numbers of different phytochemicals derived from plants are used as important medicines in many countries. The uses of herbal medicine enhanced due to its better therapeutic effects without or less side effects [2]. In the cultural cue of various indigenous societies, traditional medicine is deeply rooted. Areca catechu L. is often accomplished by a combination of treatments such as herbal remedies, physical therapies, dietary intervention with religious practices. It is one of the most popular traditional medicines with versatile medicinal properties. It is belonging to the family Arecaceae(Palm), has been used for medicinal properties for morethan 2000 years in South Asian countries. The palm is cultivated mainly

in south tosoutheast Asian countries such as India, Bangladesh, China, Indonesia, Myanmar, Thailand, Malaysia, Vietnam and Philippines.The fruit of the plant is commonly referred to as a Betel Nut [3,4]. It isanerect solitary palmthatgrowsup toaheight of 20m with diameter up to 30 cm [5]. It ismoneous palm with terminal, linear-lanceolate leaves. Flowers are orange-yellow in color wherefruits which are producedin bunches are ovoid, smooth, orange yellow in colorat ripening. Burnt inflorescence of the plant is used for easy expulsion of the placenta of post-natal woman at Nilgiri area of India [6].

Taxonomical classification [7]:

Taxonomical Rank	
Kingdom	Plantae
Order	Arecales
Family	Arecaceae
Genus	Areca
Species	*A. catechu*

Vernacular names [6]:

Assamese	Tambul
Bengali	Supari, Gua
English	Betel nut palm, Areca nut palm
Gujrati	Sopari
Hindi	Supari
Malyalam	Adakkamarom, Kamuku
Punjabi	Supari
Sanskrit	Gubak, Poogiphalam, Tantusara
Tamil	Kamugu, Pakku
Telegu	Vakka

Areca catechu nut (ACN) is found to possess carbohydrates, lipids, fibers, polyphenols, alkaloids (0.3- 0.6%), tannins (15%) and fat (14%) [8]. Generally, for chewing purposes, few slices of the nut are wrappedin the betel leaf along with slaked lime are used. Cardamom, clove, catechu orothersspicesare added for enhancingflavor and taste [7]. Surprisingly, Areca nut is chewed normally by about 10% of the world population as intoxicant. It is the fourth most ranked psycho-activejunkused after nicotine, caffeine and alcohol [9].

The variouspharmacological activities of *Areca catechu* were established in modern investigations including anti-parasitic effects, anti-fungal effects, anti-allergicanti-oxidants, anti-bacterial, anti-inflammatory, anti-aging effectsas well regulatoryeffects onblood glucoselevel[10]. Areca nut has also shown significantanti-viral activityagainst the human immunodeficiencyvirus (HIV) [11]. Catechins, important ingredientsin Areca nut,is believed to be better chemo-preventive agent against several tumors in animals [12]. There view was aimed to describe the pharmacological andmedicinal properties of *Areca catechu* L.

Pharmacological Properties

Anti-inflammatoryand anti-melanogenesis effect:

As per reports of Lee & Choi, ethanolic extract of Areca nut (ANEE) prevented the hyaluronidase enzyme. ANEE showedinhibition of delayed hypersensitivity and ear edema caused by croton oilon mice when used topically for *in vivo*experiment. The results recommended that the impact of skin problems due to inflammation and immunological regulation can be mitigated by ANEE. In B16 melanoma cells, ANEE demonstrated the capacity to inhibit the activity of the enzyme, mushroom tyrosinase and the production of melanin. This study suggests that ANEE has great anti-inflammatory and anti-melanogenesis properties, making it a promising new ingredient for cosmetic products [13].

Anti-migraine activity

In southern India, Areca nut extract (ANE) is a commonly used as folk remedy for migraines. In order to validate the traditional claim, Bhandare *et al*. conducted a study with hydro-alcoholic extract of Areca nut (HAEAN). The study's collective conclusions suggested that the extract significantly inhibited inducible nitric oxide synthase, which may be the mechanism underlying its antimigraine action. The study also established its traditional claim [14].

Anti-oxidant and analgesic activity

The potentiality of hydro-alcoholic extract of Areca nut (HAEAN) as an analgesic, anti-inflammatory and *in vitro* anti-oxidant activity was evaluated by Bhandare *et al*. Using hydrogen peroxide assay and total phenolic content method, the anti-oxidant activity of HAEAN was examined*in vitro*.In the hydrogen peroxide assay, $IC_{50}$was 83.1 µg/ml and total phenolic content was 120.6 ± 21.09 mg QE/g.These research findings suggested that the HAEAN may have the potential to be an antioxidant. Significant analgesic activity also found for HAEANthrough formalin test andhot plate in mice [15].

Hypoglycemic activity

Alkaloid Arecoline, active component of Betel nut, was found to exhibit hypoglycemic action in several animalswhen given subcutaneously. In alloxanized rabbits, Arecoline showed a notable hypoglycemia response that persisted for 4-6 hours [16]. By blocking glucosidase activity, Areca nut extract (70% ethanol extract) reduced blood glucose in postprandial hyperglycemia [17].

Anti-HIVactivity

Various extracts of *Areca catechu* nut showed HIV protease inhibitory activity, including areca tannin B1, helps forAnti-HIV activity [11, 18].

Anticancer activity

In different scientific reports, Areca nuts showed high contents of flavonoid and phenolic compounds. It was expected that the nut may have anti-cancer activity. Sari *et al*. treated 96% ethanolic extract of Areca nuts against human squamous carcinoma cell (HSC-2, HSC-3) and human keratinocyte cancer cell lines(HaCat)*in vitro*. The ethanolic extract showed cytotoxicity against both HSC-2 and HSC-3 cells but it induced higher rate of proliferation in HaCat cells. It was expected that anti-cancer activity of the extract was very selective [19].

Fan *et al.* claimed that arecoline, a significant chemical component of areca nut, that binds to and disrupts only ACAT1 tetramers of cancer cell may responsible for selective anti-cancer agent [20].

Anti-allergic activity

The semen the Arecae plant was found as most potent inhibitor of antigen-induced degranulation within the RBL-2H3 mast cells and inhibited compound 48/80-induced systemic anaphylaxis in experimental mice. Lee *et al.* suggested that Arecae semen may be helpful for treatment of various immediate or delayed allergic diseases [21].

Antibacterial activity

Areca catechu contains tannic acid, which have been suggested to reduce Gram positive and Gram negetive micro-organisms as per Al-Bayati [22]. Rural people of South Karnatakawas found tousethe husk fibers of Areca nut for cleaning their teeth. As per research findings of Cyriac *et al*. alcoholic extract of *Areca catechu* husk fibers did not inhibit bacteria like *Streptococcus mutans* and *Streptococcus sobrinus* but significantly inhibited *Candida albicans*. It can be predicted that Areca husk might improve the oral health through dentifrices action rather than antimicrobial activity when used for routine oral care [23, 24].

Antifungal activity

Anti-fungal study of hot water extract of *Areca catechu*nut was performed against *Cladosporium sp., Aspergillus niger, Candidia albicans* and *Mucor sp*. The extract is effective against *Candidia albicans*only [25].

Antidepressant activity

Mansour *et al*. assessed methanolic extract of Areca nut (ANME) for the anti-depressant activity using Mice model. Significant change was observed in the immobility for both forced swimming test as well tail suspension test. The test confirmed the antidepressant effect of the extract [26].

Anti-lipedemic activity

The chewing of this Areca nut reduces false hunger. In experiments found that betel nut extract (ANE) containing arecoline inhibited the formation of lipid droplets in adipocytes. The ANE supplemented food was also reported to decrease the concentrations of plasma cholesterol and triglycerides significantly in experimental rats thus helped to reduce obesity [27].

Effects on the nervous system

It has been claimed thatchewingof betel nut produce a sense of well-being like euphoria, salivation, heightened alertness and hot sensation within the body with increased capacity to work. Chewingof betel nut also leads to habituation, addiction as well as withdrawal syndrome [28].

The arecoline present in the Areca nut increases both brain noradrenaline and serotonin level. Through monoamine oxidase A inhibitor like property, may be responsible for Betel quid dependence (BQD) [29].

Antimalarial activity

Butanol extract of *Areca catechu* nut showed anti-malarial activity against *Plasmodium berghei in vitro.* In case of *in vivo* experiment, a 39.1% inhibition effect was observed after 4 days of suppressive and survival testing on *Plasmodium berghei*-infected mice treated with 150 mg/kg/day [30].

Anti-nociceptive activity

At varying concentrations, the methanolic extract of *A.catechu* stem demonstrated an anti-nociceptive effect by lowering the overall number of constrictions. The acetic acid induced writhing or constrictions were reduced by 30.8%, 36.6%, 40.9%, and 59.6% in response to the stem extract at doses of 50, 100, 200 and 400 mg/kg bodyweight. The percentage of inhibitions was noteworthy and in dose dependent fashion. The writhing or constrictions were suppressed by the leaf extract of the plant at the four distinct doses indicated above by 55.8%, 57.7%, 86.5%, and 88.5%, respectively, which was better than stem extract [31].

Hypertensive activity

Older people especially women chew betel quid more frequently each day. Those people who chewbetel quid without using tobacco had higher arterial pressure, diastolic blood pressure and systolic blood pressure at follow-up than those who never used it. Chewing tobacco-free betel quid was linked to both systolic hypertension and general hypertension [32].

Aphrodisiacs activity

Anthikat*et al*. administeredextract of Areca nut (ANE) at a dose of 150 mg per kg of body weight through Oral administrationin male rats. It produced significant augmentation of sexual activity. ANE significantly increased the mounting frequency, intromission latency and intromission frequency [33].

Wound healing activity

In Indian traditional medicine Areca nut is commonly used for skin ulcers. To evaluate the burn wound healing property of *Areca catechu* kernel (ACK) an ointment was made with 2% of Ethanolic extract of ACK and it was treated with normal as well as dexamethasone treated rats. In comparison with the control group, the wound healing contraction rate was consistently higher in the ACK treated group throughout all days. Epithelialization period was quicker in the extract-treated group than that of the control group. In compare to the control group, the group treated with dexamethasone exhibited a notable delay in the healing process of wounds. In the

dexamethasone-suppressed burn wound healing model, the test medication significantly reversed the pace of wound contraction and the length of the epithelializationphase [34].

Anthelmintic

The research aimed to extract arecoline from Areca nut utilizing liquid-liquid extraction technique as well as anthelmintic study of water and hexane extract of Areca nut. *In vitro* anthelmintic activity was assessed against adult earthworms (*Pheretima posthuma*) and results were compared with the standard reference, piperazine citrate. The hexane extract was the most active against earthworms, being effective at low concentrations. Both of hexane and aqueous extracts decreased the paralysis time as well as death times of earthworms. Experimental results encouraged conclusion of *Areca catechu* as an anthelmintic agent [35].

Ulcerogenic activity

Mahmood *et al.* studied the influence of *Areca catechu* L. nut ethanol extract (ANEE)for ulcerogenic activity on stomach mucosa tissues damaged by an experimental chemical agent (ethanol) onadult Sprague-Dawley rats. ANEE significantly enhanced ulcerogenic effect on ethanol-induced gastric ulcers in experimental animal. Such ulcerogenic effects was in dose dependent fashion as established by the increasing of ulcer areas of the gastric wall as well as the increasing the area of edema and leucocytes infiltration of submucosal layers of the rat [36].

In vitro cytotoxicity activity

In their work, Anajwala *et al.* tried to prove a cytotoxic effect of some extracts of medicinal plants, including *Areca catechu*, against MCF-7 and Vero cell lines*in vitro*. Methanol and aqueous extract were prepared through Soxhlation process of Areca nut. In their experiments through MTT and SRB assay, it was found that aqueous extract of *Areca catechu* was potent cytotoxic [37].

Safety Assessment in Pregnancy

Chue et al. conducted a retrospective cohort analysis of pregnant women who were migrants and refugees and who visited clinics along the Thai-Myanmar border in order to investigate the potential negative effects of Areca nut use. As per their data of the 7685 women enrolled,2484 (32.3%) only used Areca, 2284 (29.7%) never used Areca nor smoked cheroots, 438 (5.7%) only smoked cheroots, and 2479 (32.3%) used both Areca and cheroots. The researcher reported that smoking was obviously hazardousfor pregnancy but Areca nut related unfavorable pregnancy outcomes were not observed in pregnant women [38].

Anti-fertility activity

In male albino rats, the antifertility effect of *Areca catechu* was assessed. At doses of 300 and 600 mg/kg body weight, the antifertility properties of an alcoholic extract of Areca catechu were investigated. A mating test was used to determine fertility. Weight measurements were made for the body and the testicles and epididymis, the reproductive organs. *Areca catechu* alcoholic extract demonstrated antifertility efficacy at doses of 300 and 600 mg/kg body weight [39].

Hepatoprotective activity

The hepato-protective potential of aqueous extracts from Arecanut (ANAE) was examined by Pithayanukul *et al.* assessing their antioxidant capacity using four different methodologies. Those are through assessing them *in vitro* anti-inflammatory activity against 5-lipoxygenase, and

assessing their potential to prevent liver damage caused by carbon tetrachloride in rats. ANAE showed strong anti-inflammatory and antioxidant properties. When rats were treated with ANAE, CCl4-induced oxidative damage to their liver tissues was reversed. Presence of high condensed or hydrolysable tannins in the nut may provide protection against liver damage brought on by oxidative stress [40].

Anti-aging activity

Lee & Choi examined the anti-aging properties of Areca nut extract (ANE) on the skin, using animal models. ANE contains relatively high levels of protein (26%) with a significant proportion of proline (13%) in its free amino acid composition and carbohydrates (37.5%). ANE demonstrated inhibitory effects on elastase in the range of 37-98% at concentrations of 10-500 ng/ml, with IC_{50} values of 40.8 ng/ml for porcine pancreatic elastase and 48.1 ng/ml for human leukocyte elastase. From their study, Lee & Choi suggested that ANE can be utilized as new anti-aging constitutent for cosmetics [41].

Anti-platelet activity

Ghayur *et al.* studied the mechanism and the compound(s) causing the Areca nut crude extract (70% aqueous-methanol, ANAME) inhibitory effects on acetylcholinesterase (AChE) and platelets. A Lumi-aggregometer was used to quantify antiplatelet activity in human platelet-rich plasma, and spectrophotometry was used to measure *in vitro*anti-AChE activity. All the chemicals found in betel nut that were evaluated had no antiplatelet impact, with the exception of catechin, which was the most effective against aggregation caused by adrenaline. Compared to ANAME, catechin was less powerful, suggesting the inclusion of one or more additional compounds having antiplatelet activity. Tannic acid and gallic acid were discovered to have an AChE inhibitory effect; tannic acid was shown to be more efficacy than ANAME. The study demonstrated betel nut's potential as an AChE inhibitor and anti-platelet agent [42].

Anti-venom activity

Using the *in vitro* neutralization approach, the aqueous extracts Areca nut were evaluated for their ability to suppress the venom of *Naja kaouthia* (NK). In the experiments of Pithayanukula *et al*., it was found that the aqueous extracts Areca nutfound effective against snake-venom [43].

Conclusion

The review article explainedcomprehensive version of versatile pharmacological and medicinal values of different parts of *Areca catechu*with special reference to the Areca nut. Bioactive compounds of Areca are responsible for anti-inflammatory, anti-lipedemic, anti-depressant, anti-allergic, hypoglycemic activity, anti-malarial, analgesic, wound healing, hepatoprotective properties which could suggest potential therapeutic uses for certain conditions. It is safe in cosmetics butthe nut failed asanti-bacterial for oral bacteria but found suitable to stop fungus like *Candidia albicans*. Various research studies have demonstrated that it can be used to successfully treat migraines, diabetes and even HIV with promising results for inhibiting cancer cell growth more than normal cells. Indeed, the capacity of this nut to stimulate wound healing as well fight helminthesthough more research required to justify its use asregular medicine. No side effect was observed in case of pregnant women; made its safetywith added advantages but it also created many problems like stomach mucosal ulceration, enhanced blood pressure. It should be avoided during dengue due to its anti-platelet activity. Although long term use of Areca nut mayassociate

with serious complications like oral submucous fibrosis, even cancer may be due to cytotoxic effects. Hence it should be cautiously used. Although there are clear benefits seen with *Areca catechu*, more work is needed to isolate the toxic compounds and develop safer applications.

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Assessment of Nutritional status by Mid Upper-Arm Circumference (MUAC): A study among School Children (6-13 years) in South 24 Parganas District, West Bengal

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Abstract

Hunger, malnutrition, and poor hygiene continue to be the major global challenges towards the SDGs. They make people less productive and more vulnerable to disease. Preventing child malnutrition can support and accelerate progress in global commitments and agendas, including the Global action plan "*Global Strategy for Women's, Children's, and Adolescent's Health 2016–2030".* This study tries to assess the nutritional status of children (aged 6-13 years) and identify the possible determinants that are likely to be associated with malnutrition. This is a cross-sectional study and was done in seven schools managed by a non-government voluntary organization (NGVO). The schools are located in South 24 Parganas district of West Bengal. Height and Weight were measured using standard instruments, while mid-upper circumference was measured using MUAC Z-score tape. The required sample size for the study, given a prevalence rate of 28.2% in South 24 Parganas, as identified in earlier studies, is approximately 311. The study involved 463 children, out of which 256 (55%) were boys and the remaining (45%) were girls. Results show different variations in malnutrition status among both sexes and the schools. South 24 Parganas still has extensive problems of malnutrition in school-going children and proper monitoring is recommended.

Keywords: Malnutrition; MUAC score, school children, South 24 Parganas

Introduction

Health and nutrition are one the most important contributory factors for human resource development in the country. Human body requires lot of energies for day to day functions. Our body acquires energy form the food. Consumed food breaks down in our body and yields different nutrients and energy to run the bodily functions and growth and maintenance. Our diet therefore must provide all essential nutrients in the required amounts. The nutrient requirements vary with age, gender, physiological status and physical activity [1]. Dietary intakes reduce or increase the body's requirements which can lead to under nutrition (deficiency diseases) or over nutrition (diseases of affluence) respectively. Less food intake in some specific periods of life like infancy, childhood, adolescence, pregnancy and lactation and high food intake at any age can lead to harmful consequences. It is a scientifically proven fact based on research that infants who are breastfed with mothers' milk for the first six months are less likely to catch many of the illnesses in the future and to suffer chronic diseases. Nutrition is therefore one of the very important components for a child to grow physically and mentally and it should be in the right way so that every child remains healthy throughout the life. When a child does not eat proper food, there are chances of his/her not developing normally - the likelihood that some organ of his/her body may start malfunctioning, or that he/she may get some disease. Poor nutrition may also influence his / her mentality and affect social well-being. Thus the health of a child depends on the kind and the

quantity of food he/ she eats.

Malnutrition is defined as less, more or not in balance in taking energy and /or nutrients. Malnutrition covers two groups of conditions. One is **'undernutrition'**—which refers to stunting (less height in respect of age), wasting (less weight in respect of height), underweight (less weight in respect of age) and deficiencies of micronutrients. Alternatively **overweight** or obesity may lead to diseases which are not communicable (such as cardiovascular disease, diabetes, and cancer). Many factors including poverty, food insecurity, maternal health and nutritional status, mother's age and educational status, low birth weight or small for gestational age (SGA), premature births, unhealthy dietary and lifestyle patterns, health and immunization status of children, socioeconomic status of the family, environmental and household conditions, with cultural practices play an important part in reducing the growth of children at an early age.

Nutrition surveys carried out in many developing countries have shown that the diets consumed by a large majority of the population are based on mainly on cereals, and tubers, small amounts of pulses and vegetables and negligible amounts of milk and animal protein. The calorie intake in most of the developing countries are inadequate to meet the needs of the population [2, 3].

It is estimated that about 40% of children drop out at primary school. Less presence and higher school dropout rates are related to poor socio-economic conditions, child labour and lack of inspiration along with poor nutritional status of the children. According to the National Nutrition Monitoring Bureau (NNMB) survey (2006), about 40% of these children are undernourished and there is about 30% deficit in energy consumption. Sharma and Lakhawat examined 120 school-going children of 7-9 years in rural areas of Bhilwara District in Rajasthan. Information was collected by means of anthropometric estimation, clinical assessment and dietary recall method. The results in the study population showed that supplement admissions were insufficient and the anthropometrics were essentially lower than the desired reference value ($p<0.0$ 5) (4). A cross-sectional study was carried out by Mukherjee et al (2008), to decide the dietary status of 760 school children in Armed Force School, identified the prevalence of stunting as 13.81%, wasting (6.71%), and undernutrition as 9.87%. Information was also collected about affiliations of nutritional status with socioeconomic status, education status of parents, and family size, The study concluded that the mother's educational level, socio-economic status, and family size were much related with the dietary status of the child [5]. Unfortunately, most of the studies reveal that many of the Indian children including those of West Bengal suffer from undernutrition primarily due to low food intake, which could be due to poverty, ignorance etc [6]. This undoubtedly hampers their attendance & performance in their school. Therefore, nutrition support to primary education is considered as a means to achieve the objective of providing free and compulsory universal primary education of satisfactory quality to all the students less than 14 years old.

Research Objectives

One of the major studies in West Bengal, "Malnutrition Scenario among School Children in Eastern-India-an Epidemiological Study" estimated that the prevalence of undernutrition in school-going children in West Bengal was 23%, and in South 24 Parganas district as 28.2% (6). No further studies have been documented, regarding the prevalence of malnutrition among school-going children (6-13 years particularly) in West Bengal, post to the study of 2016. This study is an attempt to assess the nutritional status of children (aged 6-13 years) attending schools run by the

non-governmental voluntary organisation (NGVO), Institute of Indian Mother & Child (IIMC) in the district of South 24-Parganas of West Bengal. IIMC through registered under the Societies Act, it does not receive any benefits under the Social Welfare Department. The study also aims to find out the possible determinants that are likely to be associated with malnutrition in the study population. The information can help to understand the factors responsible for low nutritional status of school children and necessary intervention programmes can be developed based on the above information.

Research Methodology

IIMC is a non-governmental voluntary organization (NGVO), dedicated to the poorest of the poor providing primary and secondary education. The school children primarily belong to the poor and deprived rural families and seem to have suffered from poor health vis-a-vis malnutrition. This study is school-based and cross-sectional, and the study duration was from 1st January 2024 to 31st May, 2024.

The approval for the above-mentioned study was given by NSHM Institutional Ethics Committee vide approval no. no NSHMKOL/IEC/3/2024/PR-03. The required sample size for the study, given a prevalence rate of 28.2% in South 24 Parganas district in this age group, at a 95% confidence level, and a 5% margin of error, is calculated as approximately 311 participants. This study was done on 463 participants. The children were selected by random sampling method.

Study tool

The MUAC z-score Tape (approved by WHO and UNICEF) is being regularly used by countries globally) for the measurement of the status of malnutrition. Some are colour-coded that indicate the nutritional status of a child or an adult for example green for normal, yellow for moderate and red for severe acute malnutrition [7, 8]. The colour codes make them easy for the mothers and the community health workers to use (UNICEF supply division). For measuring the nutrition status in our study population, MUAC Z-score tape was used (Refer to Figure 1). The tape is a simple, inexpensive, simple, paper-based validated tool that can allow a healthcare provider to measure and assess malnutrition risk in children between the ages of two months to eighteen years [9]. Also, a pre-tested questionnaire was used to collect relevant socio-demographic information about the family and household. Information was entered in MS office-excel programme after necessary editing and are explained by SPSS using descriptive analysis.

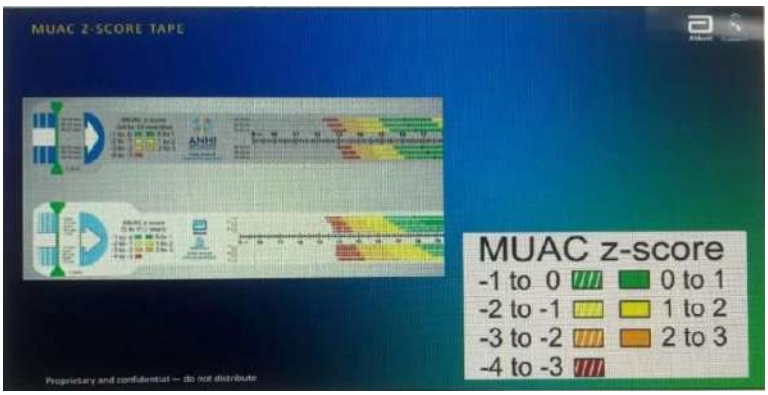

Fig 1. MUAC Z-score Tape developed by Abbott Nutrition Health Institute (ANHI)

Results and Findings

The researchers surveyed 463 children (age group 6-13 years) from seven schools located in the 24 Parganas district. The normal nutrition level was identified for 149 children (32.1%), mild undernutrition in 151 children (32.6%), moderate undernutrition in 95 children (20.5%), severe undernutrition in 64 children (13.8%). Mild overnutrition was identified in 4 children. Table 1 and Figure 2 below presents the nutrition status of the children, based on MUAC-z score readings.

Table 1. Nutrition Status of the Children (aged 6-13 years), South 24 Parganas District

Name of the School	Normal Nutrition	Mild Under Nutrition	Moderate Under Nutrition	Severe Under Nutrition	Mild Over Nutrition	Total
Path Bhawan, Chakberia.	11(19%	13(22%)	22(37%)	13(22%)	Nil	59
Hogolkuria, Joinpur,	15(31%)	18(38%)	12(25%)	3(6%)	Nil	48
Tarda, Uahapara	25(30%)	30(35%)	19(22%)	11(13%)	Nil	85
Bamangachi, Baruipur,	26(50%)	14(27%)	7(13%)	3(6%)	2(4%)	52
Shiksha Sathi, Raidighi	24(45%)	19(36%)	5(9%)	3(6%)	2(4%)	53
Purba Jata, Jogendrapur	13(32%)	15(36%)	9(22%)	4(10%)	Nil	41
Kheyadah, Challapara (1 and 2)	35(28%)	42(34%)	21(16%)	27(22%)	Nil	125
Total	149	151	95	64	4	463

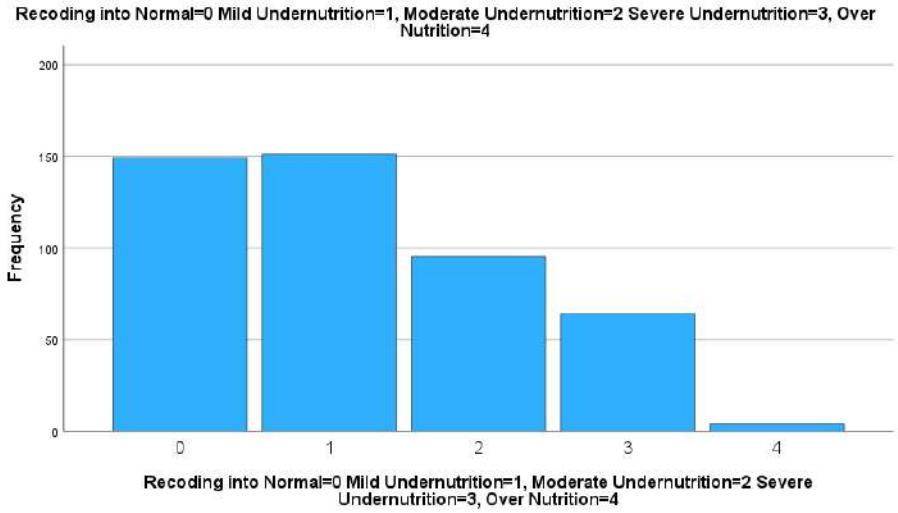

Fig 2. Nutrition Status of the Children (aged 6-13 years), South 24 Parganas District

The probable explanation for the different range of nutrition status in different school could be-

Path Bhawan, Chakberia: The area of this school is considered to be very poor in South 24 Pargana District. Most of the parents belong to the farmer community and are unaware of the importance of child nutrition which is reflected in the study of malnutrition.

Hogolkuria, Purba Jata and Ushapara: The population of these areas are mixed. People from salaried background as well as farmer background live in these areas. The salaried people are

somewhat aware of the importance of the nutrition of their children on the contrary people from farmer background are lacking about child nutrition which is reflected in the study.

Bamangachi, Baruipur and Shiksha Sathi, Raidighi: Most of the parents live in these areas are well-off and they are very much aware of the importance of proper nutrition for their children and can provide good nutrition to their children which is reflected in the study.

Kheyadah, Challapara: This village is in Kheyadah area, which is divided into two parts, lower area as Challapara 1, and upper area as Challapara 2. The lower area is dominated by the tribal population and many children of this area are deficient in nutrition. The upper area is populated by the salaried people and accordingly, their children were found to be comparatively better nourished.

The teachers informed about many children coming from very poor families and as such many of them remain sick several times in a year. Students get easily get tired during school hours, suffer from lack of concentration on studies and therefore not very attentive to their studies, as well.
Out of the 463 children analyzed, 256 (55%) were boys and the remaining (45%) were girls. Details of nutrition status in boys and girl is given in Figure 3. Pearson Chi-square test was performed to check if there were any significant differences between the nutrition status of boys and girls. Though malnutrition was detected in the study population, no significant difference was found gender wise (Refer Table 2), confirming that both the genders need equal attention regarding their food consumption pattern.

Table 2. Pearson Chi-square test

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	4.114[a]	4	.391
Likelihood Ratio	5.622	4	.229
Linear-by-Linear Association	.431	1	.512
N of Valid Cases	463		
a. 2 cells (20.0%) have expected count less than 5. The minimum expected count is 1.79.			

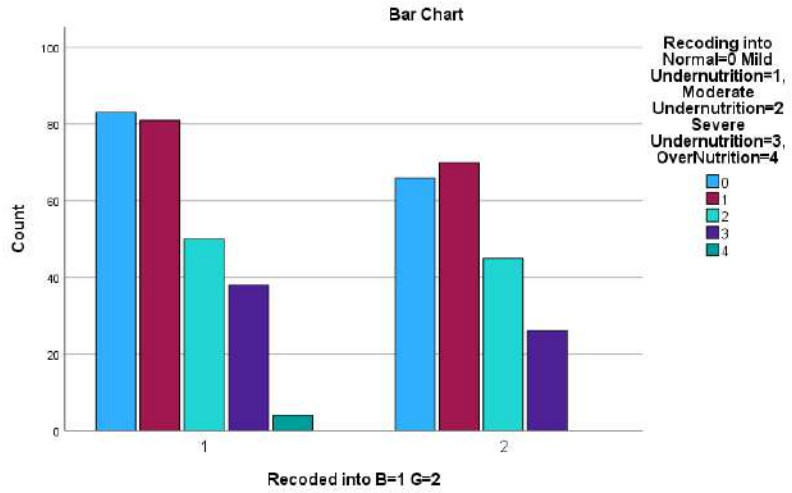

Fig 3. Distribution of Nutrition Status among Boys and Girls (aged 6-13 years)

Conclusion

South 24 Parganas has remained in focus number of development partners and government. Generation of awareness is needed [10]. There is a need for all-round political commitments from all the concerned stakeholders. Also, further application of MUAC *z*-score tape for screening in large scale and/or distinct population is essential to justify its reliability and acceptance in the Indian population.

Limitations of the study

MUAC Z-score tape though remains a cost-effective growth monitoring and malnutrition screening tool in children [11], it can have several limitations. It does not account for age or sex differences in body composition, potentially leading to underestimation or overestimation of malnutrition in certain groups, especially among older or larger children. MUAC is primarily useful for detecting acute malnutrition (wasting) and is not as effective for identifying chronic malnutrition (stunting) or micronutrient deficiencies, limiting its utility for a comprehensive nutritional assessment. It provides a single anthropometric measure without considering other important indicators like weight, height, or oedema, which are often required to fully assess the nutritional status, especially in mixed-etiology malnutrition cases [12,13,14]. Nevertheless, it can serve as a preliminary screening tool and needs to have a wider discussion in the scientific community.

Acknowledgement

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Navigating the Digital Landscape: Adolescent Mental Health

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Abstract

This study looked into how adolescents' psychological well-being was affected by their use of social media. The research design utilized a mixed-methods approach, integrating both qualitative and quantitative data collection techniques. According to the findings, there is a strong link between extensive social media use and poor mental health outcomes, such as anxiety, depression, and unsatisfactory body image. Qualitative analyses identified key themes such as cyberbullying, pressure, and social comparison to maintain a curated online image as contributing factors. The study emphasizes how critical it is to address any potential harm that social media may be causing to teenage mental health. It emphasizes the need for interventions to promote responsible social media use and provide support for adolescents who may be struggling with mental health issues. Future research should focus on developing effective strategies to mitigate the negative effects of social media and promote positive mental health outcomes among young people in the digital age.

Keywords: Digital age, mental health, technology, social media, cyberbullying

Introduction

The increasing apprehension regarding the influence of digital technology on mental health is warranted, given its substantial potential to impair an individual's emotional, social, and psychological well. Although research has linked excessive use of digital technology to poor mental health outcomes, especially in teenagers, the exact cause of these associations is still unknown [1]**.** Digital technology's constant connectivity might cause unhelpful changes in mood and social behaviour, which could endanger mental health. Furthermore, for both victims and cybersecurity professionals, cyberbullying breaches can have serious psychological repercussions such as anxiety, sadness, and burnout. It is imperative to carry out further research on this subject and support those who are experiencing mental health problems as a result of using digital technologies [2]**.** Social media's ubiquitous influence on daily life has sparked serious worries about how it can affect mental health, especially in young people. Excessive social media use has been connected in numerous studies to a number of detrimental effects on mental health, such as an increase in anxiety, despair, loneliness, and compulsive behaviours. Even though social media can be useful for connecting with people and getting information, its strong effect might make pre-existing mental health conditions worse [3]. Mental health is a serious problem in India that needs immediate care. The nation's mental health rules and laws are still insufficient, and there is a severe lack of mental health resources despite rising awareness. The COVID-19 epidemic has made the mental health issue worse, especially for vulnerable groups of elderly [2].

People's lives have been significantly shaped by social media, both positively and negatively. It has made communication and information access easier, but it has also helped false information and misinformation proliferate. Social, cultural, and economic issues all have an impact on the

complex and multidimensional effects of social media on mental health.
It has been difficult for several of India's most populous states to put in place efficient mental health policies and services. Progress has been hampered by a lack of awareness, coverage gaps, and limited resources. But there are other areas where things may be done better, like encouraging community-based mental health projects and making the most of technology's ability to provide mental health service [4].

People's mental health has been significantly impacted by the COVID-19 pandemic, especially the elderly. A study that was done during the pandemic discovered a significant relationship between elderly people's psychological suffering and their fear of dying. The epidemic has brought attention to the need for more funding for disadvantaged groups' mental health care.

The current problems in the mental health system and the negative effects of social media on mental health highlight the urgent need for all-encompassing solutions to this problem [5]. The nation may endeavour to enhance the mental health of its residents in the digital era by raising awareness of mental health issues, expanding access to mental health treatments, and putting evidence-based initiatives into practice. Teenagers living in the digital age are greatly impacted by the quick development of technology and the pervasive usage of social media. With so many avenues for self-expression and information gathering, social media platforms have emerged as essential tools for social interaction, communication, and information exchange [6].

Despite these advantages, there is rising worry about the possible harm that excessive social media may use psychological health. Examining the precise effects of social media on teenagers' mental health is crucial, as social media use is so common within this age group. The complex relationship between teenage psychological health and social media use is the focus of this study.

By addressing this research question, we can gain valuable insights into the factors influencing mental health outcomes and develop targeted interventions to promote positive mental health among teenagers in the digital age.

Positive Impacts of Technology on Mental Health

Social Connection and Support

The way individuals connect and engage with each other has changed with the advent of the digital age. Social networks have been made possible by social media platforms, online discussion boards, and messaging applications, which have also given people the chance to ask for help from others. Research has indicated that social relationships made on the internet might be advantageous for mental health, especially for people who might experience feelings of marginalisation or isolation in their offline life. For example, Twenge and Campbell's (2018) study discovered a correlation between young adults' increased usage of social media and lower rates of loneliness and despair [7].

Access to Mental Health Resources

People can now more easily access mental health options online, such as counselling services, support groups, and information. Online platforms provide a range of information and tools for treating mental health issues, including crisis hotlines, CBT programs, and mindfulness

applications. For those who reside in places where access to mental health specialists is restricted, these materials may be very beneficial [8].

Cognitive Stimulation

Technology use can present chances for mental workout and cognitive stimulation. Engaging in activities like learning new skills, solving puzzles, and playing games can enhance cognitive function and lower the risk of cognitive decline. Technology use can also foster invention and creativity, both of which are beneficial to mental health [9].

Negative Impacts of Technology on Mental Health

Cyberbullying and Online Harassment

The internet's accessibility and anonymity can serve as a haven for online abuse and cyberbullying. Through social media, messaging applications, and online forums, people may be the target of insults, threats, and other types of abuse. There are serious detrimental effects on mental health from cyberbullying and harassment, such as anxiety, depression, and even suicide ideation [10].

Social Comparison and FOMO (Fear of Missing Out)

FOMO and sentiments of social comparison might result from a steady stream of carefully chosen and perfected content on social media. When people see themselves in comparison to others, they may feel inferior or excluded. Depression, anxiety, and low self-esteem can all be exacerbated by this.

Sleep Disturbances and Addiction

Overuse of electronics, especially at night, can interfere with sleep cycles and exacerbate sleep disorders. It may be challenging to fall and remain asleep when blue light from electronics tampers with the body's normal sleep-wake cycle. Furthermore, some people may acquire technological addictions, such an unhealthy addiction to social media or gaming, which can have a detrimental effect on mental health.

Loneliness and Isolation

Technology can help people connect socially, but it can also exacerbate feelings of isolation and loneliness. Over-reliance on technology can reduce opportunities for genuine social relationships and cause a drop in in-person contacts. This may have detrimental effects on mental health, especially for those who are already vulnerable to loneliness.

Theoretical Framework

This study makes use of a number of pertinent theories to comprehend the intricate connection between teenage psychological health and social media use. According to the Social Comparison Theory, teenagers frequently compare themselves to other users on social media, which can have a detrimental psychological impact on them when they feel inadequate. The Self-Determination Theory places a strong emphasis on the role relatedness, competence, and autonomy play in fostering psychological health. Benefits and Rewards According to theory, people actively utilise

social media to find fulfilment, which might have an impact on their psychological well-being. These ideas offer a framework for comprehending the processes by which teenage usage of social media impacts mental health.

Gaps in the Literature

Although previous studies have advanced our knowledge of the connection between teenage mental health and social media use, there are still a number of unanswered questions. Studies that are tailored to the particular cultural and social contexts that influence this relationship must be conducted. In addition to quantitative methods, qualitative research can offer a more profound comprehension of the subjective experiences and viewpoints of adolescents. In addition, more investigation is required to find protective variables and practical treatments that can lessen the possible drawbacks of social media use and support teenagers' mental health outcomes.

Research Methods

A mixed-methods research approach will be used in this study to thoroughly examine the impact of teenage social media use on psychological health. A comprehensive knowledge of the phenomenon will be possible through the combination of quantitative and qualitative methodologies, enabling a deeper investigation of the experiences and viewpoints of adolescents.

Sampling

Participants will be chosen from a variety of schools using approaches for purposeful sampling. Teenagers that actively use social media platforms and are between the ages of 13 and 18 will make up the sample. In order to guarantee sufficient data collection for both the qualitative and quantitative components, the sample size will be chosen in accordance with the data saturation principle.

Data Collection

Both quantitative surveys and qualitative interviews will be used in the data collection process to capture different facets of social media use and its effects on the psychological health of adolescents.

• Quantitative Data: Based on validated measures and scales, a self-administered questionnaire will be created. This survey will evaluate teenage psychological well-being, platform kinds, and usage trends on social media. The survey may be given either in person or online.

• Qualitative Data: A subset of participants will participate in semi-structured interviews to gather detailed qualitative data on their individual experiences, viewpoints, and attitudes about using social media and how it affects their mental health.

Data Analysis

The study will employ descriptive statistics to provide an overview of the demographic traits, social media usage habits, and psychological well-being metrics. Examining potential mediating

factors, inferential statistics (correlation and regression analysis) will be used to investigate the association between teenage psychological well-being and social media use.

Result and Discussion

Quantitative Findings

Numerous important conclusions about social media use and teenage psychological health were obtained through quantitative study. A significant proportion of the participants (85%) reported using social media on a daily average for three hours. The most popular platform was Instagram, which was followed by Facebook and Twitter. In addition to sharing information and connecting with others, participants engaged in a variety of activities.
An examination revealed a strong inverse relationship between increased social media use and anxiety, despair, and unhappiness with one's body image. These results point to a negative effect of excessive social media use on the mental health of adolescents.

Qualitative Insights

In-depth understanding of teenagers' viewpoints and experiences with social media use and its effects on their psychological health was possible through qualitative analysis of interview data. Important themes that surfaced were as follows:

Social Comparison

When comparing oneself to idealised portrayals on social media, participants exhibited emotions of inadequacy and self-doubt.

Cyberbullying

Individuals reported experiencing cyberbullying, which resulted in heightened anxiety and reduced self-worth.

Pressure to Uphold a Curated Online Image

Participants experienced stress and low self-esteem as a result of feeling pressured to project a flawless online persona.

Table 1 reveals that the majority of participants in the study were **female** aged **16-18**, from **middle-socioeconomic backgrounds**. This demographic profile suggests that the sample was **representative of the adolescent population**.

- **Gender:** The higher proportion of female participants aligns with previous research indicating that females may be more likely to engage with social media and experience its associated mental health challenges.

- **Age:** The concentration of participants in the 16-18 age group is consistent with the peak years of social media usage among adolescents. This age group is likely to be more heavily engaged with social media platforms and influenced by their content.

- **Socioeconomic Status:** The majority of participants were from middle-socioeconomic backgrounds, suggesting that social media use is prevalent across various socioeconomic strata. While the study did not delve into the specific impact of socioeconomic status on mental health outcomes, it is important to consider this factor as it may influence access to resources and support.

Table 1: Demographic Characteristics of Participants

Characteristics	**Frequency**	**Percentage**
Age (Years)	13-15 16-18	59.5% 40.5%
Gender	Male Female	45% 55%
Class level	7-9 10-12	28% 72%
Socioeconomic Status	Low Middle High	17% 58% 25%

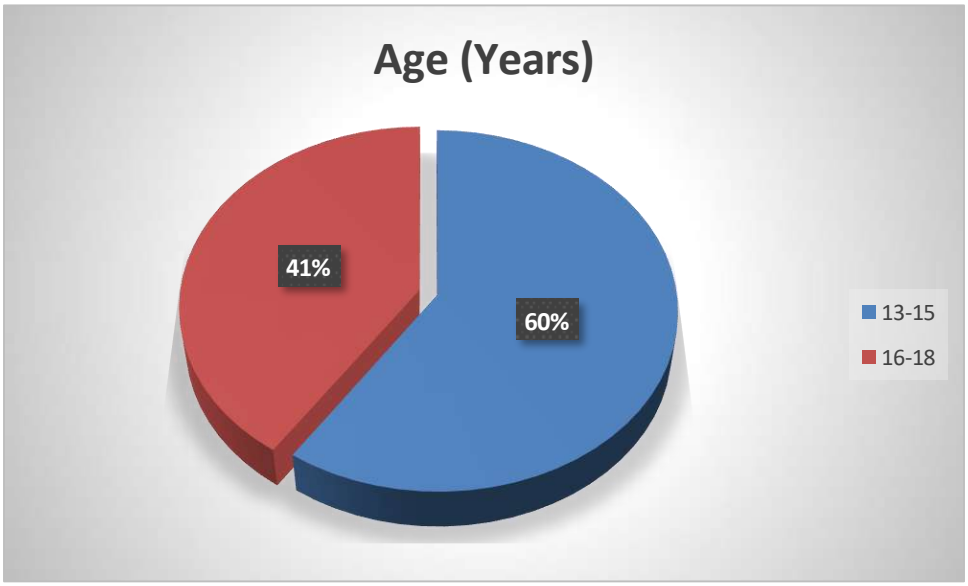

Fig 1: Pie Chart representing age of participants

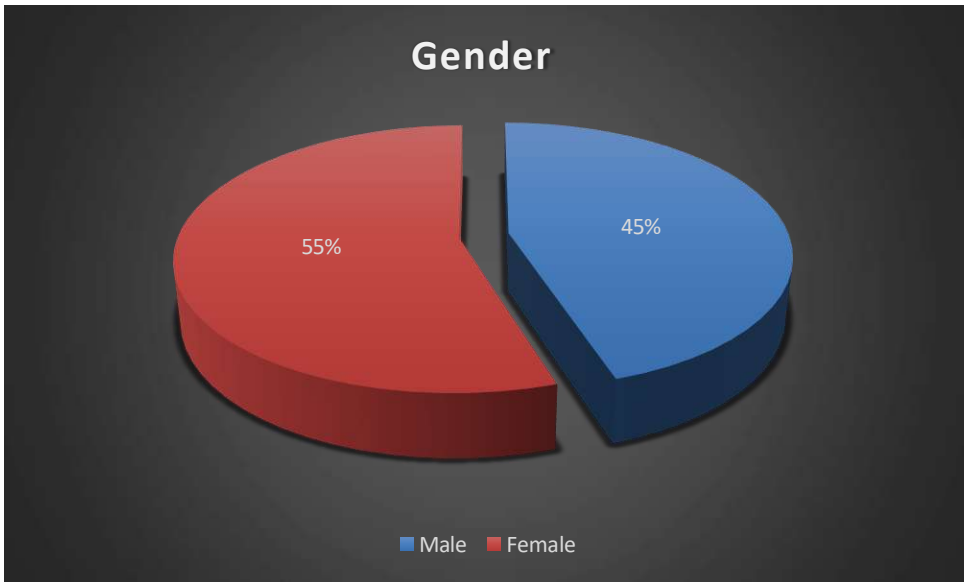

Fig 2: Pie Chart representing gender of participants

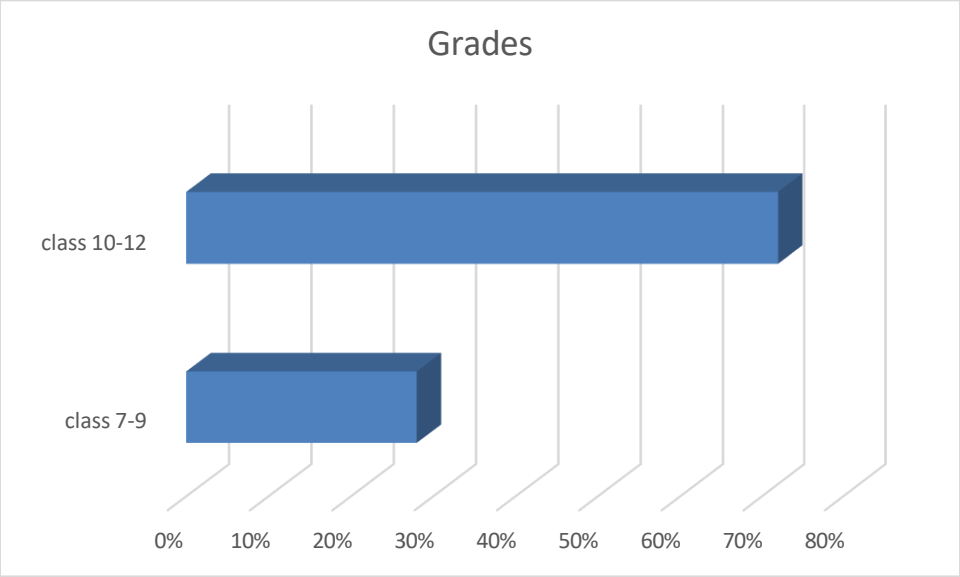

Fig 3: Bar diagram representing grades of participants

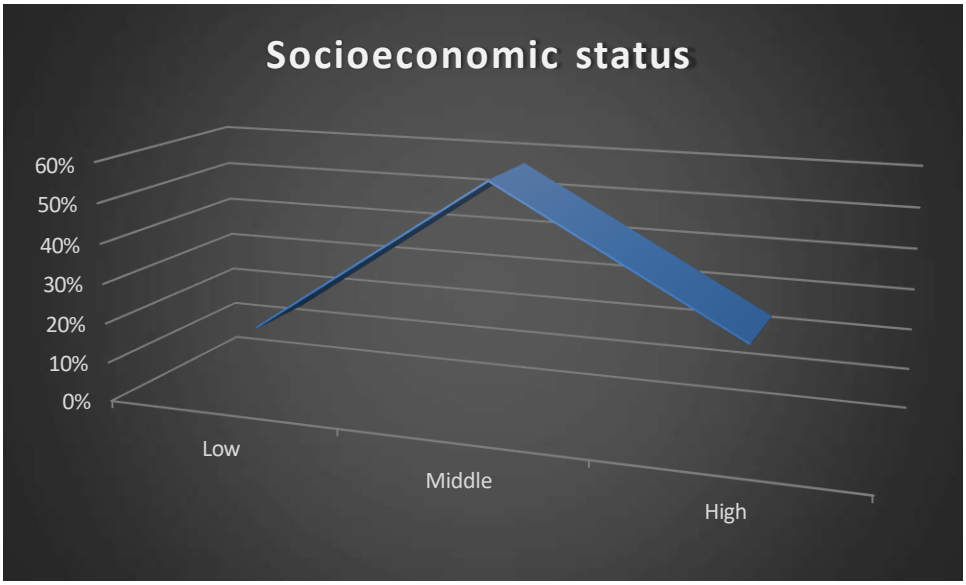

Fig 4: Line diagram representing socioeconomic status of participants

The data in table 2 indicate that social media is highly prevalent among adolescents, with a majority using multiple platforms daily. Instagram was the most popular platform, followed by Facebook and Twitter. The average daily usage of social media was 3 hours, suggesting significant time investment in these platforms.

Table 2: Social Media Usage Patterns

Platform	Frequency of Use	Average Daily Usage (hours)
Instagram	Daily	3
Facebook	Daily	2
Twitter	Daily	1
Others (e.g., TikTok, Snapchat)	Daily	1

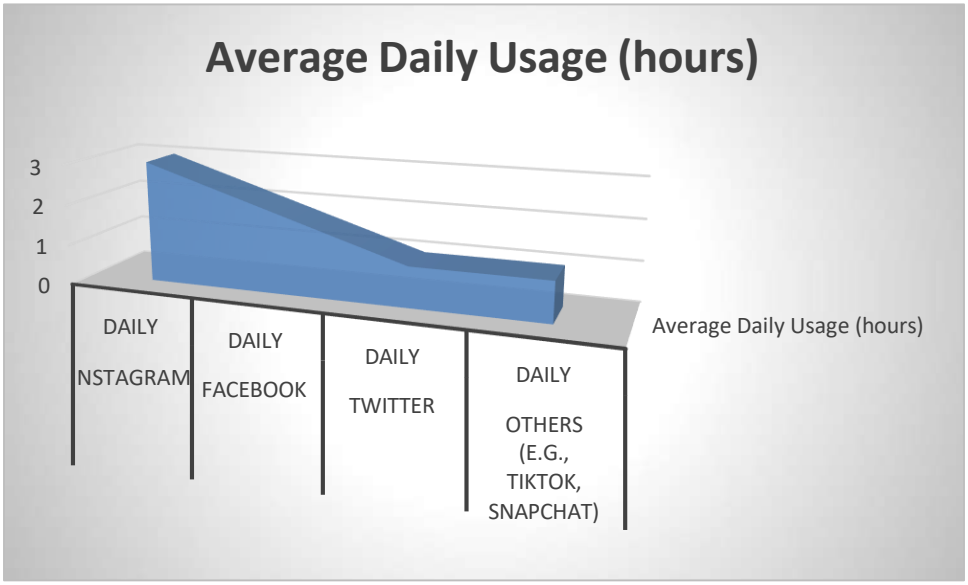

Fig 5: Line diagram representing average use of social platforms of participants

The correlation coefficients presented in the table 3 indicate the strength and direction of the relationship between social media use and the various psychological well-being variables.

Self-esteem: The correlation coefficient of -0.45 between social media use and self-esteem suggests a moderately strong negative relationship. This means that as social media use increases, self-esteem tends to decrease. In other words, individuals who use social media more frequently may be more likely to experience lower self-esteem.

Body image dissatisfaction: The correlation coefficient of 0.32 between social media use and body image dissatisfaction indicates a positive relationship, though it's not as strong as the relationship with self-esteem. This suggests that individuals who use social media more frequently may be more likely to experience body image dissatisfaction.

Depression: The correlation coefficient of 0.50 between social media use and depression indicates a strong positive relationship. This suggests that individuals who use social media more frequently may be more likely to experience depressive symptoms.

Anxiety: The correlation coefficient of 0.48 between social media use and anxiety indicates a strong positive relationship. This suggests that individuals who use social media more frequently may be more likely to experience anxiety.
Overall, the data suggest that higher levels of social media use are associated with lower self-esteem, increased body image dissatisfaction, depression, and anxiety.

Table 3: Correlation between Social Media Use and Psychological Well-being

Variable	Correlation Coefficient (r)	Significance (p-value)
Self-esteem	-0.45	<0.01
Body image dissatisfaction	0.32	<0.02
Depression	0.50	<0.01
Anxiety	0.48	<0.00

Discussion

The amalgamation of both quantitative and qualitative data offers a thorough comprehension of the impact of teenage social media usage on their psychological welfare. While the quantitative results show a negative relationship between social media use and mental health outcomes, the qualitative insights provide important background information on issues including cyberbullying, social comparison, and the pressure to maintain a well-curated online persona.

Concern over mental health in the digital era is on the rise, especially in light of how social media affects teenagers. Social media use has been related in research to psychological stress, especially when consuming excessive amounts of social media or interacting with politically heated content.

Overuse can result in addiction, which has a negative effect on mental health by causing stress, worry, depression, and loneliness. Physical problems like increases in blood pressure and hypertension are examples of long-term repercussions. Additionally, excessive social media use can cause eye health problems and sleep disturbances.

There is still little data on the connection between teenage mental health and social media use, despite growing worries. Most research has been done on adults and has yielded conflicting results. Although a recent study indicated a weak relationship between teenage well-being and social media use, it did not prove causation and had no application. Social media can, however, also have advantageous effects, such as giving people a place to socialise and get social support.

People who are struggling with mental health issues might look for resources and assistance online. Information forwarding was not significant, according to a study that looked at information-seeking and information-forwarding practices, although information seeking was positively correlated with coping results.

It is essential to inform teens and their families about the advantages and disadvantages of social media use in order to address the possible detrimental effects on adolescent mental health. In order to lessen the psychosocial risks connected with excessive social media use, parents and medical professionals should use caution and take appropriate action. Social media usage in excess and exposure to certain kinds of content can be detrimental to mental health, even if it can be a useful tool for social contact and support. Patients and healthcare providers need to be aware of these possible dangers and take preventative measures to lessen them. To fully comprehend the intricate connection between social media use and mental health, more research is required.

Limitations and Conclusion

Limitations

This study has several limitations that should be acknowledged.

- First, the cross-sectional design of the study limits the ability to establish causality between social media use and adolescent psychological well-being. Longitudinal studies are needed to examine the temporal relationship between these variables.
- Second, the study relied on self-reported data, which may be subject to biases such as social desirability bias.
- Third, the sample was limited to adolescents, which may limit the generalizability of the findings to other populations.

Conclusion

This study provides valuable insights into the relationship between social media use and adolescent psychological well-being. The findings highlight the potential negative consequences of excessive social media use, including lower self-esteem, body image dissatisfaction, depression, and anxiety. However, it is important to note that the relationship between social media use and mental health

is complex and multifaceted, and individual factors, such as personality traits, parental involvement, and cultural context, can influence the impact of social media.

Future research should address the limitations of this study, such as conducting longitudinal studies and using diverse sampling methods. Additionally, further research is needed to identify effective interventions to mitigate the negative effects of social media use and promote positive mental health outcomes among adolescents.

In conclusion, while social media can offer numerous benefits, it is essential to be aware of the potential risks and take steps to promote responsible social media use and support adolescent mental health. By understanding the complex relationship between social media and mental health, we can develop effective strategies to protect the well-being of young people in the digital age.

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Impact of Educational Intervention on Knowledge, Attitude and Practice Towards Malaria Among Graduate Students of Pharmacy

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Abstract

An assessment of the Pharmacy students' knowledge, attitudes and practices regarding malaria was carried out. Malaria is a protozoan disease caused by any one or combination of four species of plasmodia mosquito. As per World Malaria Report estimate there happened 249 million instances in 2022. The Knowledge Attitude Practice [KAP] assessment was created as a Google form and the link to it was sent to B.Pharm students of Eminent College of Pharmaceutical Technology. Following eligible submission, pre- and post-educational interventions were evaluated. 160 individuals provided appropriate responses to the KAP survey. Knowledge, attitude and practice parameters consisted of seven (07) questions each. An educational intervention led to an improvement in KAP result. In Knowledge parameter, pre-educational intervention, 32.58% correct answers were reported against 100% post-educational intervention. In Attitude parameter, prior to educational intervention, 45.89% was the percentage of correct answers against 100% post-educational intervention. In Practice parameter, pre-educational intervention again improved the number of correct responses from 25.41% to 100% post-educational intervention. Our findings indicate a significant shift in post-intervention KAP result. The study showed that less number of students knew about malaria beforehand. After educational intervention, we were able to instill into the minds of students, knowledge attitude and practice towards malaria. To increase pharmacy students' understanding of malaria, continuous educational interventions are needed.

Keywords: Knowledge, Attitude, Practice, Malaria, Pharmacy graduate students

Introduction

Five kinds of Plasmodium parasites, namely Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium knowlesi and Plasmodium vivax, are responsible for human malaria and are spread by the bite of female Anopheles mosquitoes [1]. An estimated 249 million instances of malaria were reported in 2022, according to the most recent World Malaria Report. In 2022, 608000 malaria fatalities occurred [2]. In India, in the year 2022, according to official reports, malaria positive cases stood at 176522 while deaths were 833. Fever (>92%), chills (79%), headaches (70%), and diaphoresis (64%) were experienced by most patients. Additional typical symptoms included dry cough, dizziness, myalgia, moderate diarrhoea, nausea, malaise and vomiting. Severe malaria can result in neurologic complications such as malaria of the brain.

Acute lung damage generally develops just a couple days into the illness course. Malaria can also lead to acute renal failure. Members with serious malaria often have hypoglycaemia [3,4].

The behavioral aspects are related to certain cultural behaviors that encourage mosquito reproduction and mosquito access to humans, as well as the lack of action by the population considered to be "at risk" to promptly and effectively adopt established technology for malaria treatment, control, and avoidance [5]. The existence of plasmodia, mosquito availability, and geographical or environmentally distinct variations are examples of non-behavioural variables. Both behavioral and non-behavioral determinants play an important role in the development of effective malaria interventions. Pharmacists play an important role as an observer for WHO guidelines for malaria, in context to general public. They are part of health care team whose function is to ensure the malaria disease prevention [6].

The aim of the study was to assess the impact of educational interventions on the knowledge, attitude, and practice of pharmacy students about malaria. For this study, we thus conducted a study on B.Pharm students on their KAP regarding malaria.

Method

The Knowledge Attitude Practice [KAP] questionnaire was prepared in October 2023 using Google Forms. The link to the Google form was sent to ECPT B. Pharm students using different social media platforms in Semesters 1, 3, 5, and 7. Exclusion criteria were ex-students of ECPT and other college students. They were instructed to fill out the online form using only their own knowledge and without assistance from any other source. The students received notification that the link labeled as "accepting responses" would close after 72 hours. The link was shut down after 72 hours, and the pre-intervention data was compiled. The respondents were then given the intervention consisting of correct answers along with notes. A week after the intervention, only participants who had previously completed the same Google form were given access to it once more. The same timeline and approach as before were used. Proper and appropriate validation of the KAP questionnaire was completed prior to its distribution via Google Form. Google Form consisted of two parts. First part was consisted of identity of the respondent. Second part consisted of Knowledge Attitude Practice questions, each 07 numbers.

Results

After explaining the study to the students, a total of 160 pharmacy students of the college representing both genders participated in this study, who answered the KAP questionnaire accordingly. Accordingly means those respondents who filled all parts of the questionnaire in proper time line.

For knowledge parameter, pre educational intervention only 02 out of 160 gave correct response for question number 2 which was regarding the scientific name of the parasite responsible for the disease. Post education intervention, all participants gave correct response. Correct answer percentage increased from 01.25% to 100%, post educational intervention. For knowledge parameter, pre educational intervention only 03 out of 160 gave correct response for question number 3 which was regarding continent which bore highest number of the disease. Post education

intervention, all participants gave correct response. Correct answer percentage increased from 01.87% to 100%, post educational intervention. (Table 1)

Regarding whether malaria is a preventable disease or not, attitude parameter question number 6, pre educational intervention only 05 gave correct response. Participants giving correct response increased to 160 out of 160, post education intervention. Positive swing in correct answer percentage from 03.12% to 100% was noticed after counseling. Regarding effectiveness of methods of malaria prevention, attitude parameter question number 3, pre educational intervention only 07 gave correct response. Participants answered correctly increased to 160 out of 160, post intervention and its percentage change was from 04.37% to 100%. (Table 1)

The very first question in the practice domain was whether the subject uses mosquito net or not. Only 08.75% respondents out of 160 used bed nets for prevention of mosquito bite. After intervention, all agreed on the benefit of usage of nets. Students indicated that they did not receive any formal patient oriented clinical training on malaria. Hence this intervention proved the need of the day as after the education all indicated satisfaction in this practice parameter. (Table 1)

Table 1. KAP responses

Knowledge	**Pre-Educational Intervension (160 Nos.)**		**Post-Educational Intervesion (160 Nos.)**	
	Correct Response	**Percentage (%)**	**Correct Response**	**Percentage (%)**
1. What is the primary vector responsible for transmitting malaria?	160/160	100%	160/160	100%
2. Which parasite causes malaria in humans?	2/160	1.25%	160/160	100%
3. Which continent has the highest burden of malaria?	3/160	1.87%	160/160	100%
4. What is the most common symptom of malaria?	30/160	18.75%	160/160	100%
5. Which anti malarial medication is derived from the bark of the cinchona tree?	6/160	3.75%	160/160	100%
6. Which age group is most vulnerable to severe malaria?	4/160	2.5%	160/160	100%

7. What is the recommended preventing measure for travelers to malaria endemic areas?	160/160	100%	160/160	100%
ATTITUDE	--		--	
1. On a scale of 1 to 4 how concerned malaria in your community?	13/160	8.12%	160/160	100%
2. Do you believe that malaria is a serious health threat in your area?	160/160	100%	160/160	100%
3. Do you trust the effectiveness of malaria prevention methods?	7/160	4.37%	160/160	100%
4. Are you willing to take steps to protect yourself and your family from malaria?	160/160	100%	160/160	100%
5. Are you satisfied with the accessibility of malaria treatment in your community?	9/160	5.62%	160/160	100%
6. Do you think malaria is a preventable disease?	5/160	3.12%	160/160	100%
7. Are you willing to participate in malaria control initiative, such as spraying camping?	160/160	100%	160/160	100%
PRACTICE	--		--	
1. Do you or your family members use bead nets to prevent mosquito bites?	14/160	8.75%	160/160	100%
2. Have you ever taken anti-malaria medications for prevention ?	121/160	NA	0/160	NA

3. How often do you seek medical care when you suspect you have malaria ?	18/160	11.25%	160/160	100%
4. If diagnosed with malaria, did you complete the prescribed treatment ?	42/160	26.25%	160/160	100%
5. Do you know how to recognize malaria Symptoms in yourself on others?	153/160	95.62%	160/160	100%
6. Have you received education on training on Malaria prevention and treatment?	0/160	0%	160/160	100%
7. Have you ever been use mosquito repellent?	117/160	10.62%	160/160	100%

Discussion

The study showed that less number of students knew about malaria. After educational intervention, we were able to instill into the minds of students, knowledge attitude and practices towards malaria.

A KAP study was conducted by Gupta et al., 2019. The survey had 250 respondents in total, of which 148 (59.2%) and 102 (40.8%) were men and women, respectively.14.8% of respondents had ever experienced malaria, while nearly 98.4% of respondents had heard of the illness. Approximately 96% of participants were aware that mosquito bites cause malaria, and 94% were aware that malaria is preventable. The majority of responders (72%) were even aware that malaria may be lethal. Of the responders, 131 (52.4%) reported having a fever and chills, whereas 29 (11.6%) were unaware of the signs of malaria. The majority of responders (79.2%) were aware that standing water serves as a mosquito's resting location. TV (45.6%) was the primary source of information on malaria, followed by hospitals (22.4%).Nearly 90.4% of respondents had a favourable attitude towards mosquito control methods, and the majority of participants (80.4%) thought that malaria was a severe health issue. The majority of respondents (69.6%) did not use bed nets, while 86.4% of them routinely cleaned their surroundings. The majority of responders (65.2%) used windows mesh or nets as their primary mosquito control method, followed by coils (29.2%)[7].

A KAP study was conducted by Chand G and Soan V, 2019. 239 respondents were interviewed in the study. Merely 37.6% of participants were aware that mosquitoes carry malaria. The majority of respondents (94.2%) said that anyone may have malaria and did not know that it is a deadly illness. Just 27.2% of those surveyed believe that fever is caused by malaria. Only 14.6% of people knew about the medicine chloroquine, and very few people knew about the

malaria medication. The respondents' primary prophylactic method against mosquito bites is smoking. Just 5% of those surveyed said they used a mosquito net. The majority of respondents was aware of the significance of pesticide spraying and preferred to have only the cow shelters treated[8].

A KAP study was conducted by Ismail N and Jimam N, 2019. Study comprised of 239 respondents. Approximately 89 out of every 100 patients were aware that malaria is primarily spread through mosquito bites. Additionally, 88.5 of them knew that children and pregnant women face a higher risk of contracting the illness. Furthermore, 86.9% of the patients could correctly identify a rise in body temperature as a symptom of uncomplicated malaria, and 84.2% recognized body weakness as another accurate indicator of the disease. Regarding their expertise on medicines used to treat malaria, the majority (53.2%) were aware that the recommended treatment for uncomplicated malaria is an artemisinin-based combination therapy. However, many (45.0%) were uncertain about the role of sulfadoxine-pyrimethamine in managing the disease. While 32.5% believed it was the recommended drug for uncomplicated malaria, 22.5% disagreed with this. Additionally, a significant percentage (35.3%) of participants rejected the idea that chloroquine is the recommended anti-malarial drug, with a similar proportion (34.6%) agreeing that it is the recommended treatment[9].

A KAP study was conducted by Lopez AR and Brown CA, 2020. Study comprised of 316 respondents. The majority of participants (85.4%) possessed a solid understanding of malaria. The preferred option for seeking treatment was the health center/clinic (50.6%). Notably, all respondents expressed a willingness to seek treatment within 24 hours. Mosquito coils were the most popular choice (58.9%) for protection against mosquito bites. However, a significant proportion of households (58.5%) lacked bed nets, and the usage of bed nets was low (10.1%). Encouragingly, nearly half of the respondents (49.4%) exhibited a positive attitude towards malaria, and 40.5% demonstrated good practices[10].

In our study all 100% respondents knew the primary vector responsible for transmitting malaria. This is in line with the works done by Ismail N 2019 and Gupta 2019 where 89% and 96% respondents gave correct answer. 18.75% respondents in our pre-interventional stage answered correctly the most common symptom of malaria. In the work done by Gupta 2019, 10.8% respondents identified malaria symptom as fever with chills and body pain. Malaria is a serious health threat. In our study all 100% respondents expressed their concern. Around 80% respondents agreed to this in Gupta 2019 study. 8.75% respondents in our study said they use bed nets against Gupta 2019 report of 30.4%.

Conclusion

The KAP of students of pharmacy towards malaria may increase with the provision of professional patient oriented training and educational awareness.

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Conflict of Interest

The authors declare there is no conflict of interest.

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Formulation and Evaluation of Gastroretentive Floating Tablets of Plumbagin

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Abstract

The present research study focuses on the formulation and evaluation of gastro-retentive floating tablets of Plumbagin by wet granulation method with combination polymers. To achieve the necessary floating features and controlled drug release, the floating tablets were manufactured utilizing a wet granulation process with different polymers. Sodium bicarbonate and citric acid were used to release CO_2 gas to maintain buoyancy. To achieve the necessary floating features and controlled drug release, the floating tablets were manufactured utilizing a wet granulation process with different polymers. Based on a zero-order kinetic model, the findings showed that the optimized formulation had a floating lag time of less than 2 min and sustained drug release for 9- 10 h. The floating tablets showed promise for better stomach retention and increased Plumbagin bioavailability throughout the release period by remaining buoyant and intact. With a longer gastric residence period and regulated drug release, F4 formulation demonstrated potential as an efficient gastroretentive tablet. It is recommended that more *in vivo* research be done in order to validate these results.

Keywords: Plumbagin, floating tablets, gastroretentive drug delivery system, gastric residence time, controlled drug release

Introduction

Over the past few decades, various advancements have been achieved in the development of novel drug delivery systems, thereby resolving issues such as short gastric residence time (GRT) and unpredictable stomach emptying time. By addressing these obstacles, researchers have developed gastro-retentive formulations that enable the targeted delivery of drugs with a restricted 'absorptionwindow', or those that are only absorbed in specific gastrointestinal tract (GIT) regions [1]. Oral drug administration is the most promising and recommended route of delivering any drug to the systemic circulation [2].The drugs that have short half-lives and are readily absorbed via GIT are quickly removed from the systemic circulation. Such drugs are required to be dosed frequently in order to produce the intended therapeutic effect. In an attempt to overcome this limitation, oral sustained release formulations were developed, which release the drug gradually into the GIT andmaintain plasma concentration of drug for a considerable amount of time [3].

Gastro-retentive drug delivery systems (GRDDS) extend GRT of the drug and increase the bioavailability of drugs with either systemic or local effects that are absorbed at a specific site in the stomach or upper GIT. Prolonged stomach retention increases drug solubility, lowers dosage requirements, and enhances bioavailability of drugs that are poorly soluble in the high pH environment of the small intestine [4,5]. Floating tablets are a type of GRDDS that floats in the stomach and remains buoyant for prolonged duration without retarding the stomach emptying rate [6]. In the current study, the wet granulation method was used to formulate gastro-retentive floatingtablets of Plumbagin by combining two distinct polymers, Xanthan gum and HPMC E 15 LV, in varied ratios. The produced tablets were further assessed for hardness, *in vitro* drug release, swelling, buoyancy, weight variation, friability, content uniformity and stability analysis [7,8].

Materials and Methods

Materials

Plumbagin was purchased from P.C. Chem, Mumbai, Maharashtra, India. Hydroxypropyl methyl cellulose (HPMC E 15 LV), Magnesium stearate, Talc, and Xanthan gum were procured from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India. Rest other chemicals were used of analyticalgrade.

Methodology

Determination of UV absorption (λ_{max}) of drug

Drug stock solutions containing (1 mg/100ml) in 0.1 N HCl (pH 1.2) were produced. Then the solution was scanned at the range of 200-800 nm by using UV-visible spectrophotometer for determining λ_{max} of the drug.

Drug excipients interaction study

The Fourier transform infrared (FTIR)spectrum of Plumbagin (drug) was compared with the spectra of physical mixture of drug with all the polymers using FTIR Spectrophotometer in the range of 4000-400 cm^{-1}. The base line correction was done using dried KBr. By using KBr disc method, drug, HPMC E 15 LV, PVP K-30, Xanthan gum, physical mixture, and Plumbagin floating tablet were analyzed. No shifting of any characteristics peaks was observed in case of the physical mixture of drug and polymers, which suggests that drug and polymers used in the tablets do not chemically interact with each other. The occurrence of the characteristic peaks assures theauthenticity of the materials employed in the study [9].

Determination of melting point

The determination of melting point of Plumbagin was carried out by using capillary tube technique.To determine the melting point of the drug, melting point apparatus was used. A10-15 mm long capillary tube was loaded with fine powdered drug and it was sealed at one end previously. The capillary tube was tied to a thermometer and was subjected to elevated temperature, and the temperature at which the drug melted was recorded [9].

Solubility studies

To determine the solubility of the drug,1 g of drug was accurately weighed and transferred to the volumetric flasks containing varying solvents such as methanol, ethanol, water, 0.1N HCl and DMSO, respectively [10]. The solutions were then filtered through 0.45µ membrane filter and the filtrate was suitably diluted with the solvents and analyzed using UV Spectrophotometry at 265 nm.

Preparation of Plumbagin floating tablets

By conventional wet granulation method, the different batches of the floating tablets were formulated by implementing Digicon tablet punching machine. The drug and other ingredients in each formulation were weighed accurately and mixed properly in mortar with pestle, for 5 min without magnesium stearate and talc. PVP K-30 ethanolic solution was used as granulating agent. The solution was added dropwise until suitable mass was produced. By using sieve 40# the wet mass was passed. The granules were dried at 50°C in hot air oven for 30 min and then mixed with magnesium stearate and talc in the weight proportion as mentioned in table 1 and then compression was done with tablet punching machine (Digicon pharma machinery, Ahmadabad, India).

Table 1. Formulation composition of Plumbagin floating tablets

S.No.	Ingredients	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)
1.	Plumbagin (Drug)	20	20	20	20
2.	HPMC E 15 LV	45	50	55	60
3.	Xanthan Gum	55	50	45	40
4.	Citric acid	15	15	15	15
5.	Sodium bicarbonate	30	30	30	30
6.	PVP K-30	05	05	05	05
7.	Magnesium stearate	05	05	05	05
8.	Talc	10	10	10	10
	Total weight (mg)	185	185	185	185

Post compression parameters of Plumbagin floating tablets

Thickness

The diameter and thickness of the tablets was evaluated by using calibrated Vernier calliper. Five tablets from each batch were chosen at random and their thicknesses were determined separately. In the tablet formulation, the extent of deviation should not exceed the limit of ± 5% of their determined values [11,12].

Hardness

The hardness of the tablets was examined using Monsanto hardness tester. Ten tablets were chosen at random and their hardness (kg/cm^2) was examined. The hardness of the tablets should be in the range of 4-10 kg, as per IP [12, 13].

Friability

The friability of all the tablet formulations was examined by using USP friability test apparatus (Roche Friabilator). From each formulation ten tablets were chosen at random; initial weight (w_1) was noted and tablets were placed in the Roche Friabilator for 4 min at 25 rpm. Subsequent to 100 revolutions, the tablets were removed, de-dusted, and weighed again(w_2). Percentage friability of less than 1% is considered to be within limit [12]. The percentage friability (%F) was calculated by equation:

$$\%F = [(w_1 - w_2)/w_1] \times 100$$

Weight variation

The weight variation of the tablets was evaluated by selecting 10 tablets at random from each batch.The tablets were then weighed in a digital balance. The average weight was noted and was compared with the individual weight of the tablet. Not greater than weight of two individual tabletsshould deviate from the average weight by more than ±7.5% [11, 13].

Drug content uniformity test

The drug content in each batch was evaluated by randomly selecting 20 tablets; each individual tablet was weighed and then triturated using mortar and pestle. The weight of powder equivalent to the weight of one tablet was transferred into a 100 ml volumetric flask and volume was made up to the mark using 0.1 N HCl [12]. After 24 h, the sample was withdrawn (about 1 ml) and diluted with 10ml of 0.1NHCl in another volumetric flask. The sample was then filtered through 0.45µ membrane filter. Using 0.1 N HCl as a blank, the filtered solution was analyzed for drug content at 265nm by UV-visible spectrophotometer (Shimadzu, UV-1900) [14].

In vitro buoyancy study

The buoyancy of the tablets was assessed by floating lag time method. The tablets were transferred in a 100 ml beaker which contains 0.1N HCl. The time taken by the tablet to float upto the surface was considered as floating lag time (FLT) and the total duration upto which the tablet remained buoyant is considered as total floating time (TFT) [12].

Swelling study

To determine the swelling index of the tablet batches, the individual weight of the floating tablets was noted (W_0) and placed in different glass beaker of 100 ml with 0.1 N HCl and incubated at 37 °C ± 1 °C. After every hour, the tablet was removed from beaker and carefully wiped off excess surface liquid using the tissue paper and weighed again, upto 8 h [12, 15]. The tablets were further re-weighed (W_t) and swelling index (SI) was determined by the following formula:

$$SI\% = [(W_t - W_0) / W_0] \times 100$$

Where, SI = swelling index,

W_t = Weight of tablet at time, t

W_0 = Weight of tablet before immersion

In vitro drug release study

The *in vitro* dissolution study of drug loaded floating tablets was analyzed by using USP type II (Paddle type) dissolution testing apparatus (Electro lab). By using 900 ml of 0.1N HCl at 37± 0.5 °C temperature and 50 rpm speed, the dissolution test was performed. From the dissolution testingapparatus, 10 ml sample solution was withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12h andsame volume of fresh dissolution medium was replaced after each withdrawal. Then the samples were filtered through a 0.45µ membrane filter and diluted to a suitable concentration with 0.1 N HCl. The absorbance of these solutions was measured at 265 nm using a UV-visible spectrophotometer (Shimadzu, UV-1900). The cumulative percentage drug release was determined by using an equation which was obtained from a standard curve [16, 17].

Stability analysis

According to ICH and WHO guidelines, stability study was carried out. All the tablets were sealedwith aluminium packing lined with polyethylene on the inner side, and samples were kept in humidity chamber (Thermo labs, Mumbai) at 40°C ± 2°C and 75% ± 5 % RH for 3 months. Subsequently, the samples were examined for hardness values, drug content, floating behavior, and *in vitro* dissolution studies [13].

Results and Discussion

Determination of UV Absorption (λ_{max}) of Plumbagin

The UV-spectrophotometric method was used for estimation of Plumbagin. Plumbagin stock solution (10 µg/ml) was scanned by UV-Visible Spectrophotometer (UV-VIS 1900, Shimadzu) in the wavelength of 200-800 nm. The obtained spectrum had the wavelength of maximum absorption (λ_{max}) at 265 nm in 0.1N HCl (pH 1.2) (Fig.1).

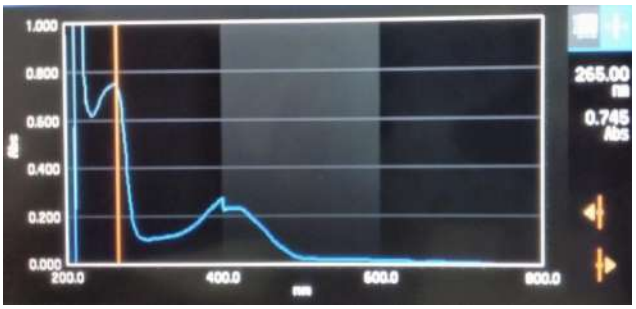

Fig.1. UV spectrum of Plumbagin in 0.1 N HCl

Fourier transform infrared (FTIR) spectroscopy

The Fourier transform infrared (FTIR) spectroscopy analysis was carried out by FT-IR Perkin Elmer spectrometer in KBr dispersion in the range of 4000-400 cm^{-1} to assess any potential chemical interactions between the drug and excipients.

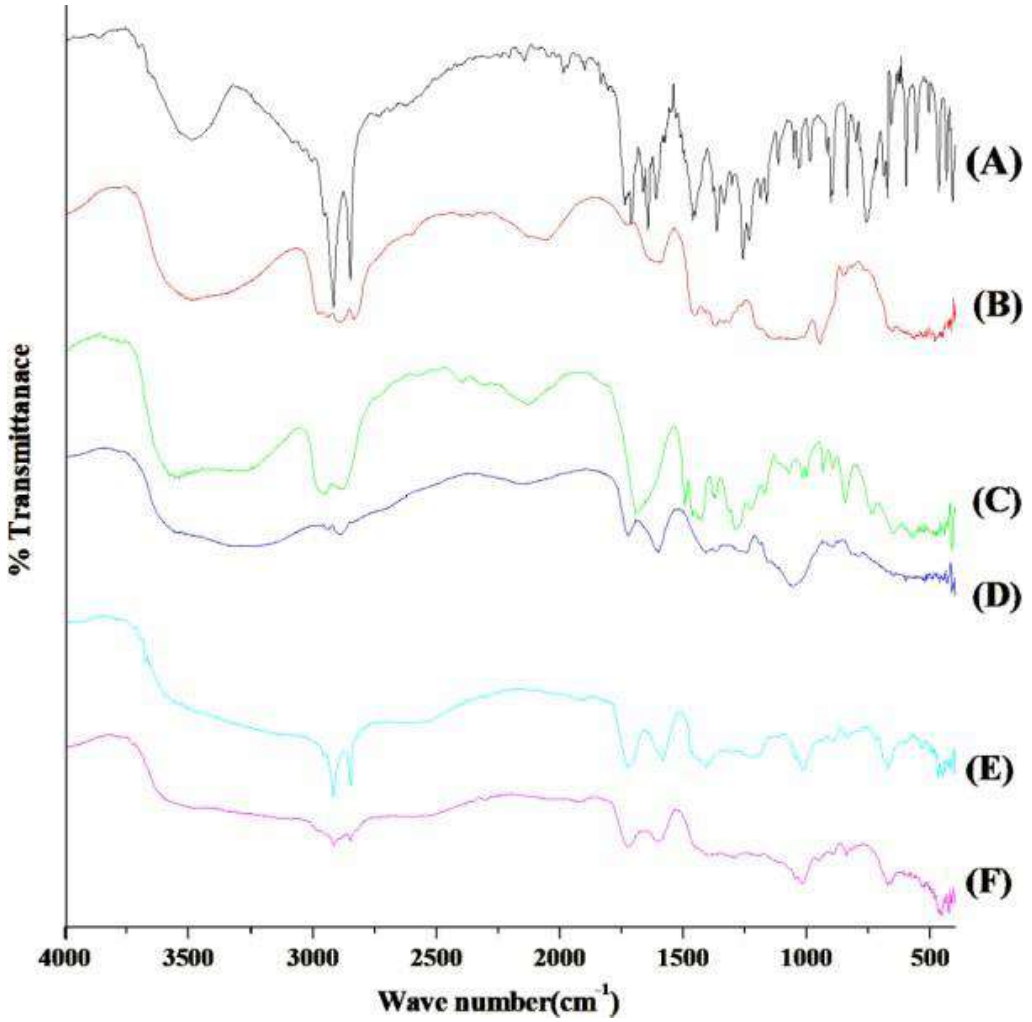

Fig.2. FTIR spectra of (A) Drug (Plumbagin) (B) HPMC E 15 LV (C) PVP K-30 (D) Xanthan Gum (E) Physical mixture (F) Plumbagin floating tablet

The infrared transmittance spectrum analysis of drug revealed the presence of alcohols and phenols, O-H (strong broad signal at 3439 cm^{-1}), alkanes group, H-C-H (asymmetric and symmetric stretch at 2858.28, 2922.34 cm^{-1}), ketone group, C=O (sharp signal at 1648.25, 1751.03 cm^{-1}), diethyl ether, C-O (stretch signals at 1045.34, 1112.76, 1256.71 cm^{-1}), amine group, N-H (stretch signalsat 1454.40 cm^{-1}) (Fig.2).

The IR spectrum of drug, excipients, physical mixture and floating tablet reveled that no detectable interaction of the excipients with drug was observed. In the floating tablet spectra, it was clearly observed that the major characteristic IR peaks of the drug was present, indicating that the molecular structure of drug was maintained completely intact in the tablet formulation. Hence, it could be concluded that the drug and the selected excipients did not interact in any way that was noticeable.

Determination of melting point

By using capillary tube method, the melting point of Plumbagin was determined. It was found to be quite identical to the already reported melting point of Plumbagin, and was found to be 75-77 °C.

Solubility studies

The solubility studies of the drug in different solvents (organic and aqueous) were carried out. It was noticed that the drug was readily soluble in organic solvents such as methanol (4.8 mg/ml), ethanol (4.9 mg/ml), and DMSO (10 mg/ml) and very slightly soluble in water (0.6 mg/ml).

Pre-compression parameter of Plumbagin granules

The pre-compression parameters results are tabulated in (Table 2). All the pre-compression evaluation parameters were within the USP Pharmacopoeia limits. The bulk density of granules was in the range of 0.575 ± 0.048 to 0.624 ± 0.043 gm/ml and the tapped density of granules was found to be between 0.679 ± 0.057 to 0.691 ± 0.053, respectively. Hausner's ratio ranged from 1.107 ± 0.075 to 1.18 ± 0.011 and Carr's index was found to be between 9.69 ± 1.74 to 15.44 ± 2.30, indicating good flow characteristics. The results of angle of repose ranged from 24.625 ±
0.374 to 29.653 ± 0.784 indicates good flow property of the granules. All formulations revealed satisfactory flow properties.

Table 2. Flow properties of Plumbagin powder blend

Formulation	Bulk density (gm/ml)	Tapped density(gm/ml)	Hausner's ratio (HR)	Carr's Index(%)	Angle of repose(θ)
F1	0.624 ± 0.043	0.691 ± 0.053	1.107 ±0.075	9.69 ±1.74	28.561 ± 0.380
F2	0.575 ± 0.048	0.680 ± 0.061	1.182 ±0.011	15.44 ±2.30	24.625 ± 0.374
F3	0.605 ± 0.086	0.682 ± 0.049	1.127 ±0.055	11.29 ±1.10	29.653 ± 0.784
F4	0.611 ± 0.048	0.679 ± 0.057	1.111 ±0.071	10.01 ± 1.41	28.462 ± 0.850

Post-compression parameters of Plumbagin floating tablets

By using wet granulation technique, all the tablet batches were prepared. All the formulated tablets were subjected to the post compressional evaluation parameters such as hardness, thickness, diameter, friability, weight variation, drug content, swelling studies, *in vitro* buoyancy studies, *in vitro* dissolution studies, and stability studies.

Characterization of Plumbagin floating tablets

The shape of all the formulated tablets were round, flat-faced with no visible cracks, smooth and remained yellow (Fig.3).

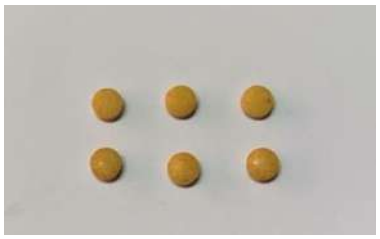

Fig.3. Visual appearance of Plumbagin floating tablets

All the formulated tablets diameter and thickness was measured by Vernier calipers and was in the range of 5.7 ± 0.8 mm to 6.5 ± 0.1 mm, and 3.72 ± 0.8 to 4.62 ± 0.2 mm, respectively. The hardness of all formulations was found to be in the range of 5.8 ± 0.26 to 6.8 ± 0.5 kg/cm^2. By using Monsanto hardness tester (Indian Equipment Corporation (IEC) Mumbai, India), the hardness of the tablets was measured. The standard deviation values show that the hardness of all the formulations was almost uniform and possess satisfactory mechanical strength. The weight of the tablets varied between 178 ± 2.43 mg to 180 ± 0.55 mg for different formulations, indicating uniformity of weight. The weight variation test is passed by all formulations because the percentage of weight variation was within the ±5% of the weight pharmacopoeial limitations. The friability was measured by Roche Friabilator and the friability was found to be 0.1 ± 0.12 to 0.22 ± 0.01%. All the values are below 1%, it can be concluded that all formulations of tablets have high compactness and sufficient resistance to abrasion and mechanical shock. The percentage drug content was analyzed by UV method and was found to be in the range of 98.21 ± 0.81 to 99.62 ± 0.40% which ensure express good consistency in drug content in various formulations. All the data are represented in Table 3.

Table 3. Post compression parameters of Plumbagin floating tablet

Formulation	Average weight, W_t in (mg)±SD	Hardness (kg/cm^2) ±SD	Thickness (mm) ±SD	Diameter (mm) ±SD	Friability (%)	Drug content (%)±SD
F1	180 ± 0.55	6.5 ±0.3	4.62 ±0.2	6.5 ±0.1	0.16 ±0.02	98.21 ±0.81
F2	179 ± 1.45	6.0 ± 0.1	4.32 ±0.3	6.2 ±0.3	0.18 ±0.04	99.23 ±0.82
F3	178 ± 2.43	5.8 ± 0.26	3.72 ±0.8	5.9 ±0.6	0.22 ±0.01	98.87 ±0.23
F4	180 ±1.53	6.8 ± 0.5	3.86 ±0.76	5.7 ±0.8	0.1 ±0.12	99.62 ±0.40

In vitro buoyancy studies

The *in vitro* buoyancy studies were carried out with 0.1N HCl (Table 4, Fig.4). Sodium bicarbonate and citric acid were used as effervescent agents. When it comes into contact with an acidic medium, CO_2 is generated. By reducing the density of the tablet below 1 g/cm^3, the gas produced is contained and shielded within the gel that is produced by the hydration of polymer, allowing the

tablet to float. The floating characteristics of each formulation, such as total floating time and floating lag time, were investigated. The test results were documented in Table 4. All the formulations floated within 76 sec and total floating time was around 9 to 9.5 h.

Table 4. Floating properties of Plumbagin floating tablets

S.No.	Batch code	FLT (sec)	TFT (h)
1.	F1	76	9
2.	F2	75	9.5
3.	F3	76	9.5
4.	F4	75	9.5

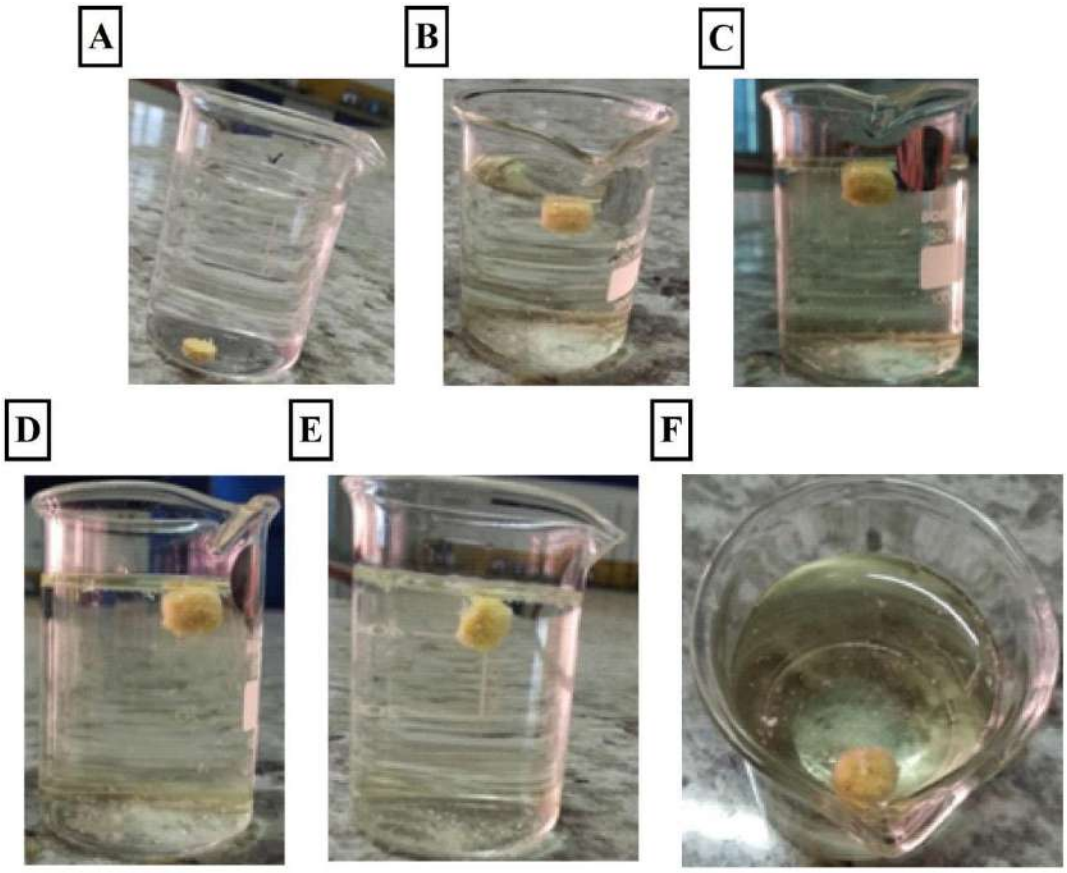

Fig.4.*In vitro* buoyancy study of Plumbagin floating tablet at (A) 0 sec, (B) 40 sec, (C) 76 sec, (D) 3 h, (E) 6 h, and (F) 9.5 h

Swelling index

The percentage swelling index was analyzed with respect to time. As the rate of hydration increased, the weight of the tablets was also increased. Subsequently, it steadily decreased because the gelled layer on the outside of the tablets had dissolved into the dissolution medium. The swelling index for each formulation was calculated and graph of %swelling index versus time (h) is illustrated in Fig. 5.

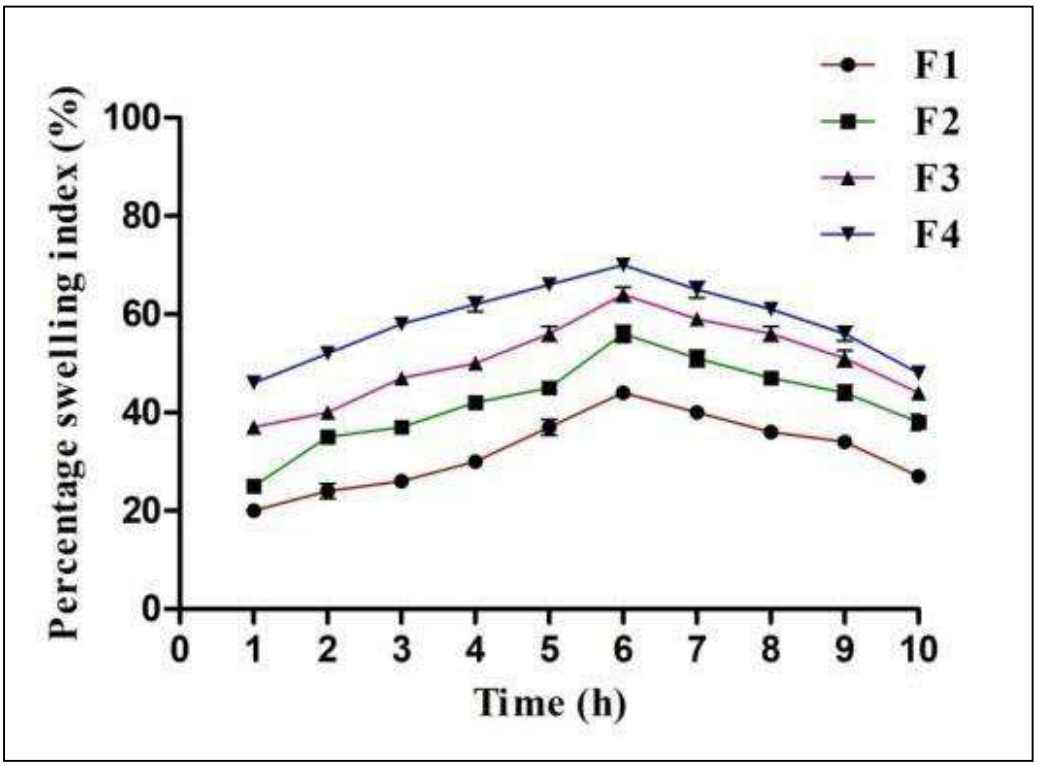

Fig.5. Swelling index of Plumbagin floating tablet

In vitro dissolution studies

The *in vitro* drug release study was performed for all batches up to 12 h in 0.1N HCl. The cumulative percentage of drug release from formulation F1, F2, F3, F4 was 96.92±3, 91.9±2.8, 84.98±2.7, 78.31±2.5 in 12 h (Fig.6). The lag time was less than a minute for each of the four formulations (F1, F2, F3, and F4). Formulation F4 was considered to be the most effective of all of them, since it significantly retards the rate of drug release. F4 follows zero order kinetics.

In case of all the tablet batches (F1–F4), it was observed that the rate of drug release decreases withincreasing the rate of polymer. High drug release is observed in F1 batch, since it contains low concentration of polymer (HPMC E 15 LV). The dissolution study led to the conclusion that swelling and erosion of polymers mainly governs the drug release from the matrix.

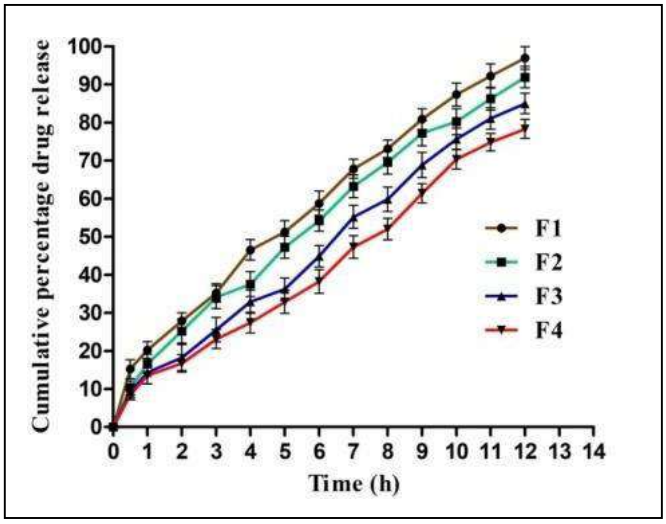

Fig.6. *In vitro* dissolution profile of Plumbagin floating tablets

To determine the drug release mechanism from floating tablet matrices, several kinetic models like zero order kinetics, first order kinetics, Higuchi model, Korsemeyer-Peppas and Hixson Crowell kinetic models were employed (Table 5). From every kinetic model, the best fit model was found by comparing the values of the regression coefficient (R^2). When comparing the R^2 values of various models, it was found that for the majority of formulations, the best fitting to zero ordermodel was represented by the highest regression value (R^2 = 0.982 to 0.994), which is close to 1. It was suggested that the drug was released from the floating tablets in a controlled manner.

Table 5. Drug release kinetics data of Plumbagin floating tablet

Sl.No.	Formulation code	Zero order	First order	Higuchi	Korsmeyer-Peppas	Hixson Crowell
1.	**F1**	y = 6.361x + 3.218 R² = 0.991	y = -0.103x + 2.097 R² = 0.895	y = 28.99x - 8.232 R² = 0.978	y = 0.919x + 1.030 R² = 0.612	y = 0.237x - 0.045 R² = 0.970
2.	**F2**	y = 7.351x + 8.319 R² = 0.986	y = -0.080x + 2.056 R² = 0.948	y = 27.88x - 10.13 R² = 0.973	y = 0.976x + 0.950 R² = 0.678	y = 0.202x - 0.026 R² = 0.985
3.	**F3**	y = 7.606x + 11.15 R² = 0.982	y = -0.064x + 2.049 R² = 0.951	y = 25.92x - 12.11 R² = 0.943	y = 0.986x + 0.880 R² = 0.710	y = 0.174x - 0.059 R² = 0.978
4.	**F4**	y = 6.967x + 4.277 R² = 0.994	y = -0.052x + 2.035 R² = 0.947	y = 23.49x - 11.37 R² = 0.927	y = 0.966x + 0.848 R² = 0.714	y = 0.148x - 0.046 R² = 0.970

Stability study

The tablets were examined at 40°C± 2°C / 75±5% RH for 3 months in stability chamber (Thermo labs, Mumbai). Based on the data, it was found that the formulation was stable in the specified conditions, since there was no discernible change in the physical appearance, weight variation, hardness, friability, percentage amount of drug content, *in vitro* drug dissolution, *in vitro* buoyancy and stability analysis of the tablets (Table 6). Consequently, it was discovered that the Plumbagin floating tablets remained stable for at least three months in these storage conditions.

Table 6. Stability study of F4 optimized formulation

Sl.No.	Parameters	1st month	2nd month	3rd month
1.	**Physical appearance**	yellow, smooth, flat faced	yellow, smooth, flat faced	yellow, smooth, flat faced
2.	**Weight variation (mg)**	180 ± 0.55	180 ± 0.53	180 ± 0.52
3.	**Hardness (kg/cm^2)**	6.8 ± 0.5	6.8 ± 0.1	6.7 ± 0.2
4.	**Friability (%)**	0.10 ± 0.01	0.10 ± 0.02	0.10 ± 0.03
5.	**Drug content (%)**	99.62 ± 0.40	99.61 ± 0.42	98.60 ± 0.44
6.	**Buoyancy Lag Time (sec)**	76 s	75 s	74 s
7.	**Total Floating Time (h)**	9 h	9 h	8.5 h
8.	**Buoyancy on disturbing**	float	float	float
9.	***In vitro* drug release (%)after 12 h**	96.92 ± 3	95.51 ±4	95.22 ±6

Conclusion

In this study, Plumbagin floating tablets were developed by wet granulation technique and various evaluation parameters were assessed. Based on the results of this investigation, it was determined that the floating tablets of Plumbagin will enhance patient compliance and product quality. In this method, HPMC E 15 LV used as a polymer, in different concentrations and sodium bicarbonate and citric acid were used as gas generating agent. The formulated Plumbagin floating tablets can increase the bioavailability as well as gastric residence time. Different pre-compression parameters like angle of repose, Carr's index, Hausner's ratio indicate good flow properties of granules. The formulations were evaluated for the various parameters like hardness, thickness, diameter, weight variation, friability, *in vitro* buoyancy study, *in vitro* drug release, etc. Depending on different evaluation parameters, formulation of batch F4 was considered to be an optimized formulation. From the results, it was clearly understood that the increase in the concentration of HPMC E 15 LV polymer in the tablet formulation, decreased the rate of drug release from the formulation. Formulation F4 was found to possess good swelling capabilities based on the swelling study. All parameters were found satisfactory and therefore, it was concluded that our formulation was fairly

stable at an elevated temperature upto 40 °C. Moreover, further *in vivo* investigation on animal model is required to get a clearer picture regarding the therapeutic efficacy of our formulated floating tablets.

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Conflict of interest

The authors declare no potential conflict of interest.

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Decrypting the crosstalk mechanisms between cGAS-STING and TBK1 signaling pathways in cancer immunotherapy: A comprehensive review

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Abstract

In the tumor microenvironment, the cytosolic DNA sensing, cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway is a crucial regulator of immune response. The cGAS of innate immune system detects cytoplasmic DNA by catalyzing cyclic GMP-AMP (cGAMP), which subsequently activate STING pathway. STING activation leads to the phosphorylation of TANK-binding kinase 1 (TBK1) and recruitment of key transcription factor IRF3, notably particularly to start the synthesis of Type-I interferons and pro-inflammatory cytokines. This cascade enhances antigen presentation and primes cytotoxic T-cells, leading toinduce anti-tumor immunity. Harnessing this axis in cancer immunotherapy has emerged as a promising strategy, offering synergy with immune checkpoint inhibitors, and CAR-T cells therapy. However, fine-tuning activation of this pathway is essential to balance immune responses, and marking the cGAS-STING/TBK1 pathway as a key target for innovative cancer treatments. The review has enlightened upon the crosstalk mechanisms of cGAS-STING and TBK1 pathway in tumor microenvironment. Subsequently, this study delivered an outlook of cGAS-STING/TBK1 pathway in cancer immunotherapeutic arsenal, these may shed insight on cancer therapy options and aid in our understanding of their possible involvement in tumor immunity.

Keywords: cGAS-STING, TBK1, Tumor Microenvironment, Immunity, Cancer Immunotherapy

Introduction

One of the most-deadly, life-threating illnesses in the world is cancer. According to the GLOBOCAN database, estimated for around 20 million new cases and 9.7 million deaths in the year 2022 [1]. Cancer is a genetic abnormal disorder encouraged through the alteration of numerous factors. The cancerous proliferation is commenced by the mutations of oncogenic drivers. The accumulation of oncogenic proteins mutation provides the cancer cells' selective advantage populace through augmenting the extent of genetic variation and quickening their rate of evolution [2,3]. This variety may set cancer cells apart from healthy cells, which immune systems are more likely to identify as foreign invaders. Cancer immunotherapy has been a major advance in the treatment toolbox for oncology due to immune activation. Chemotherapy, radiation, and surgery continue to be the mainstays of the traditional therapeutic strategy, but they significantly reduce patient survival rates [4-6]. With the recent development of our knowledge of tumoral immunity, immunotherapy has evolved into a potent new tool in the fight against carcinogenesis.

In the 20th century, the idea of cancer immunotherapy reappeared and gained substantial momentum with the introduction of new technologies associated with immune system stimulation [7]. The immune system can maintain the defensive mechanism against the infected antigens and self-antigens by an apposite balance between stimulation and inhibition of immune response. Fundamentally, this procedure encompasses the receptor-ligand binding amongthe antigens and various immune cells to modify T-cells activation, that simplifying the development of tumorigenesis and anti-tumor immune response[8,9].Intriguingly, tumor microenvironment actively induces T-cell tolerance, resulting in an immunosuppressive action that facilitate tumoral recurrence and progression[10,11].Recent studies have demonstrated that numerous cancers suppress the native immune responses by hindering effective anticancer immunity, as demonstrated through findings of reduced lymphocyte counts, apoptosis of cytotoxic T-cells, T-cells exhaustion and anergy formation, down-regulation of antigen presentation in tumor cells, and increased proliferation of regulatory T-cells and tumor-associated macrophages[12].In cancer immunology, strategic efforts have focused on developingimmunotherapy that target the intricate interactions between the cancer cells and immune cells. Cancer immunotherapy explores the impact of radiation treatment and modern cytotoxic chemotherapy on the host immune system, grounded in a deep understanding of the cancer-immune system interaction. In recent eons, a significant advancement has been developed in different immune-based treatments, includes immune checkpoint blockade (ICB) therapy, adoptive cellular therapies through CAR-T cells and CAR-NK cells, administration of specific cytokines, and cancer vaccines, as new paradigm of cancer armamentarium[13-15].The most widely used of these as an efficient treatment for various solid tumors and hematological malignancies is immune checkpoint inhibitors (ICIs).ICB therapy are associated by immune checkpoint inhibitors as drugs which unleash effector T-cells activity via attenuating immune checkpoint such as cytotoxic T-lymphocyte antigen 4 (CTLA-4)/B7 and programmed cell death 1 (PD-1)/ligands (PD-L1/PD-L2) interactions, located on the cellular membrane of T-cells and tumor cells[16].Moreover, adoptive cellular treatments are based on the patient's body being infused with immune cells that attack tumors.Cytokine treatment includes the infusion of immunomodulatory cytokines to stimulate the immune system, and cancer vaccines might be created to give preventive or therapeutic efficacy[17].

An innate immunological sensor called cyclic GMP-AMP synthase (cGAS) is able to identify different cytoplasmic double stranded DNAs [18].In order to affect the expression of Type-1 interferons (IFNs), interferon stimulator genes (ISGs), and other pro-inflammatory cytokines, cGAS interacts with stimulator of interferon genes (STING) and activates downstream pathways. This activationstronglyenhances the host immune responses which aiding in the suppression and elimination of tumors [19,20]. Recent evidence increasingly suggests that cGAS-STING pathway is closely allied to the onset, propagation, and retreat of cancer. The cGAS-STING signaling exhibits pro-tumorigenic or antitumorigenic activity via modulating different stages of the cancer immunity cycle (CIC). This includes the release of tumor antigens, antigen presentation, T-cell priming, activation and, trafficking as well as T-cell infiltration into tumor tissues, and thereby augmenting immunogenic tumor cell death [21,22]. Furthermore, TBK1 (TANK-binding kinase 1) performs significant part in cancer immunology through its interaction with the cGAS-STING pathway, a critical component of the innate immune responses. TBK1 is a key kinase of cGAS-STING pathway to facilitate downstream signaling processes via phosphorylation of IRF3 (interferon regulatory factor 3), leading to transcription of interferons and other immune modulators. In contrast, TBK1 and cGAS-STING pathway could lead to debilitate immune responses, that contributing to initiate immune evasion by tumors [23,24].Therefore,

understanding the role of cGAS-STING and TBK1 pathways in cancer immunotherapy is essential for optimizing and developing novel therapeutic strategies to overcome resistance mechanisms. This review article sheds lighton the crosstalk mechanisms between thecGAS-STING pathway and TBK1 in cancer immunotherapy, hoping to provide a direction for exploring new cancer immune mechanisms and therapeutic approaches, particularly through the development of selective inhibitors targeting cGAS, STING, and TBK1.

cGAS- STING Pathway: Molecular Mechanism

Structural biology of cGAS-STING

cGAS, an approximately 520 amino acid containing protein belongs to nucleotidyl transferase (NTase) superfamily made up of two catalytic domains [25,26]. One is an N- terminal positively charged domain that controls cGAS dimer stabilization and another is C-terminal catalytic domain required for enzymatic activity and a Mab21 domain for DNA binding. Structurally, Mab21 domain composed of two lobes disunited by wide rift. One is N-terminal lobe, made up of β sheets and edged by α helices, another is C-terminal lobe containing helix bundle with a conserved zinc region. By using two step the c-GAS binds to the ATP and GTP simultaneously to form the end product 2'3'c-GAMP. In the first step using ATP as the donor and the 2'-OH on GTP as the acceptor, a linear dinucleotide,5'-pppG (2'-5')pA formed.This intermediate product then flips over in the catalytic pocket during the second step, the 3'–5' phosphodiester bond, placing the AMP moiety at the acceptor position and the GTP moiety at the donor position[26].The cytosolic dsDNA is sensed by cGAS and form 2:2 cGAS-dsDNA complex, each molecule of cGAS is bound to two molecules of ds DNA through the primary and secondary binding site. Site A, the primary binding site for DNA in cGAS interacted with sugar phosphate backbone of DNA and causes conformational change of cGAS. Site B, made up of many surface-exposed loops and a helix is important for cGASsignaling (Fig. 1A)[27].

STING, a 40 kDatiny protein present in endoplasmic reticulum (ER) membrane, has four transmembrane (TM) helices and N-terminal cytosolic portion [27]. The crystal structure of STING cytoplasmic domain is a highly organized hydrophobic dimer. In STING dimer there is a C-terminal tail (CTT) that binds to the TANK-binding kinase 1 (TBK1) necessary for downstream signaling and a cytoplasmic ligand-binding domain (LBD) that is crucial for dimerization and cGAMP binding [26]. The four TM helices in the STING dimer are arranged into two layers: the perimeter, made by TM1 and TM3 and the centre layer, formed by TM2 and TM4. When 2'3'-cGAMP binds to LBD conformational changes occur. Following cGAMP-induced activation, CTT binds to TBK1 and cause STING-CTT phosphorylation. After phosphorylation, STING recruits IRF3, which is phosphorylated by TBK1. Phosphorylated IRF3 dimerizes and enter nucleus to activate IFNs (Fig. 1B) [27].

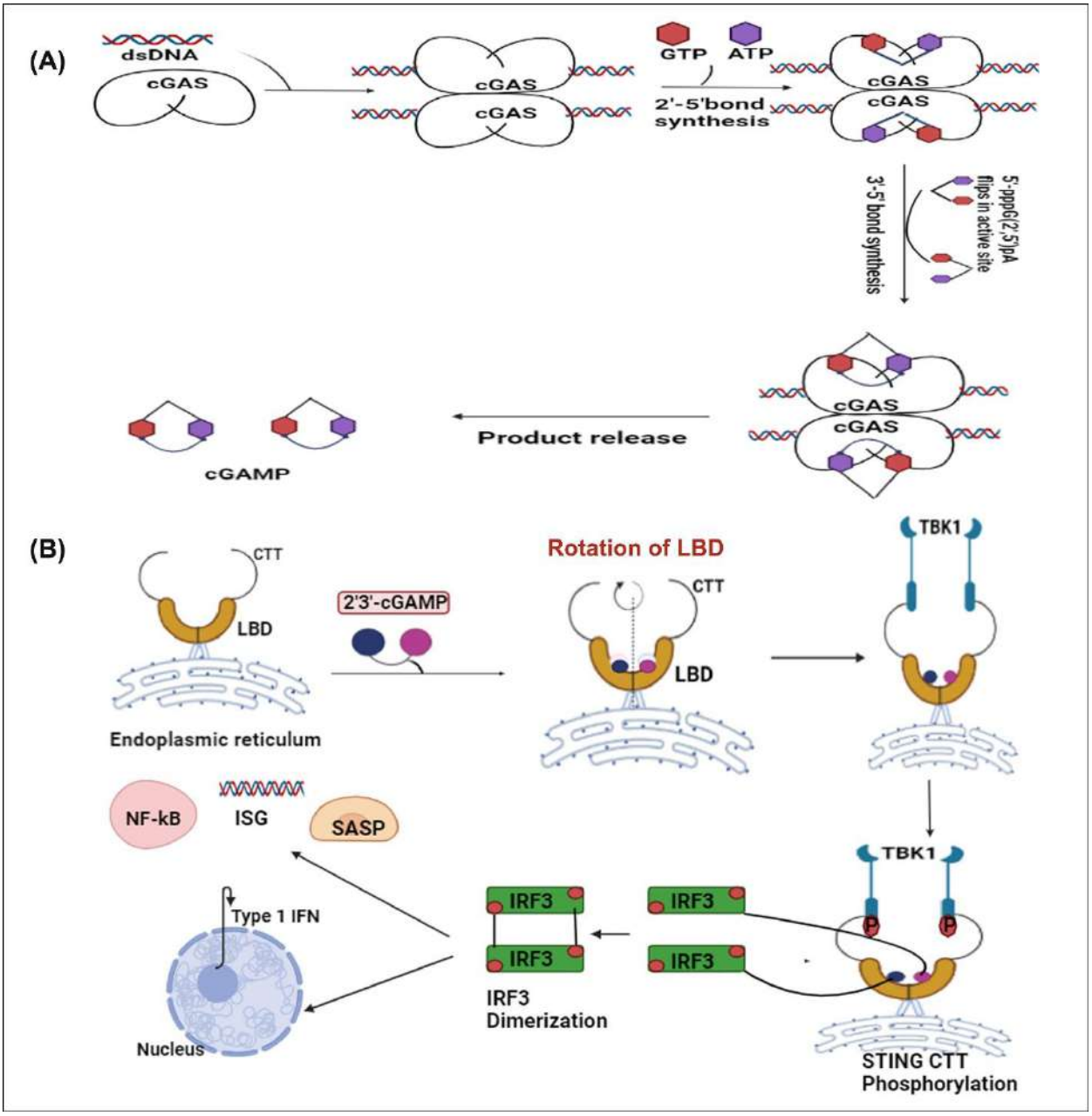

Figure 1: Structural biology of cGAS-STING

Role of cGAS-STING in Tumor microenvironment

cGAS-STING stimulates the growth of tumors

The cGAS-STING signaling pathway, which upregulates IFN production and promotes anti-tumor effects through innate immune responses, but in other studies it has been found that it can promote tumor development and induce carcinogenesis driven by inflammation[28]. Metastasis is mediated in a cell-autonomous manner via cGAS–STING activation. Through gap junctions, cGAMP produced by cGAS is transported to astrocytes, where it activates astrocyte STING and starts the production of inflammatory cytokines to support the growth of tumors and the survival of metastatic cancer cells in the brain (Fig. 2A)[29]. The metastasis of human brain cells is linked to

chromosomal instability (CIN), which is brought on by chromosome mis-segregation during cell division. In metastatic models, CIN promotes the growth of micronuclei and the cGAS–STING pathway, eliciting non-canonical NF-κBsignaling but not type I IFN signaling. CIN-driven metastasis is dependent on STING and NF-κBsignaling, and it is also correlated with the activation of genes related to inflammation and the change from epithelialtomesenchymal tissue[30]. Research on STING's function and mechanism in Lewis's lung cancer (LLC) has revealed that abnormally high STING expression significantly promotes LLC growth and multiplication. When tumor cells are damaged by carcinogens, significant amounts of DNA are released into the cytoplasm, which keeps the STING signaling pathway active and encourages the synthesis of chemokines in the tumor cells. In order to promote the growth of tumors, these chemokines attract a large number of inflammatory cells, including immunosuppressive cells like M2 tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). Studies showed that STING increases MDSCs proportion in mouse melanoma. As a result, treating these malignancies with STING agonists runs the risk of over-stimulating the STING signaling system and accelerating the growth of tumors(Fig. 2A)[28].

cGAS-STING in Tumor inhibition

Cytosolic DNA, produced by a number of DNA damage process, including oxidative stress, radiation, hyper activation of oncogene signaling, low chromosomal instability is widespread in cells constituting the tumor micro environment[29]. Cyclic GMP-AMP synthase (cGAS) recognized the abnormal exogenous and endogenous DNA in cytoplasm and produces an endogenous second messenger 2'3'cyclic GMP-AMP (2'3'-cGAMP) which binds directly and activates stimulator of interferon genes (STING), which in turn activates IFN response and produces pro-inflammatory cytokines (Fig. 2B)[31,32].When cGAMP binds to a specific pocket on the STING dimer, STING is transported from the endoplasmic reticulum (ER) to perinuclear microsomes through the Golgi apparatus[30]. Activation of the cGAS-STING pathway increases the production of type I IFN and senescence-associated secretory phenotypic factors (SASP) and promote the cell senescence. Intumor cell type 1 IFN triggers the apoptotic factors like caspase 9, caspase 3, Bax and ultimately cause apoptosis.Thus, activation of cGAS-STING pathway inhibits the growth of cancer by causing cell senescence[28]. The cGAS-STING pathway which is also activated in dendritic cell (DC) plays a crucial role in tumor inhibition. DCs produce type I IFN that primes CD8+ T cells against immunogenic malignancies (Fig. 2B)[30].Natural killer (NK) cells, which activate the cGAS-STING signaling pathway and upregulate the production of NKG2D ligand, are essential components of the STING-mediated antitumor immune response, in addition to T cells and DCs. The binding of increased NKG2D ligands on the surface of tumor cells to NKG2D receptors on the surface of NK cells facilitates the killing of tumor cells by NK cells. The tumor microenvironment is largely composed of stromal cells that express STING genes, such as fibroblasts and endothelial cells.

Apart from that cGAS-STING is also involved in tumor metastasis alongside to its involvement in oncogenesis and maturation. When STING is activated in tumor cells, the environment surrounding cancer is altered to cause cell death via NF-κBsignaling, which effectively stops tumor migration and metastasis[28].

Role of cGAS-STING in immune regulation

Tumorigenic cell clearance by the immune system first depends on production of type I interferon (IFN) by dendritic cell (DC) and the recruitment of CD8+ T lymphocytes, which encourage the

targeted killing of such aberrant cells[31].It was discovered by researchers through an unclear mechanism that tumor-derived DNA and cGAMP activate the cGAS-STING pathway in DCs, which in turn enables cross-presentation to DCs and engages CD8+ T lymphocytes for direct, non-spontaneous tumor eradication (Fig, 2B)[33]. Type I IFN, a key player in the STING pathway, is involved in the production of chemokines like CXCL9 and CXCL10, which are essential for cytotoxic T lymphocyte metastasis. This pathway also plays a role in immunosuppression of regulatory T cells[34].Dendritic cells (DCs) are thought to be able to accept tumor-derived DNA or cyclic GMP-AMP (cGAMP) through gap junctions or endocytosis. Following this, the expression of cell surface co-stimulator molecules is aided by the stimulation of the STING signaling pathway. Moreover, it improves DC antigen presentation and fosters DC maturation. Additionally, tumor cells, stromal cells, and immunological cells such T cells, macrophages, and NK cells express STING (Fig. 2B)[28]. To eradicate tumors, NK cells require STING expression in tumor cells. Large volumes of IFNs are produced by activated immune cells, and these IFNs stimulate antitumor immunity by directly or indirectly interacting with IL5 and IL15 receptors in NK cells, ultimately killing tumor cells to create effects that decrease tumor growth[33]. STING-mediated autophagy may work in conjunction with canonical NF-κBsignaling and IRF3 to prevent or inhibit the growth of cancer through an unidentified mechanism. Furthermore, the cGAS-STING pathway facilitates the regulation of antitumor immunity through interaction between immune cells and tumor cells[29].

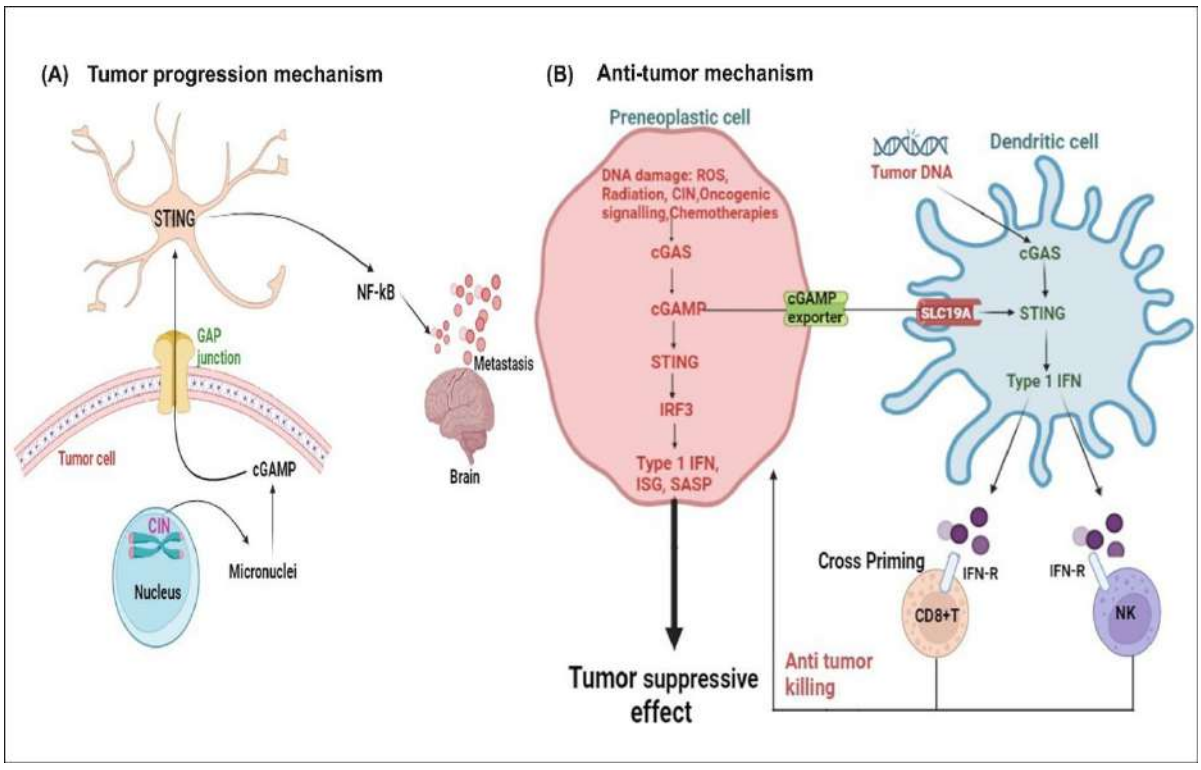

Figure 2: Dual mechanisms of cGAS-STING in tumor. (A) Tumor progression mechanism (B) Anti-tumor mechanism

TBK1 Signaling pathway: molecular insight

Biology of TBK1

TRAF-associated NF-kB activator, or TANK Serine/threonine kinase binding kinase 1 is a 729 amino acid member of the nuclear factor-kb (IkB) kinase (IKK) family of non-canonical inhibitors [24]. TBK1gene is located on chromosome 12 which is constitutively expressed in all tissues along with fibroblasts, skin, adipocytes, CNS (central nervous system) and cancer cells [35]. TBK1 contains four key domains: an N-terminal kinase domain (KD; residues 1-307), a ubiquitin-like domain (ULD; residues 308-384), and two coiled-coil domains (CCD1/SDD (scaffold/dimerization domain); residues 407-657 and CCD2; residues 658-713). The KD is essential for phosphorylating various substrates, including IRF3, and contains two lobes (N-terminal and C-terminal) with an active site between them. The activation loop (residues Leu164-Gly199) within the KD includes Ser172, whose phosphorylation triggers structural changes that enable substrate binding [36,37]. The ULDregulates kinase activity by interacting with the KD and other proteins, and it plays a crucial role in maintaining TBK1's kinase activity. Conserved residues within the ULD, such as Leu316, Ile353, and Val382, are critical for protein-protein interactions, with mutations in this region potentially halting downstream signaling. TBK1 forms homodimers or heterodimers with IKKi through interactions involving the SDD/CCD1 domains, KDs, and ULDs. This dimerization is necessary for TBK1 activation and subsequent signaling. The CCD2 domain in the C-terminus contains an adaptor-binding motif that enable interactions with proteins like TANK,NAP1(NAK–associated protein),TBKBP1(TBK1-binding protein 1; also known as SINTBAD) optineurin(OPTN), determining TBK1's subcellular localization and signaling specificity[37].TBK1 activation can be regulated by Oncogenic kinases, inflammatory cytokines, pathogen-associated molecular patterns (PAMPs; released by invasive bacteria or viruses), damage-associated molecular patterns (DAMPs; released by injured tissues), including activated K-RAS/N-RAS mutants[24].TBK1 integrates responses from a variety of extracellular and intracellular stimuli and regulates different signaling pathways by regulating expression of interferon regulatory factor 3 and 7 (IRF3/7), key transcription factors of type I interferons (IFNs; IFN-α/β),while also participating in different biological processes like immunity, inflammation, autophagy, mitochondrial metabolism, specifically mitophagy and xenophagy, energy homeostasis and cell death[35,36].

TBK1 in tumor microenvironment

TBK1 have a very heterogenous role in the tumor microenvironment. TBK1 in cancerous cells drives tumorproliferation by activating key pathways involved in survival and proliferation, including MYC, JAK/STAT, p62/autophagy, NF-κB, and AKT-mTOR1.It also induces tumorigenic cytokines like IL-6, supporting autocrine cell survival. In KRAS-mutant cancers, TBK1 promotes survival by enhancing NF-κB and mTOR1 pathways, which prevent apoptosis and boost protein synthesis. TBK1 activates NF-κB through phosphorylation of key regulators and interacts with components of the AKT-mTORC1 pathway to promote mTORC1 signaling which is essential for tumor growth (Fig. 3) [38]. Interestingly, TBK1 performs a dual function in immune regulation, sometimes promoting immune suppressive action and in other way enhancing immune responses. For instance, deletion of TBK1 in dendritic cells can enhance T cell activation and antitumor immunity, while in other contexts, TBK1 promotes an immunosuppressive environment by influencing cytokine production including IL-6, TNFα, IFNβ, and CXCL10 (IP-10) and T cell function. The STING-TBK1 pathway, crucial for activating CD8 T cells via dendritic cell priming,

highlights TBK1's complex role in the immune response against tumors (Fig. 3). However, TBK1's role is context-dependent and varies across different cancers[39]. In prostate cancer, TBK1 inhibits mTOR signaling in bone marrow niches, contributing to cancer cell dormancy and resistance to conventional treatments. In contrast, in renal cell carcinoma (ccRCC) and breast cancer, TBK1 supports tumor survival and progression through pathways like autophagy and estrogen receptor signaling. Through pre-metastatic niche TBK1 also interact with in situ tumorsas well as distant metastatic tumors. Thus, through persistent inflammatory activation, the TBK1 pathway not only initiates tumor formation but also enhances anti-tumor surveillance [39].

TBK1 in immune regulation

One early effector in the innate immune system is TBK1 and its Primary role is to induce type I interferons (IFN-α/β) in immune cells, through phosphorylation of IRF3at Ser386 and Ser396 and IRF7 at Ser477 and Ser479 is important for their activation. When phosphorylated IRFs attach to IFN-stimulated response elements (ISRE) in gene promoters like IFNB and RANTES, they form dimers and go into the nucleus. TBK1 mediates the activation of the IRF3/IRF7 and NF-Kb pathways in cytosolic DNA-induced/STING-dependent manner, thereby inducing the production of inflammatory cytokines TNFα, IL-8, and IL-1β, as well as Type 1 interferon. Additionally, TBK1 promotes proliferation by regulating genes involved in survival and proliferation, such as BCL-xL,XIAP,Cyclin D1, andRelB[40].An adaptor protein which is Stimulator of Interferon genes (STING, TMEM173) activate in response to dsDNA (via cGAS-STING), involves generation of the cyclic dinucleotide 2′3′-cGAMP, a second messenger generated by cyclic GMP-AMP synthase (cGAS) and induce autophagy (Fig. 3). The immune defense response against tumors is mediated by the activation of both the NF-κB and IRF3 pathways. When paired with the IL-6R/JAK signaling pathway, this activation increases macrophage phagocytosis and triggers apoptosis. STING is essential for polarizing macrophages and produces IFN-β[41]. Several studies demonstrated that TBK1 reacts to signals from many receptors, including B cell receptor (BCR), T cell receptor (TCR), and members of the TNF receptor (TNFR) super family, which maintain adaptive immune responses and homeostasis. In B cells, TBK1 prevents TNFR superfamily members including CD40 and BAFFR from noncanonically activating NF-κBby phosphorylating NIK, leading to its ubiquitin-dependent degradation and control IgA class switching [42].By Controlling IFN-induced signaling is likely a key mechanism by which TBK1 controls dendritic cell function in T cell homeostasis and activation. This function involves phosphorylation of STAT3 at serine 727, which negatively regulates gene expression and STAT1 activation caused by Type-I IFN (Fig. 3)[24].

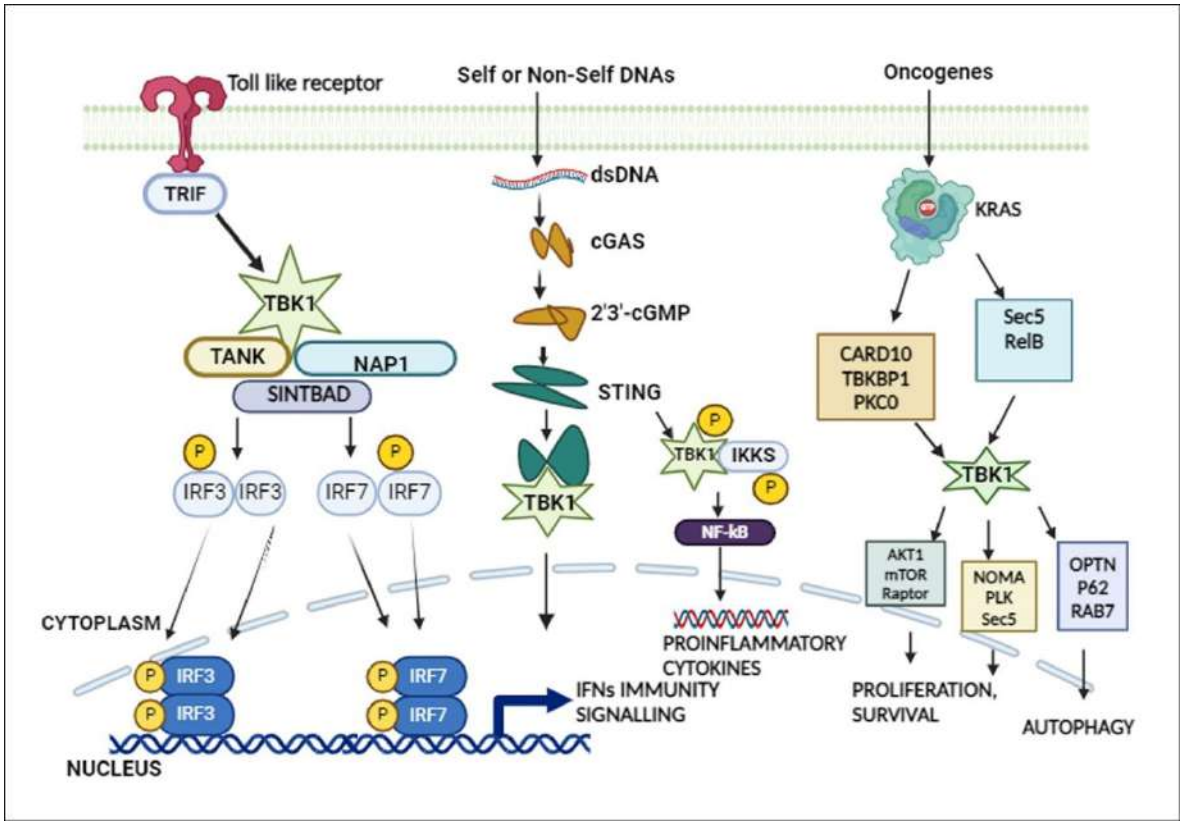

Figure 3: TBK1 role in tumor microenvironment and immune regulation

Crosstalk between cGAS-STING and TBK1 pathway in tumorigenesis

The key cellular sensor for cytosolic double-stranded DNA (dsDNA) is the cGAS-STING pathway, which helps the innate immune system fight off infections, inflammation, and cancer [43,44].Both intrinsic and extrinsic self-DNA sensing can participate to the activation of cGAS-STING pathway. Sequence-independent upstream contact between dsDNA and the cGAS enzyme causes cGAS toconformationally alter, which catalyzes the synthesis of 2C,3′-cyclic GMP-AMP (cGAMP), a cyclic dinucleotide made of phosphodiester linkages from both 2′-5′ and 3′-5′[45-47]. The cGAMP unveiling as a second messenger whichactivates STING expression in endoplasmic reticulum, and subsequently translocate into Golgi apparatus to form tetramer by higher-order oligomerization(Fig. 4)[48-50].Palmitoylationof STING within the Golgi apparatus is significantly encouraged to facilitate the recruitment of TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3)[51,52].In addition, the tetramerization of STING stimulates and draws in TBK1 dimers, which thentransphosphorylate STING at its C-terminal domains, activating IFR3.Subsequently, IFR3 translocation into nucleus drives the upregulation of immune stimulated genes (ISGs) and Type-1 interferons production (Fig. 4). This modulation improves the body's immune system's ability to identify and eradicate cancer cells by promoting the development of dendritic cells and the infiltration of cytotoxic T-lymphocytes (CTLs) at specific areas[53], thus improving the body's own immune system to recognize and eliminate cancer cells. As an alternative, STING activation can also promote the production of nuclear factor-kappa B (NF-κB), a transcription factor that is necessary for cell survival and carcinogenesis development [54].

Conversely, the downregulation of cGAS-STING / TBK1 transduction often exploited by tumors to evade the immune response. Cancer cells could accomplish this signaling via the suppression of cGAS or STING expression, and promotion of negative regulators within cGAS-STING / TBK1 pathways[55].The suppression of cGAS-STING / TBK1 pathway dampens the production of Type-I interferons and weakens the immune systems to battle tumors, facilitating tumor progression and metastasis (Fig. 4)[56,57]. Understanding the dynamic regulation of cGAS-STING-TBK1 axis in different tumors is critical for developing targeted therapies that can either enhance or restore their function, thereby improving the effectiveness of cancer immunotherapy.

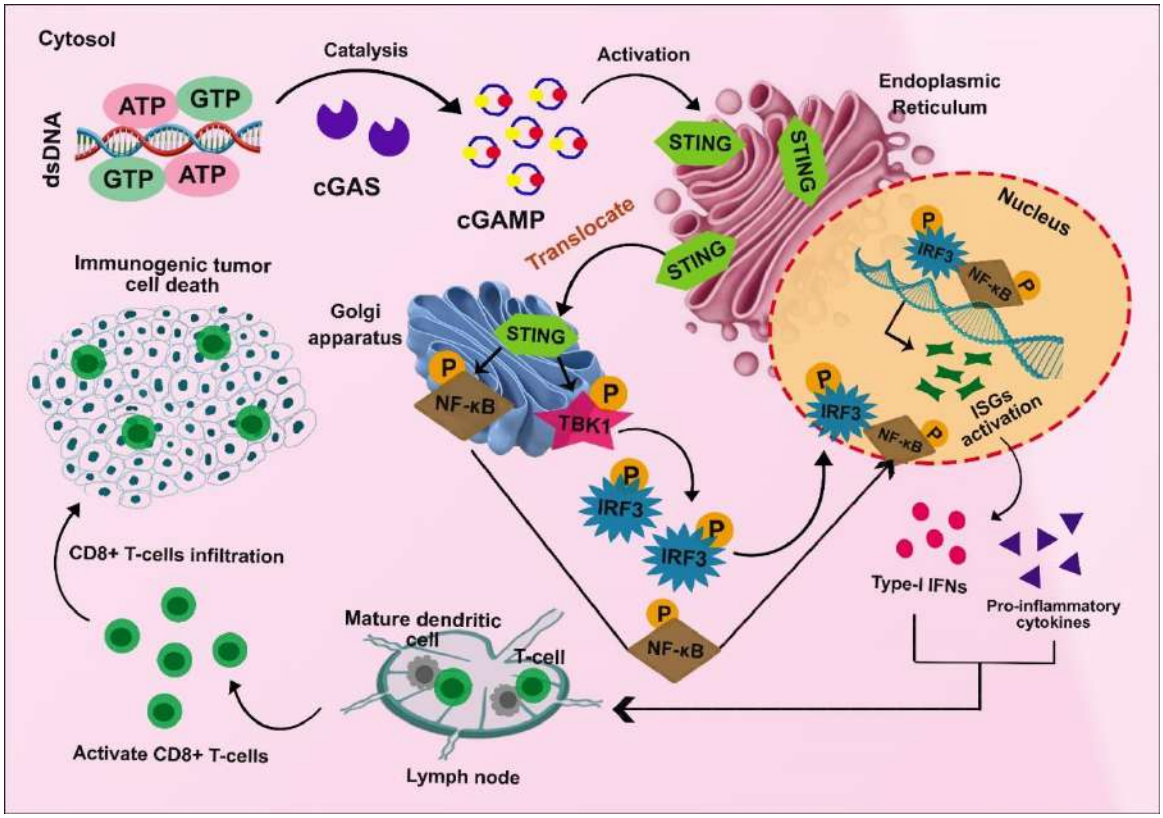

Figure 4: Crosstalk mechanism of cGAS-STING-TBK1 in cancer immunotherapy

Application of cGAS-STING/TBK1 pathway in cancer immunotherapy

ThecGAS-STING/TBK1 signaling plays a pivotal role in cancer immunotherapy by triggering innate immune responses against tumors. This pathway is initiated when cGAS detects cytosolic DNA from tumor cells which increases the generation of cGAMP, leading to activate STING in endoplasmic reticulum. Following stimulation, STING enlists and activates TBK1, which helps phosphorylate IRF3. This activation leads to the production of Type-I interferons (IFNs)and other pro-inflammatory cytokines that promote immune cells infiltration and enhance antitumor immune responses[58,59]. By engaging the cGAS-STING and TBK1 axis, cancer immunotherapy can boost tumor cell recognition via immune cells activation, facilitating immunogenic tumor cell death(Fig. 4)[60,61].However,cGAS-STING/TBK1 axis role in inducing immunogenic cell death and overcoming immune evasion has also made a potential biomarkerfor enhancing the efficacy

of existing treatments[62,63], such as immune checkpoint inhibitors, CAR-T cells therapy, cancer vaccine, and oncolytic immunotherapy.

Application of STING in cancer immunotherapy

The appropriate adjuvant plays an important factor in overcoming tolerance and enhancement of immune responses in tumor, and innate immune systemwhich leads to boost antigen presentation, it makes tumor-associated antigens(TAAs) more immunogenic[64].With both cancer cells secreting cyclic dinucleotides (CDNs) and granulocyte-macrophage colony-stimulating factor (GM-CSF), STINGVAX has emerged as the first STING-based cancer vaccine[65,66]. Research on STINGVAX injection in B16 transplanted melanoma, demonstrated that STINGVAX is prominently reduced the tumor proliferation in dose dependent manner, increase T-cell infiltration in tumor tissues[67].In addition, Miao and associates created and produced a successful STING-dependent cyclic lipid nanoparticle (LNP) adjuvant for the delivery of antigen-specific mRNA vaccines. Through the use of a one step-component procedure, this research team created several synthetic lipid structures. The STING pathway may be activated by the cyclic amino head group of lipids. Using this combinatorial LNP in a mouse model shown a significant increase in survival rate by activating STING and inducing antitumor immune responses[68].

Recently, several STING agonists have been utilized in clinical trials as an anticancer agent, and STING/ICIs combinations were also established[69]. STING signaling and Type-I interferons plays an essential role in substantial T-cell action by cross-presents of CD8α+ DCs, influence intra tumoral T-cell infiltration[70].Furthermore, using anti-CTLA-4 therapy can lower the threshold for T-cell activation.Harding and his research team observed that in the absence of STING, the combination of ionizing radiation and anti-CTLA-4 therapy failed to induce abscopal tumor regression and reduce the amount of CD8+ T-cell infiltration in tumor tissues[71].Similarly, Ager'sgroup conducted a related study with comparable findings. Their outcomes demonstrated that the combination therapy of anti-PD-1, anti-CTLA-4 and agonistics anti-4-1-BB persuade bilateral tumor suppression while STING agonist added, which remarkably intercept the bilateral tumors in 75% of mice model. Moreover, the combination of CAR-T cells and cyclic di-GMP (cdGMP, a STING agonist) activates host APCs and lymphocyte responses, which effectively eradicates malignancies[72].Although theprecise processesby which the corelease of STING agonist and CAR-T cells activates the host immune system remain unclear, this CAR-T/cdGMP combination reflected long-lasting antitumor immune responses.

Application of TBK1 in cancer immunotherapy

TBK1 is increasingly recognized for its role in cancer immunotherapy due to involve in multiple key pathways that influence tumor progression and immune responses.Serine/threonine kinase, TBK1 regulates Type-I-interferon responses and modulates inflammation, both of which help to activate the innate immune system[73]. Recent studies have highlighted the dual mechanisms of TBK1 in cancer, such as initiation of antitumor immune responses by enhancing the activation of interferon stimulated genes and contributing to the immune surveillance of tumors.Additionally, it can also facilitate tumor progression and resistance to therapy through the modulation of different signaling pathways, including NF-κβ, and autophagy[39,74]. For instances, research by Zhang and his colleagues demonstrated that TBK1 inhibition can enhance the efficacy of immune checkpoint blockade therapy by overcoming resistance mechanisms and promoting a more robust immune responses against tumors[75].Furthermore, it has been shown by Sun and his colleagues that TBK1 is a gene involved in immune evasion. By sensitizing tumor cells to effector cytokine-

induced cell death, targeting TBK1 can improve responsiveness to PD-1 inhibition. This work demonstrated that TBK1 targeting sensitizes tumors to immune challenge using patient-derived ex-vivo models and syngeneic mouse tumor models[76]. Based upon the recent research, targeting TBK1 could be promising strategy to improve outcomes in cancer immunotherapy by bolstering antitumor immune responses and overwhelming resistance to existing therapies.

Therapeutic targeting cGAS-STING and TBK1

The understanding of innate immunity has expanded, with a focus on the recognition of cancer-encoded messages by pattern recognition receptors[67]. The cGAS-STING pathway is a key component of both innate and adaptive immunity in response to foreign DNA derived from pathogens. It is recruited and activated by the presence of dsDNA in the cytoplasm, which is converted into a second messenger, cyclic GMP-AMP (cGAMP), which binds to and activates STING. cGAS recognizes tumor-specific DNA fragments, such as those derived from chromosomal instability and genomic breaks[77].cGAS-STING and TBK1 pathway can be targeted in a number of ways for cancer treatment, including small molecule inhibitors, gene editing, STING agonists, and combination therapies (Table 1, 2, and 3).

Small molecule inhibitor

STING, a protein involved in the Golgi network, is a potential anticancer target due to its role in phosphorylation, dimerization, and trafficking to the Golgi network. It forms a complex with TANK-binding kinase 1 (TBK1), leading to IFN secretion via the type I IFN pathway. However, none of STING inhibitors have been approved for clinical use. Several preclinical and clinical phases are investigating the effectiveness of STING inhibitors (Table 1). DITTRIN-10, DITTRIN-11, and DITTRIN-12 are dimerization inhibitors that reactivate IFN activation via the cGAS-STING pathway after electric or electro-optical stimulation administration with an mRNA vaccine[78]. CRISPR-Cas9, an effective and highly potent agonist for the induction of IFN, has been found to be effective in osteosarcoma cell line B14. More experimental studies are needed to fully understand STING's potential in cancer treatment[79].

Table 1: Small molecule inhibitors targeting STING

Small molecules	Mechanisms	Affinity	References
H-151	It binds covalently to Cys91 in the hSTING protein ⟶ prevent palmitoylation brought on by STING activation ⟶ prevents the hSTING protein from assembling into multimeric complexes in the Golgi and suppresses downstream signaling pathways.	NA	[80]
SN-11	It works in vivo as a potent inhibitor of the cGAS/STING pathway exhibits in the ⟶ Trex1 KO mice model comparable efficacy to the covalent molecule H-151.	NA	[81]
C-178	It attaches covalently to Cys91 in the mSTING protein to prevent palmitoylation brought on by STING activation ⟶ prevents the protein from assembling into multimeric complexes at the Golgi and inhibits signaling pathways downstream.	NA	[80]

C-176	It binds covalently to Cys91 in the mSTING protein, it prevents palmitoylation brought on by STING activation ⟶ prevents the protein from assembling into multimeric complexes at the Golgi and inhibits signaling pathways downstream.	NA	[80]
C18	It has some inhibitory effects on the production of IFN-β generated via cGAMP.	IC5068nM	[80]
EGCG	It selectively targets G3BP1 to efficiently suppress DNA-induced cGAS activation and type I interferon production.	NA	[80]
Aspirin	It efficiently suppressescGAS-mediated immune responses by directly acetylating cGAS.	NA	[80]
Astin-C	It may prevent IRF3 from joining the STING signalosome, obstructing subsequent signaling cascades.	Kd∼53 ± 14nM	[80]
RU365	It adopts an active conformation in the DNA-induced "open pocket", cGAMP with cGAS ⟶ benzimidazole ring and portion of the pyrazole ring in RU365 partially stack with Arg 364 and Tyr 421 form the key intermolecular interactions.	NA	[82]
RU521	It shows that the two chloro moieties insert deeper in the catalytic pocket of cGAS ⟶ increases the stacking surface of the compound with Arg 364 and Tyr 421 resides in the cGAS catalytic site prevent it from binding GTP and ATP.	NA	[82]
J001 & G001	These two compounds are potent than m-cGAS subsequently, based on both found compounds, they ran an optimization program for medicinal chemistry to increase h-cGAS activity and selectivity.	NA	[82]

Table2: STING agonists in clinical trials for cancer therapy

Agent (drug)	Combination therapy	Indication (cancer type)	Phase	Status	Route	References
Ulevostinag (MK-1454)	Monotherapy or combined with pembrolizumab	Advanced/metastatic solid tumors or lymphomas	I	Completed	Intratumorally	[83]
Ulevostinag (MK-1454)	combined with pembrolizumab	Metastatic or unresectable, recurrent head and neck squamous cell carcinoma	II	Completed	Intratumorally	[83]
E7766	Monotherapy	Advanced solid tumors or lymphomas	I	Completed	Intratumorally	[83]
MK-2118	Monotherapy or combined with pembrolizumab	Advanced/metastatic solid tumors or lymphomas	I	Completed	Intratumorally	[83]

TAK-676	Combined with radiotherapy and pembrolizumab	Non–small-cell lung cancer, triple-negative breast cancer, or squamous cell carcinoma of the head and neck	I	Recruiting	Intravenously	[83]
SB 11285	Monotherapy or combined with pembrolizumab	Melanoma head and neck squamous cell carcinoma advanced solid tumors	I	Recruiting	Intravenously	[83]
IMSA101	Monotherapy or combined with immune checkpoint inhibitor (ICI) or immuno-oncology (IO) therapy	Advanced treatment-refractory malignancies	I / II	Recruiting	Intratumorally	[83]
GSK3745417	Monotherapy	Myeloid malignancies Including acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (HR-MDS)	I	Recruiting	Intravenously	[83]
BMS-986301	Monotherapy or combined with nivolumab and ipilimumab	Advanced solid cancers	I	Active, not recruiting	Intratumorally, Intramuscular, or intravenously	[83]

Table 3: Small molecule inhibitors targeting TBK1

Small molecule	Mechanisms	Affinity	References
BX795	Originally designed to be a moderately powerful inhibitor of 3-phosphoinositide-dependent protein kinase 1 (PDK1), this drug also exhibits significant activity against a variety of other kinases, such as MLK1-3 (mixed lineage kinase 1-3), IKKε, Aurora B, and MARK1e4 (AMP-activated protein kinase 1-4).	NA	[82]
MRT67307	It is derived from BX795 with improved selectivity for TBK1 and IKKε over other kinases	NA	[82]
CYT387	It is derived from momelotinib, a JAK1/2 inhibitor that is therapeutically used to treat myelofibrosis.	NA	[82]
K252a, dovitinib & oxindole	These compounds can form similar H-bonding network in the kinase hinge binding region ⟶often function as multi-target inhibitors, TBK1 included. They concentrated on tozasertib, an Aurora kinase inhibitor with moderate TBK1 efficacy	NA	[82]
GSK8612	This compound inhibits IRF3 phosphorylation in Ramos cells inhibits⟶the release of IFN-b in THP1 cells treated with ds-DNA-containing viruses or 2C,3′-cGAMP..	NA	[82]

Gene editing

Gene editing (GE) is the ability to delete, change, or frame a specific DNA sequence by an intended targeted mutation in a living organism. New gene editing technology from site-specific artificially defined nucleases (TALENs), zinc finger nucleases (ZFNs), and the newest technology for clustered regularly interspaced short they repeat sequences (CRISPR) has opened new research areas in life sciences, including phenotypic experiments and genetic scanning. These tools will significantly enhance the ability to decode the functions of genes, increasing the speed of mutation creation, trait optimization, and trait regeneration. Gene editing technique utilizing clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) may lessen the side effects of immune checkpoint blockade medication [84].

Although both short interfering RNA and CRISPR-Cas9 might down regulate gene editing, the knockdown strategy based on CRISPR-Cas9 has the benefits of permanently silencing the target gene, high efficiency for correct gene editing, and reduced off-target chance. Specifically, the CRISPR-Cas9 gene editing technique targets the genome sequence with sgRNA (Guide RNA), which enables the Cas9 protease to effectively eliminate the target gene. Hollow manganese dioxide (HMn) is an appropriate nanocarrier for drug administration because to its large loading abilities, great response to the tumor microenvironment (TME), and minimal toxicity [85].This HMn was further coated with hyaluronic acid (HA) and loaded with STING agonist MSA-2 and CRISPR-Cas9 plasmid for PD-L1 silencing. The nanoplatform was referred to as HMnO2-MSA-2-PD-L1@HA, or HMnMPH for short. Through the CD44 receptor and the EPR effect, the

HMnPMH nanoplatform may be able to enter cancer cells. Once inside, it will breakdown to release Mn2+, MSA-2, and the Cas9/sg-PD-L1 plasmid in the TME in conjunction with GSH/pH.Furthermore, MSA-2 recruits and activates interferon regulatory factor 3 (IRF3) and tank-bound kinase 1 (TBK1) to start downstream signaling processes. Mn2+ can also be used for magnetic resonance imaging (MRI) to direct therapy. Proinflammatory cytokines including IFN-β and interleukin-6 (IL-6) as well as type I interferon (IFN) were developed and secreted in order to activate the STING pathway. The maturation and antigen presentation of dendritic cells were then facilitated by type I interferon. Additionally, by altering the PD-L1 gene, CRISPR-Cas9 triggered the development of cytotoxic T lymphocytes from immunosuppressed T cells and triggered a series of powerful cellular immune responses that prevented cancer[86].

Emerging therapeutic strategies

The traditional cancer treatments, e.g., chemotherapy, radiotherapy, and surgery, have been established for decades. Recently, immunotherapy against shape-tumor immunity has become a hot spot of biomedical research and clinical practice and has achieved obvious and efficient long-term therapeutic effects in some tumors. Both small molecular chemicals and biologics have been developed to selectively activate CDNs (cyclic dinucleotides) and STING through PKR purification, among which harmful STING agonists have higher potency and can induce strong and durable antitumor responses. Harmful agonists are administered through systemic, local, or vaccination delivery to attract and activate immune cells of the patient, thus achieving increased frequency and efficiency of tumor regression, preventing tumor metastasis, and enhancing durability of response. Among the most widely studied STING agonists, DMXT Dimer, which is an efficient macrofollicle delivery product, has achieved significant benefit in combination immune therapy in mice and demonstrates the positive immune response in the tumor[87].

Nowadays, the exploration of harmful agonists has extended to human beings. ADUS100 and ADUS1008, two novel agonists, have been synthesized, which can induce strong type I interferon activation in both human primary peripheral blood mononuclear cells and HEK293 cell lines. In particular, AGT's ALRN-6924 is currently in preclinical testing for evaluation of safety, pharmacologic, and pharmacodynamics profile suitable for intratumor injection. Additionally, the vaccines have been developed to enhance the antitumor immune response, which is maximized by co-targeting STING in the cDC (conventional dendritic cell) to make DC vaccine or STING agonist vaccine. Among them, NeuVax is one preclinical STING agonist vaccine that has the Phase II Trials, which induces a 24.1% pERB2-specific cellular immune response. Immune markers that show T cell activation, T cell proliferation and migration, and NK cell-mediated killing can be used to investigate the effective immune response. These markers enable successful antitumor responses in a variety of solid tumor models[88,89].

Challenges and opportunities

The pathway known as cGAS-STING has become a critical regulator of tumor immunity, with its activation demonstrating the potential to impede initial neoplastic progression through the upregulation of ISGs and the mediation of the release of cytokines, chemokines, and proteases associated withthe secretory phenotype linked to senescence, collectively restricting tumorigenesis[77]. Notably, cancer cells withChromosome mis-segregation occurs frequently in unstable genomes during cell division, leading to the generation of micronuclei that canburst and

allow the cytosol to contain the genetic contents, subsequently detected by cGAS. Moreover, the transfer of tumor-cell-derived cGAMP into immune cells can activate the cGAS-STING pathway, facilitating antigen-specific priming of T-cells. Preclinical models have further underscored the significance of cGAS-STING signaling in cancer, as evidenced by enhanced tumorigenicity and decreased CTL infiltration in tumors with lost or reduced STING expression.

While the potential of STING agonists in strengthening current immunotherapies and increasing anti-tumor immunityis well-supported, several challenges exist in the clinical targeting of the cGAS-STING pathway[90]. The administration of first-generation CDN STING agonists intratumorally limits their use to accessible tumors, prompting efforts to developsubstances with enhanced systemic delivery capabilities. Concerns over the potential induction of pathologic inflammation and off-target effects on immune cells, as well asthe cGAS-STING pathway's selective inactivation in some cancers, highlight the need for a comprehensive comprehension of the mechanisms causing abnormalities in STING signaling and the development of remedial proceduresto boost the tumor microenvironment's response to STING agonists.

Future direction

Future directions regarding the use of STING antagonists in cancer immunotherapy were discussed. The potential of STING agonists as potent drugs for immunotherapy has been demonstrated, but there are still challenges in clinically targeting the cGAS-STING pathway. One significant barrier is the limited use of first-generation CDN STING agonists due to their requirement for intratumoral administration, which restricts them to accessible tumors. Efforts are being made to develop compounds with improved properties for systemic delivery in order to overcome this limitation[90].However, concerns remain about the potential induction of pathologic inflammation and off-target effects on immune cells, particularly T cells, as a result of systemic incorporation of STING agonists. Additionally, the potential negative feedback loops and resistance mechanisms that may dampen the effect of STING agonists need further exploration.

Another area of future research is understanding the detailed understanding of the underlying molecular pathways defining sensitivity to STING agonism and identifying suitable biomarkers to determine sensitivity. This is essential for choosing the right patients to participate in clinical trials using synthetic STING agonists[83]. Furthermore, because of their susceptibility to the reactivation of STING and its downstream signals, particularly STAT1, cancer types linked to the inactivation of the cGAS/STING pathway, including KL NSCLC, may be attractive therapeutic targets for STING agonists.

Concluding remarks

The cGAS-STING pathway has been acknowledged as a noteworthy immune regulatory pathway to identify cytosolic DNA. The burgeoning interest in the cGAS-STING pathway utilizing individual patient immune system for eradicating tumors, Intriguingly, STING pathway acting as a critical immune sensor to controlDNA sensing from tumors and T-cell priming. This evaluate highlighted the crosstalk of cGAS-STING and TBK1 signaling pathway in cancer immunotherapy, which reveals a pivotal convergence of innate immunity and tumor eradication, offering profound implications for therapeutic interventions. This axis orchestrates a finely-tuned immune response by sensing cytosolic DNA through cGAS, which catalyzes the production of cyclic GMP-AMP(cGAMP). The subsequent activation of STING receptor triggers TBK1 and IRF3 signaling pathway, resulting in the production of Type-I interferons and pro-inflammatory cytokines. This

immunomodulatory cascade enhances antigen presentation, promotes dendritic cells maturation, and activates cytotoxic T-lymphocytes, facilitating robust anti-tumor immunity. Notably, cGAS-STING/TBK1 pathway demonstrated dual mechanism to foster immunogenic cell death and suppress tumor progression, an emergent therapeutic target in cancer immunotherapy to increase the therapeutic efficacy when combined with immunotherapy from cancer vaccine to ICIs, and CAR-T cells immunotherapies. This intricate signaling network presenting the opportunities and challenges for the development of next generation cancer immunotherapeutic.

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Sero-prevalence of syphilis among Anti Natal Care clinic attendees in three hospitals in Eastern India: A retrospective study

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Abstract

***Introduction*:** Syphilis along with a group of bacterial diseases like chancroid, and gonorrhoea remains treatable Sexually Transmitted Infections. Acquired syphilis specifically during pregnancy carries an inherent risk of fetal malformation like non-hemolytic hydrops. The risk of congenital syphilis increases with the time the mother develops the disease if it is acquired during the present pregnancy it increases a lot. Therefore, early diagnosis during the 1st contact in an antenatal care visit is essential as per the guidelines of MoHFW.

NACO's National AIDS Control Program since 1992 had a stellar performance in containing the HIV spread through gender mainstreaming, working on HIV Care and support, and containing the spread of sexually transmitted infections through targeted intervention programs.

***Methods*:** Three hospitals in Eastern India were selected. Selected hospitals have been a medical college in North 24 Parganas, a teaching hospital in the West District of Tripura, and a primary health center in the Darjeeling district.

***Results*:** During the financial year 2023-24, data obtained from a medical college was 1, 7 and 6 seropositive cases out of 3217, 2018,2008 cases respectively

***Discussion*:** This medical college hospital is part of the Kolkata Metropolitan A, teaching hospital in Tripura caters to a vast area of the West District and the PHC mentioned caters to a diverse population. Hence the estimated prevalence of 0.16% is an indication that the primary objective of sexually transmitted infection containment has succeeded and values have remained stable.

Keywords: Syphilis, antenatal care, MoHFW, seropositive case

Introduction

Congenital syphilis was described by Gasper Torella in 1497. It is caused by vertical transmission of Treponema pallidum and has adverse effects on the fetus. This syphilis constitutes the group of treatable sexually transmitted infections. The effect of untreated syphilis on maternal and neonatal health outcomes is immense. Institute of Health Metrics mother-to-child transmission causes globally 3.6 Million $ Disability Adjusted Life Years and costs 309 $ million in costs worldwide [1].

Syphilis has been recorded in human antiquity. Primary lesions in females go unnoticed in most cases. Mode of presentation for syphilis is primary chancre over coronal sulcus in males and females it is primary chancre over labia majora in females which may go unnoticed [2].

The incubation period of syphilis is 10 to 90 days [average is 21 days). There is a small abrasion on the skin and lymphatic vessels are invaded. The lesion may be so innocuous that people may forget it or go unnoticed.

The secondary lesion appears as a maculopapular rash. Tertiary syphilis and congenital syphilis are the outcomes of untreated syphilis. It has a poor prognostic outcome. With the advent of penicillin, and the progress of syphilis it has been possible to halt the progress of syphilis and the dreaded complication which has been mentioned can only be found in the annals of medical history. Perhaps the best-recorded movie onthe importance of syphilis treatment control versus no treatment is "Miss Evers' Boys". One does need to visit the pathology museums to see the outcome of tertiary and congenital syphilis. Therefore, syphilis has been regarded as a dreaded disease [3].

A note on the pathogenesis of syphilis

Trepanoma pallidum a spirochete is an etiologic agent of syphilis. There are three diseases caused by spirochetes. Other diseases caused by species of Trepanoma are Pinta and Bejel. It has been possible through sustained efforts it has been possible to eliminate these diseases.

Trepanoma – syphilis (sexually transmitted infectious disease), non-venereal disease Yaws, Pinta

Borrelia – Lyme disease and relapsing fever

Leprosaria- Leptospirosis

The organism is extremely small and rarely visible by a light microscope. It is identified by distinctive undulating movement by a dark field microscope [3].

It is rarely visible in vitro thus transmission almost always requires direct contact with the infectious lesion. WHO estimates that 12 million are infected every year. Screening and early detection can lower the costs of treatment. Centre for Disease Control recommends that those who have syphilis should be tested for HIV as they coexist. The mechanism of transmission remains the same, ulcer produced by syphilis may bleed which increases the risk of transmission. Chances increase the risk of HIV transmission to 2 to 5 times. Studies done in Bhopal have shown the prevalence of syphilis in rural has been 37.7% while urban population it is 63.3%. The exact prevalence of STI specifically among Antenatal women in India is not readily available in NFHS 5. In 2014 prevalence was 9.07% among high risk in a study from High Prevalence to 21.9% among truckers driving and working in Central India. In the same period, the prevalence of ANC is 1.9%.

Syphilis is classified as

a. Primary
b. Secondary
c. Latent
d. Congenital

Congenital syphilis may present as cerebral palsy, hydrocephalus, sensorineural deafness, and musculoskeletal deformity [3].

Diagnosis of congenital syphilis

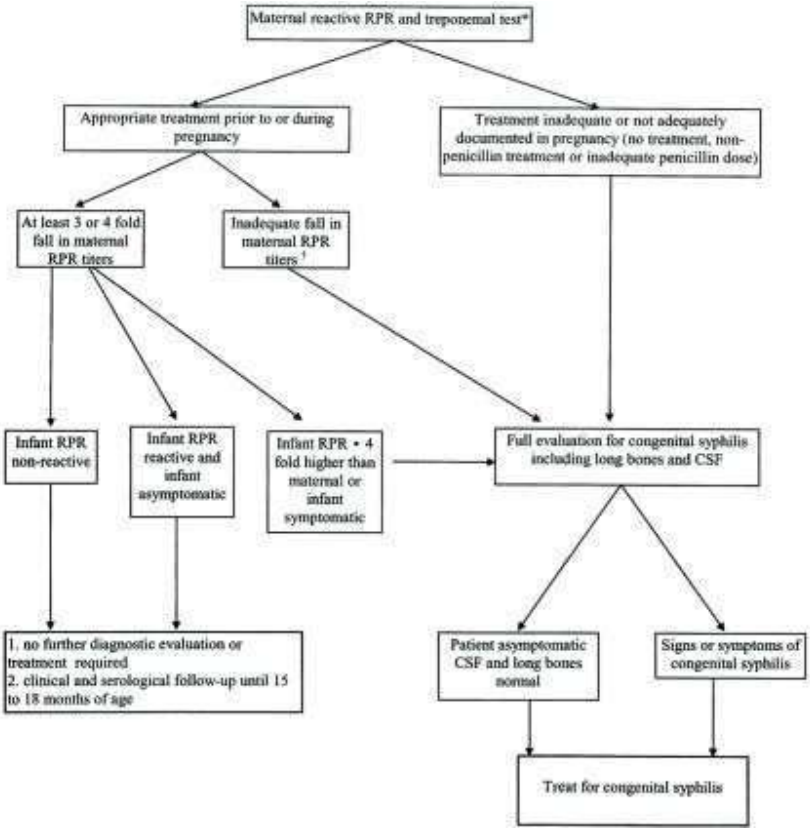

Fig. 1. Diagnosis of congenital syphilis

Ministry of Health and Family Welfare in 2014 brought out a guideline for screening syphilis in pregnant women. The ministry has reiterated its commitment to bringing down the Maternal Mortality Rate ratio in these guidelines. From 1990, which aligns with the Government of India's commitment to achieving sustainable development goals [4,5,6,7]

Guidelines for diagnosis of syphilis by the Ministry of Health and Family Welfare

Guidelines put forward are simple and cost-effective measures and have been aimed in achieving eliminating congenital syphilis as a part of SDG. As with any guideline developed by the ministry lot of stakeholders are involved. Development partners, faculties of medical college, NACO. Syphilis being a sexually transmitted infection has the same mode of transmission as HIV/AIDS. So if diagnosis of syphilis is done early it is possible to diagnose and eliminate HIV transmission. The reason is that the screening for syphilis and HIV is being done in the same sitting during Village Health and Nutrition Day or the same Antenatal Clinic Day (Points of Care). Kit for the same are carried in the same specially adapted cold box. This is part of the RMNCH+A strategy of the Ministry of Health and Family Welfare [4]

Based on HIV sentinel surveillance in 2010-11 average seroprevalence of syphilis was found to be quite high. In West Bengal it was found to be 1.91% and Tripura [0.63%}; the two states are being highlighted among other states that would be part of the narrative in this article. As has been

previously mentioned elimination of syphilis is part of sustainable development goals. This is best described as a three-pronged strategy

1. Elimination of syphilis will result in reduced incidence of low birth weight, perinatal death and congenital syphilis (SDG 4) [8, 9]
2. Reduction of incidence of stillbirth and spontaneous abortion will help in improving maternal outcome and reduce maternal morbidity [SDG 5]
3. This will also reduce HIV transmission through behaviour change counseling (SDG 6) [8,9]

Protocol for investigation and management of syphilis

During the 1st ANC visit the women should be tested for syphilis using RPR [Rapid Plasma Regain test}. Women who test positive for syphilis also do antibody titer. Estimation of antibody titer would decide the course of treatment for newborns.

A repeat of syphilis would be undertaken in the following circumstances.

a. history of STI
b. Women with more than one sexual partner
c. Sex workers
d. Injecting drug users
e. Residing in anarea which has a high prevalence of syphilis

There is a mandate for spouse testing, testing of newborns and treatment for newborn. Pregnant women tested again in the third trimester and newborns test should also be tested.

The drug of choice remains Penicillin during pregnancy [8,10].

Health facility where the study has been done

It has been done in one of the medical colleges in the northern fringes of the city.

North 24 Parganas is a part of the urban agglomerate of Kolkata. The area has a large number of municipalities. Therefore,

District Hospital- Secondary Health Care facility now upgraded to a Medical College [tertiary care facility} Thisis a part of Kolkata Metropolitan Agglomerate. There are 27 municipalities in NORTH 24 PARGANAS [11,12]

Primary Health Care is situated in the Darjeeling district. It is part of the greater Siliguri Metropolitan Area. Therefore, this area is an urbanized village. It is now best described as a cosmopolitan nature of this area [13].

This hospitalis one of the major hospitals in the West District of Tripura [14].

ANC women can be considered a low risk in sentinel surveillance sites. Therefore, assessing the sero prevalence of VDRL in pregnant women during the antenatal period is highly necessary for a healthy state and also important for this study [6].

Syphilis and HIV coinfection are particularly prevalent in MSM. Having syphilis can increase a person's risk of contracting HIV, syphilis may progress faster and be harder to treat, although these effects are more common in people with high viral load and low CD4 count

Incidence and prevalence of syphilis before and after the introduction of guidelines

WHO made a sustained effort in 2007, and launched a global initiative to eliminate mother-to-child transmission. The outcome of the effort would be eliminating less than 50 cases per 100,000 live births.

Between the period 2012-2016, global maternal syphilis prevalence in 2016 was 0.69% [95% confidence interval: 0.57–0.81%} resulting in a global CS rate of 473 [385–561} per 100,000 live births and 661,000 [538,000–784,000} total CS cases, including 355,000 [290,000–419,000}

Adverse birth outcome (ABO)is defined as stillbirth, live birth characterized by congenital syphilis like failure to thrive or deficient limb movement as a proven radiological sign in a child less than 2 years of age. Such adverse outcomes of childbirthis evident if the mother is not adequately treated [6].

WHO estimates that globally there has been a drop in active mother to child syphilis transmission.

Objective of this study

A review of seroprevalence data on Syphilis diagnosed through VDRL or RPR has been obtained from HMIS data or hospital records in three hospitals of Eastern India.

Methodology

It is a retrospective study based on Health Management Information Study [HMIS} data. The data manager of the hospital provided the data from the HMIS portal which is available in the public domain.

Ethical Clearance was obtained from the NSHM Institute Ethical Clearance

Results

The state district hospital is now upgraded to a medical college hospital hence a tertiary care hospital.

Total number of pregnant **cases** 3217 cases were pregnant 1 tested positive

There has been 1 reported case in the years 203-24. The test done has been the Rapid Plasma Regain Test

Table 1. Tertiary Care Center and State Hospital of Tripura located in Agartala

	Month	Pregnancy cases	Seropositive cases
	April	333	3
	May	337	1
	June	267	1
	July	356	0
	August	328	0
	September	231	1
	October	227	1
	November	304	0
	December	97	0
	January	212	0
	February	23	0
	March	99	0

Total number of pregnancy cases was 2817.

This data has been obtained from the ANC-registered clinic Seropositive cases is 7.

Table 2. Results from Primary Health Center in Darjeeling district.

	Month	Pregnancy cases	Sero- positive cases
	April	150	1
	May	170	0
	June	212	0
	July	152	1
	August	144	2
	September	243	1
	October	220	0
	November	172	0
	December	242	0
	January	120	0
	February	51	0
	March	**132**	**1**

Discussion

HMIS data has been analyzed from three different hospitals. Serology was done using either VDRL or RPR in these hospitals

In Village Health Nutrition Day or Urban Health Nutrition Day}sessions or in Antenatal Clinics it is necessary to have screened for syphilis. Hence in the Barasat Medical College, ANC clinics out of 3127 pregnant cases 1 particular case was found to be positive. In West District hospital likewise, 2769 pregnant mothers 7 were found to be positive. Apple and oranges are not been compared in this study it shows the proportionally higher number of cases are found to be positive though coverage at the Medical College mentioned here caters to a larger population load [it has 27 municipalities} and larger population and is part of larger Kolkata Urban Agglomerate. The figures may be underreporting.

The hospital in West District is also a tertiary care center. It serves people from the West, South and outskirts of the neighboring Sephijala district. The client base is best described from both rural hospitals [14].

Centre for Disease Control has said rise in syphilis cases around the world. The year 2020 has been stated as the year of the pandemic when there was a lock down across the world. CDC reported that in the USA, there has been an increase in trend of three treatable STI cases of Congenital Syphilis, Syphilis, Gonorrhea, and Chlamydia increase in number from 2016. Hence they have advocated for good surveillance. Even in 2021, it showed an increasing trend. There were reports of 2.5 million cases. The cases were chlamydia, gonorrhea and syphilis.

In countries like Ethiopia a study was done to the prevalence of 4 sexually transmitted infections using Hepatitis B surface antigen, Anti- HCV, HIV, Syphilis proportion of positive cases are 56.3%, 27.1%, 10.4%and 6.3%. This data was taken from youth friendly services clinic of city in this country.

Though there is no data to compare between previous years but first case of HIV India started the National AIDS Control Program in 1992 The 1st phase of the program was meant for awareness generation. Focus shifted to involvement of NGO and mainstreaming of network of people of HIV. During the phases of National AIDS control program has been mitigation of AIDS and also lowering the burden of Sexually Transmitted Infections through intervention in the high risk group and finally doing a sentinel surveillance. There are few studies based on evaluated data regarding prevalence of syphilis in India. BK Das et al. has analyzed the trend of syphilis between the year 2002 and 2012. They analyzed the data of 73642 cases overall 393 cases tested positive. In this study there was 83.3% cases were from ANC clinic [pregnant women and their spouses].

India's strategy to control sexually transmitted infection has been a multipronged approach in counselling. Counseling helps the people to make people make informed decision. Sexually transmitted infections is associated with stWest District hospital a. Isolation, discrimination, rejection and discrimination. The West District hospital a associated with STI have a gender dimensions. Khan S et al studies done in South India ha s shown a decline in sero- prevalence of syphilis cases from 0.88 [2008} to 0.66% in 2008 which has found to be statistically significant. Sero-positivity cases highest in 20-30 years age group. Estimated prevalence is 2.0 to 4.8%. Screening of surveillance has led to development of syphilis screening guidelines as a booklet by the MoHFW in 2014. However, there needs to be word of caution for all stake holder Recent data from ANC clinic [2014 to 2024} was not found. In the study done in Jawaharlal Nehru College and Hospital sero prevalence was 0.15%. This data is of 2014. In these three hospitals the total number of pregnant cases is 7428, while the number of syphilis cases is 12 is 0.16%.

Result is similar in Aligarh. It can be admitted social and cultural is not similar but it does make sense that program has been optimally successful but however in three divergent social milieu this low prevalence is extremely encouraging. Data collected is regarding sero prevalence of syphilis in ANC clinic is an interesting one. ANC clinic are considered low risk population. Proportion of cases among ANC mothers in area of high migratory population [PHC of Darjeeling district PHC}, number of pregnant women 2008, and Sero Positive Cases 6 cases, West District Hospital[2814 cases 7 cases} is a signature that the program has been well sustained.

Success of any campaign is its sustainability. Therefore, there is a need to continue the work of advocacy and action for syphilis screening among low risk group.

WHO had set the targets for reduction of gonorrhea and syphilis between 2018 and 2030. When a broader area of South East Asia is concerned, there are decline in countries like Sri Lanka and Thailand. There has been decline in many regions of India but recent data is lacking in the public domain to substantiate this. However, targeted intervention programs launched in earlier years does appear to have a sustainable impact. It has lowered the prevalence of sexually transmitted infection and hence slowed the HIV transmission

Limitation

There was no scope for speaking with partners of ANC pregnant women as it is done on secondary data analysis from clinic record or HMIS data. So detailed history of sexual behavior could not be ascertained.

Conclusion

It is indeed responsibility of the State AIDS control continue the work of health promotion and awareness generation on targeted intervention program for control of sexually transmitted infection. It may be a long way for the triple elimination of HIV, Syphilis and hepatitis B.

Ethical Clearance has been obtained from NSHM Institute Ethics Committee.

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Exploring the Link between Sleep, Chronotype, Diet, and Polycystic Ovarian Syndrome in Indian Women of Reproductive Age (15-45 Years): A Cross-Sectional Study

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Abstract

PCOS, a common hormonal imbalance in women, often leads to metabolic issues and difficulty conceiving. This research explored the potential connections between PCOS and individual differences in sleep, circadian rhythms, and food choices among Indian women. Among the participants, 51 were diagnosed with PCOS and 52 served as healthy controls (HC). Data were collected through self-reported and validated questionnaires covering sociodemographic, medical history, age at menarche, sleep quality, chronotype, and dietary habits. Findings revealed that 31.4% of women with PCOS experienced early menarche (before age 8), and 37.3% reported inadequate night-time sleep and daytime napping. Many women in the PCOS group experienced morning headaches, a potential sign of obstructive sleep apnoea. Women with PCOS were more likely to have a preference for later chronotype ($p < 0.00001$). Additionally, PCOS was linked to poor dietary habits, including high consumption of fried foods, carbonated beverages, and red meat, contributing to weight gain, lethargy, and depressive symptoms. Addressing PCOS requires a holistic approach, including early diagnosis and the implementation of lifestyle strategies, to improve overall well-being and quality of life.

Keywords: PCOS, Menarche, Sleep Quality, Chronotype, Mediterranean Diet, Dietary Habits, Lifestyle

Introduction

Polycystic Ovarian Syndrome (PCOS) is a multifaceted endocrine disorder affecting 5-20% of women of reproductive age, though up to 70% of cases remain undiagnosed. First described in 1935, PCOS is characterized by irregular menstrual cycles, excessive hair growth, and enlarged ovaries with numerous small follicles [1]. Over time, our understanding of PCOS has evolved, recognizing it as a complex condition linked to metabolic imbalances, infertility, and a range of other health concerns, including psychiatric, cardiovascular, and cosmetic issues. [2].

The exact causes of PCOS remain unclear, though low-grade inflammation and oxidative stress are believed to play a role. It likely results from environmental, polygenic, and epigenetic interactions [3]. PCOS is diagnosed using the Rotterdam criteria, which require two of the following: hyperandrogenism, oligo- or anovulation, and polycystic ovaries. Hyperandrogenism, present in 80% of cases, is a key feature, and PCOS is more prevalent in women with obesity,

affecting 38-88% [4]. The surge in PCOS cases parallels the increase in early puberty, particularly idiopathic central precocious puberty (ICPP), which involves premature ovarian and uterine maturation due to early activation of the hypothalamic-pituitary-gonadal axis [5]. This leads to increased luteinizing hormone (LH) activity before age eight. PCOS is associated with a hypothalamic shift marked by increased GnRH and LH pulses [6]. Women with a history of ICPP often exhibit higher rates of biochemical hyperandrogenism, leading to symptoms like hirsutism and acne [7].

Despite available treatments, women with PCOS often struggle to meet health goals, partly due to sleep disturbances like obstructive sleep apnea (OSA). OSA is more common in PCOS and causes fatigue, restless sleep, and headaches [8]. Guidelines recommend screening for OSA to improve sleep and address related issues [9]. Short sleep duration in women with PCOS is linked to higher obesity risk, reduced insulin sensitivity, increased hunger, and menstrual irregularities [10]. Chronotype, or an individual's sleep and activity preference, is categorized into morning, evening, and neither type [11]. Evening chronotypes are linked to poorer dietary adherence [12], higher obesity rates [13], and increased insulin resistance [14]—issues common in women with PCOS [15]. Physiological functions like body temperature and hormone secretion vary by chronotype [16], with evening types often exhibiting unhealthy lifestyles, poor dietary habits, and higher risks of health problems [17]. Given the role of lifestyle and nutrition in managing PCOS, chronotype is now recognized as a key factor influencing food preferences and chronic disease risk [18].

Eating habits are linked to conditions like cardiovascular diseases and type 2 diabetes [19]. Obesity and PCOS share mechanisms, with obesity worsening PCOS and increasing the risk of metabolic syndrome [20]. Unhealthy eating, reduced physical activity, and irregular sleep contribute to PCOS by promoting obesity, disrupting gut flora, triggering inflammation, increasing insulin resistance, and driving androgen overproduction, leading to hormonal imbalances [21].

The primary aim of this study was to evaluate sleep variables and chronotype categories in women with PCOS using previously validated questionnaire and compare these with age-matched controls. Additionally, this study sought to explore the relationship between general eating habits and the occurrence of PCOS. The findings underscore that adopting a healthier lifestyle and maintaining good dietary habits have positive effects on physical well-being in women with PCOS.

Materials and Methods

Study Population

The study focused on women between the ages of 15 to 45 years. Data were collected from two groups: women diagnosed with PCOS (case group) and women without PCOS (control group).

Study Considerations

- **Subjects:**
  Women aged 15-45 years from various regions across India.
- **Place of Study:** Data were gathered from participants all over India.

- **Duration of Study:** 9 months (September 2023 – May 2024).
- **Study Design:** Observational, cross-sectional study.
- **Sampling Method:** Participants who fell within the specified age range and were willing to participate were included in the study.

Study Tools

Information on sociodemographic characteristics, medical history, menstrual history, and age of menarche was self-reported by participants. Additionally, participants self-reported their height and weight, which were used to calculate Body Mass Index (BMI). The following tools were used to evaluate sleep quality, chronotype categories, and dietary habits:

Variables	Equipment and Materials used
Sleep quality assessment	Pittsburgh Sleep Quality Index (PSQI) [22]
Chronotype Categories	Munich Chronotype Questionnaire (MEQ) [23]
Dietary habits	Food Frequency Questionnaire

Statistical Analysis

Statistical analysis was conducted using the widely-used software package SPSS version 16.0.

Results

Table 1. Demographic Profiles of Women with PCOS and a Healthy Control Group

Attributes	PCOS (N = 51)	HC (N = 52)
Age	19-40 years	18-40 years
Mean age	24.39 years	23.78 years
Education status		
▪ Under-graduate	5 (9.8%)	16 (30.8%)
▪ Graduate	34 (66.7%)	21 (40.4%)
▪ Post-graduate	12 (23.5%)	14 (26.9%)
▪ PhD	0 (0%)	1 (1.9%)

Occupation		
▪ Accountant	0 (0%)	2 (3.8%)
▪ Athlete	0 (0%)	1 (1.9%)
▪ Dietitian	0 (0%)	2 (3.8%)
▪ Digital creator	1 (2.0%)	0 (0%)
▪ Engineer	12 (23.5%)	4 (7.7%)
▪ Entrepreneur	0 (0%)	1 (1.9%)
▪ Government employee	2 (3.9%)	0 (0%)
▪ House-maker	0 (0%)	2 (3.8%)
▪ Researcher	0 (0%)	1 (1.9%)
▪ Sales employee	4 (7.8%)	0 (0%)
▪ Student	30 (58.8%)	37 (71.2%)
▪ Teacher	2 (3.9%)	2 (3.8%)
Marital Status		
▪ Un-married	42 (82.4%)	47 (90.4%)
▪ Married	9 (17.6%)	5 (9.6%)
Body Mass Index (BMI)		
▪ Underweight	2 (3.9%)	3 (5.8%)
▪ Normal Range	12 (23.5%)	22 (42.3%)
▪ Overweight	11 (21.6%)	9 (17.3%)
▪ At risk	10 (19.6%)	5 (9.6%)
▪ Obese I	7 (13.7%)	10 (19.2%)
▪ Obese II	9 (17.6%)	3 (5.8 %)

Onset of Menarche and Its Association with PCOS

Table 2. Association between age of menarche among PCOS and HC group

Type of study sample	**Mean Age of menarche (years)**	**p-value**
PCOS (N = 51)	8.9412	**0.000***
HC (N = 52)	12.0192	

*Chi-square test demonstrated a highly significant relationship between the age of menarche and PCOS ($p < 0.00001$).

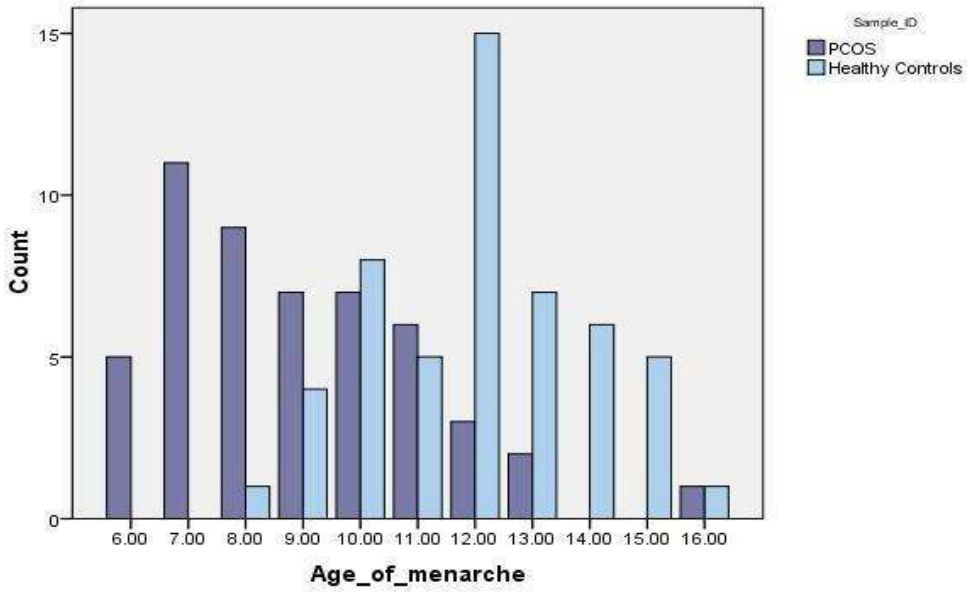

Figure 1. Bar diagram illustrating the age of menarche for study subjects, including both the PCOS and Healthy Control (HC) groups.

Association Between Sleep Quality and PCOS

Table 3. Sleep pattern in women with PCOS and HC group

Sleep Parameters	PCOS (N= 51)	HC (N= 52)	p-value
1. Hours of sleep/night			
▪ **Very less (4 hrs or less)**	19 (37.3%)	4 (7.7%)	
▪ **Less than normal (5-6 hrs)**	24 (47.1%)	6 (11.5%)	**0.000***
▪ **Normal (7-8 hrs)**	8 (15.7%)	42 (80.8%)	
2. Troubled sleep during past month			
▪ **Never**	24 (47.1%)	28 (53.8%)	0.138
▪ **Yes**	21 (41.2%)	23 (44.2%)	
▪ **Yes and regularly**	6 (11.8%)	1 (1.9%)	

Because of – **2.a) Awakenings in middle of night**			
▪ **Not during past month**	12 (23.5%)	34 (65.4%)	**0.000***
▪ **Less than once a week**	7 (13.7%)	7 (13.5%)	
▪ **Once or twice a week**	24 (47.1%)	9 (17.3%)	
▪ **Three or more times a week**	8 (15.7%)	2 (3.8%)	
2.b) Cannot get to sleep within 30 minutes			
▪ **Not during past month**	14 (27.5%)	34 (65.4%)	
▪ **Less than once a week**	9 (17.6%)	14 (26.9%)	**0.000***
▪ **Once or twice a week**	20 (39.2%)	4 (7.7%)	
▪ **Three or more times a week**	8 (15.7%)	0 (0%)	
2.c) Troubled breathing			
▪ **Not during past month**	36 (70.6%)	48 (92.3%)	
▪ **Less than once a week**	8 (15.7%)	3 (5.8%)	**0.034***
▪ **Once or twice a week**	5 (9.8%)	1 (1.9%)	
▪ **Three or more times a week**	2 (3.9%)	0 (0%)	
2.d) Having pain			
▪ **Not during past month**	22 (43.1%)	40 (76.9%)	
▪ **Less than once a week**	4 (7.8%)	4 (7.7%)	**0.002***
▪ **Once or twice a week**	22 (43.1%)	8 (15.4%)	
▪ **Three or more times a week**	3 (5.9%)	0 (0%)	
2.e) Other reasons			
▪ **Not during past month**	20 (39.2%)	48 (92.3%)	
▪ **Less than once a week**	10 (19.6%)	4 (7.7%)	**0.000***
▪ **Once or twice a week**	11 (21.6%)	0 (0%)	
▪ **Three or more times a week**	10 (19.6%)	0 (0%)	

This table reveals noticeable differences in sleep patterns between women with PCOS and those without the condition. Women with PCOS tend to get less sleep, with many reporting under the recommended 7-8 hours, often struggling with issues such as difficulty falling asleep, frequent awakenings, and disturbances due to breathing issues, pain, or other factors. Only 15.7% of women with PCOS reported achieving normal sleep, compared to 80.8% in the healthy group, with many sleeping just 4-6 hours or less. Significant p-values for several factors, including sleep duration, awakenings, and breathing issues, suggest these sleep disturbances are particularly prevalent among women with PCOS, highlighting a greater challenge in achieving restful sleep compared to the control group.

Table 4. Sleep quality analysis of women with PCOS and HC group

Sleep Parameters	PCOS (N= 51)	HC (N= 52)	p-value
1. Sleep Quality			
▪ **Very good**	8 (15.7%)	19 (36.5%)	
▪ **Fairly good**	28 (54.9%)	29 (55.8%)	**0.008***
▪ **Fairly bad**	11 (21.6%)	4 (7.7%)	
▪ **Very bad**	4 (7.8%)	0 (0%)	
2. On medication for sleep			
▪ **Not during past month**	39 (76.5%)	46 (88.5%)	
▪ **Less than once a week**	4 (7.8%)	2 (3.8%)	0.304
▪ **Once or twice a week**	6 (11.8%)	4 (7.7%)	
▪ **Three or more times a week**	2 (3.9%)	0 (0%)	
3. Had trouble staying awake while driving, eating meals or engaging in social activities			
▪ **Not during past month**	24 (47.1%)	48 (92.3%)	
▪ **Less than once a week**	13 (25.5%)	2 (3.8%)	**0.000***
▪ **Once or twice a week**	2 (3.9%)	2 (3.8%)	
▪ **Three or more times a week**	12 (23.5%)	0 (0%)	

4. Problem in keeping up enough enthusiasm to get things done ▪ **No problem at all** ▪ **Only a very slight problem** ▪ **Somewhat of a problem** ▪ **A very big problem**	33 (64.7%) 10 (19.6%) 7 (13.7%) 1 (2.0%)	39 (75.0%) 11 (21.2%) 2 (3.8%) 0 (0%)	0.229
5. Episodes of disorientation/confusion during sleep ▪ **Not during past month** ▪ **Less than once a week** ▪ **Once or twice a week** ▪ **Three or more times a week**	26 (51.0%) 8 (15.7%) 16 (31.4%) 1 (2.0%)	49 (94.2%) 2 (3.8%) 1 (1.9%) 0 (0%)	**0.000***
6. Had morning-headache ▪ **Not during past month** ▪ **Less than once a week** ▪ **Once or twice a week** **Three or more times a week**	7 (13.7%) 17 (33.3%) 19 (37.3%) 8 (15.7%)	48 (92.3%) 3 (5.8%) 1 (1.9%) 0 (0%)	**0.000***

This table highlights key differences in sleep quality and related issues between women with PCOS and a healthy control group. Women with PCOS reported lower overall sleep quality, with only 15.7% rating it as "very good," compared to 36.5% in the control group. PCOS participants also reported more frequent drowsiness or difficulty staying awake during daily activities, such as driving or eating, with 23.5% experiencing this three or more times a week, while no one in the control group reported this frequency. Issues like disorientation during sleep and morning headaches were also much more prevalent among those with PCOS; 31.4% experienced disorientation once or twice a week, and 37.3% reported morning headaches, compared to minimal or no reports in the control group. Although both groups had similar rates of slight difficulties in keeping up enthusiasm, women with PCOS seemed more frequently affected by these sleep-related challenges, as evidenced by significant p-values for sleep quality, daytime alertness, disorientation, and morning headaches, underscoring the broader impact of sleep disturbances among women with PCOS.

Chronotype Categories and Their Association with PCOS

Table 5. Chronotype Comparison: PCOS vs. Healthy Controls

Chronotype Categories	PCOS (N = 51)	HC (N = 52)	p-value
Extreme early type	0 (0%)	3 (5.8%)	**0.000***
Moderate early type	2 (3.9%)	14 (26.9%)	
Slight early type	12 (23.5%)	13 (25.0%)	
Normal type	4 (7.8%)	12 (23.1%)	
Slight late type	14 (27.5%)	6 (11.5%)	
Moderate late type	8 (15.7%)	3 (5.8%)	
Extreme late type	11 (21.6%)	1 (1.9%)	

Table 5 shows a significant relationship between PCOS and chronotype categories ($p < 0.00001$). Most women with PCOS were classified as slight late type (27.5%), with significant portions as extreme (21.6%) and moderate late types (15.7%). Few were slight early type. In contrast, women in the Healthy Control group were mostly moderate (26.9%) and slight early types (25%), with a notable proportion as normal type (23.1%).

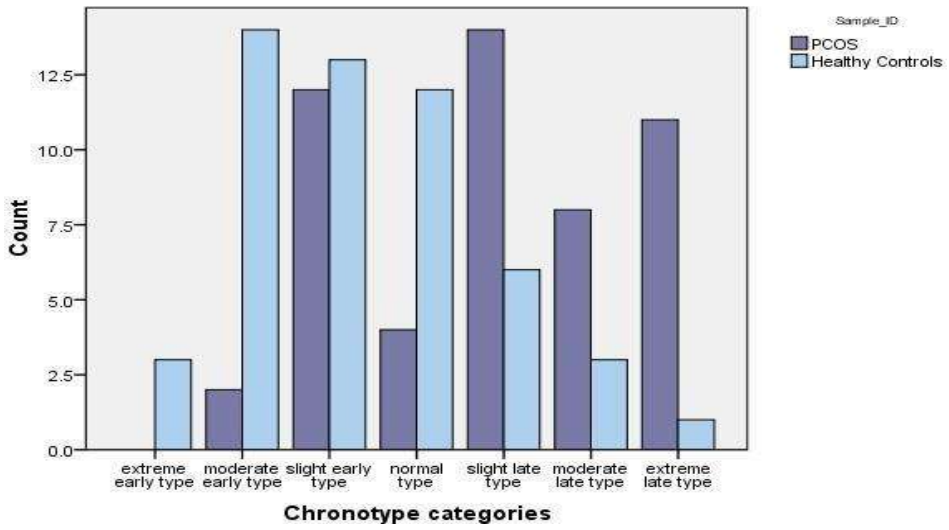

Figure 2. Bar Diagram Illustrating Chronotype Categories Among Study Subjects, Including PCOS and Healthy Control (HC) Groups

Table 6: Dietary Patterns: A Comparative Analysis of PCOS and Healthy Controls

Food Groups	Frequency	PCOS (N = 51)	HC (N = 52)	p-value
Processed cereal	Once a day	29 (56.9%)	29 (55.8%)	**0.020***
	Twice a day	15 (29.4%)	7 (13.5%)	
	Thrice a day	4 (7.8%)	1 (1.9%)	
	4-6 days a week	2 (3.9%)	6 (11.5%)	
	3-5 days a week	1 (2.0%)	5 (9.6%)	
	1-2 days a week	0 (0%)	3 (7.7%)	
	Never	0 (0%)	0 (0%)	
Unprocessed cereal	Once a day	29 (56.9%)	20 (38.5%)	0.067
	Twice a day	7 (13.7%)	16 (30.8%)	
	Thrice a day	0 (0%)	1 (1.9%)	
	4-6 days a week	2 (3.9%)	9 (17.3%)	
	3-5 days a week	3 (5.9%)	4 (7.7%)	
	1-2 days a week	0 (0%)	2 (3.8%)	
	Occasionally	7 (13.7%)	0 (0%)	
	Never	3 (5.9%)	0 (0%)	
Pulses	Once a day	20 (39.2%)	17 (32.7%)	0.889
	Twice a day	0 (0%)	0 (0%)	
	4-6 days a week	8 (15.7%)	12 (23.1%)	
	3-5 days a week	9 (17.6%)	10 (19.2%)	
	1-2 days a week	8 (15.7%)	5 (9.6%)	
	Occasionally	5 (9.8%)	3 (5.8%)	
	Never	0 (0%)	5 (9.6%)	
Roots and tubers	Once a day	12 (23.5%)	24 (46.2%)	**0.001***
	Twice a day	26 (51.0%)	12 (23.1%)	
	Thrice a day	10 (19.6%)	2 (5.8%)	
	4-6 days a week	2 (3.9%)	9 (17.3%)	

	3-5 days a week	1 (2.0%)	4 (7.7%)	
Green leafy vegetables	Once a day	18 (35.3%)	24 (46.2%)	0.059
	Twice a day	5 (9.8%)	7 (13.5%)	
	4-6 days a week	4 (7.8%)	8 (15.4%)	
	3-5 days a week	8 (15.7%)	10 (19.2%)	
	1-2 days a week	8 (15.7%)	2 (3.8%)	
	Occasionally	4 (7.8%)	1 (1.9%)	
	Never	4 (7.8%)	0 (0%)	
Other vegetables	Once a day	17 (33.3%)	20 (38.5%)	0.354
	Twice a day	17 (33.3%)	17 (32.7%)	
	Thrice a day	1 (2.0%)	2 (3.8%)	
	4-6 days a week	9 (17.6%)	3 (5.8%)	
	3-5 days a week	6 (11.8%)	5 (9.6%)	
	1-2 days a week	1 (2.0%)	3 (5.8%)	
Fruits	Occasionally	0 (0%)	2 (3.8%)	0.335
	Once a day	21 (41.2%)	27 (51.9%)	
	Twice a day	2 (3.9%)	5 (9.6%)	
	4-6 days a week	7 (13.7%)	9 (17.3%)	
	3-5 days a week	4 (7.8%)	4 (7.7%)	
	1-2 days a week	6 (11.8%)	3 (5.8%)	
	Occasionally	10 (19.6%)	4 (7.7%)	
	Never	1 (2.0%)	0 (0%)	
Fish	Once a day	15 (29.4%)	14 (26.9%)	0.277
	Twice a day	2 (3.9%)	4 (7.7%)	
	4-6 days a week	8 (15.7%)	10 (19.2%)	
	3-5 days a week	6 (11.8%)	9 (17.3%)	

	1-2 days a week	7 (13.7%)	1 (1.9%)	
	Occasionally	2 (3.9%)	5 (9.6%)	
	Never	11 (21.6%)	9 (17.3%)	
	Once a day	14 (27.5%)	13 (25.0%)	
	Twice a day	1 (2.0%)	2 (3.8%)	
	Thrice a day	1 (2.0%)	0 (0%)	
Egg	4-6 days a week	13 (25.5%)	8 (15.4%)	0.487
	3-5 days a week	10 (19.6%)	11 (21.2%)	
	1-2 days a week	8 (15.7%)	7 (13.5%)	
	Occasionally	2 (3.9%)	3 (5.8%)	
	Never	2 (3.9%)	8 (15.4%)	
Red meat	4-6 days a week	1 (2.0%)	0 (0%)	**0.000***
	3-5 days a week	17 (33.3%)	0 (0%)	
	1-2 days a week	17 (33.3%)	2 (3.8%)	
	Occasionally	13 (25.5%)	25 (48.1%)	
	Never	3 (5.9%)	25 (48.1%)	
Milk	Once a day			0.142
	Twice a day	15 (29.4%)	10 (19.2%)	
	4-6 days a week	2 (3.9%)	4 (7.7%)	
	3-5 days a week	3 (5.9%)	6 (11.5%)	
	1-2 days a week	4 (7.8%)	7 (13.5%)	
	Occasionally	1 (2.0%)	5 (9.6%)	
	Never	8 (15.7%)	11 (21.2%)	
Fats and oil	Once a day			**0.000***
	Twice a day	5 (9.8%)	27 (51.9%)	
	Thrice a day	17 (33.3%)	11 (21.2%)	
	4-6 days a week	29 (56.9%)	0 (0%)	

	3-5 days a week	0 (0%)	11 (21.2%)	
Sugar	Once a day			**0.000***
	Twice a day	21 (41.2%)	16 (30.8%)	
	4-6 days a week	29 (56.9%)	6 (11.5%)	
	3-5 days a week	0 (0%)	3 (5.8%)	
	Occasionally	0 (0%)	1 (1.9%)	
	Never	1 (2.0%)	19 (36.5%)	
Carbonated beverages	Once a day			**0.000***
	4-6 days a week	16 (31.4%)	2 (3.8%)	
	3-5 days a week	10 (19.6%)	0 (0%)	
	1-2 days a week	7 (13.7%)	4 (7.7%)	
	Occasionally	5 (9.8%)	10 (19.2%)	
	Never	13 (25.5%)	31 (59.6%)	
Cake and pastries	4-6 days a week			**0.000***
	3-5 days a week	8 (15.7%)	0 (0%)	
	1-2 days a week	12 (23.5%)	1 (1.9%)	
	Occasionally	16 (31.4%)	9 (17.3%)	
	Never	15 (29.4%)	32 (61.5%)	
Other snacks	Once a day	10 (19.6%)	0 (0%)	**0.000***
	4-6 days a week	13 (25.5%)	0 (0%)	
	3-5 days a week	13 (25.5%)	1 (1.9%)	
	1-2 days a week	11 (21.6%)	9 (17.3%)	
	Occasionally	4 (7.8%)	32 (61.5%)	
	Never	0 (0%)	10 (19.2%)	
Consumption of salad	Everyday			**0.000***
	Sometimes	10 (19.6%)	29 (55.8%)	
	Never	33 (64.7%)	23 (44.2%)	

Regular consumption of fried foods	Yes	50 (98.0%)	10 (19.2%)	**0.000***
	No	1 (2.0%)	42 (80.8%)	

This table compares dietary habits between women with PCOS and a healthy control group, showing significant differences in the consumption of various food groups. Women with PCOS reported higher daily intake of processed cereals, fats, and oils, with 56.9% consuming fats and oils 4-6 days a week, compared to none in the control group. PCOS participants also had a higher frequency of consuming sugar, carbonated beverages, and snacks like cakes, pastries, and other fast foods multiple times per week. Conversely, the control group showed a higher intake of healthier options, like unprocessed cereals, fruits, vegetables, and salads, with 55.8% consuming salads "sometimes" and only 44.2% avoiding them altogether, compared to 64.7% avoidance in the PCOS group. Significant p-values for items like red meat, fried foods, and sugary beverages emphasize that PCOS participants have a diet richer in processed and fried foods and poorer in fresh produce, suggesting dietary patterns that could impact metabolic health differently in these groups.

Discussion

This study evaluated the association between irregular sleep patterns, chronotype categories, and dietary habits with the occurrence of Polycystic Ovary Syndrome (PCOS) in comparison to age-matched healthy controls (HC).

A key finding in this study was that women with PCOS experienced menarche earlier than healthy controls, with a mean age of 8.94 years compared to 12.01 years in controls. Notably, 31.4% of women with PCOS reported menarche before age 8, whereas none of the controls did. This aligns with previous research linking early menarche and idiopathic central precocious puberty (ICPP) to an increased risk of PCOS later in life [24]. Prior research has indicated that 4-32% of adolescents with a history of ICPP develop PCOS, with hyperandrogenism (HA) being a common feature in adult women with a history of ICPP [25]. This study further reinforces the notion that early menarche may be a risk factor for PCOS, highlighting the importance of monitoring hormonal and reproductive health in women with early menarche.

The study revealed significantly poorer sleep quality in women with PCOS compared to healthy controls, including lower sleep efficiency, prolonged wakefulness, and frequent awakenings. Notably, 37.3% of women with PCOS slept less than 4 hours per night, and 52.9% reported daytime sleepiness, compared to 7.6% of controls. Difficulty falling asleep and night-time awakenings were strongly linked to PCOS ($p < 0.00001$). Symptoms like disorientation during sleep and morning headaches suggest obstructive sleep apnea (OSA), which aligns with research indicating higher daytime sleepiness and potential links to elevated androgen levels in PCOS [26].

High testosterone and psychiatric comorbidities, such as anxiety and depression, may also be related to disrupted sleep [27].

Chronotype analysis in the study showed a significant association between late chronotypes and PCOS. Women with PCOS were more likely to be classified as late chronotypes, with 27.5% identified as slight late types and 21.6% as extreme and 15.7% as moderate late types. In contrast, the majority of women in the healthy control group were classified as moderate or slight early types or normal types (23.1%). The finding that women with PCOS tend to have an evening chronotype aligns with existing literature, which links evening chronotype with obesity, unhealthy lifestyle choices, and adverse metabolic outcomes [28]. Individuals with a late chronotype often exhibit poor dietary habits, reduced physical activity, and are more prone to behaviors such as alcohol consumption and smoking, all of which can worsen metabolic profiles and increase the risk of obesity and insulin resistance [29]. Given that PCOS is already a risk factor for insulin resistance, circadian misalignment in women with PCOS may further exacerbate their metabolic health [30].

Women with PCOS exhibited significantly poorer dietary patterns compared to healthy controls. Most women with PCOS frequently consumed fried foods, baked goods, and sugary snacks, with only 19.6% eating salads regularly. Additionally, 31.4% drank carbonated beverages daily. Significant associations were found between PCOS and the consumption of red meat ($p < 0.00001$) and chicken ($p < 0.05$), which are high in saturated fats and linked to obesity and chronic conditions [31]. Unhealthy eating habits included a preference for fried foods (51%), street foods (45.1%), and restaurant meals (15.7%). These patterns align with previous research linking poor dietary choices to worsened PCOS symptoms [32].

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Marine Anti-Oxidants: A Brief Review

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Abstract

Anti-oxidants are reactive oxygen species (ROS) inhibitors which play vital roles in safe guarding us from ROS mediated damages responsible for development of several diseases and ailments. A number of anti-oxidants are found in many natural sources as exemplified by the anti-oxidant vitamins like vitamin C and alpha tocopherol. Beta carotene is another important example. Amongst synthetic anti-oxidants probucol, BHA and BHT are well known. Marine world is well known for its vast reserve of numerous unexplored anti-oxidants. It has been reported that there are different sources of anti-oxidants in seas and oceans. Several marine macroorganisms like sea cucumbers, sea weeds, fishes, sponges, corals, etc and microorganisms like fungi, bacteria, and microalgae are promising sources of many unexplored anti-oxidants Proper exploration and exploitation of them could be beneficial for serving mankind in a large sense.

Keywords: Anti-oxidants, marine source, macroorganisms, microorganisms

Introduction

Worldwide attempts to find new, biologically active products—which are acknowledged as the foundation of the future bioeconomy and, in particular, of potential drugs—are centered on marine species and their metabolites. Unusual, structurally varied chemicals created by marine organisms can occasionally have enough potentiality compared to those originating from terrestrial sources. When the marine organism comes in contact with stressful situations, it develops defense mechanisms that result in formation of the widely recognized metabolites of secondary type and other macro-molecules having anti-oxidant activity. Research on the exploration of sea anti-oxidants and their prospective application in pharmaceutical, cosmetic, nutraceutical, and other industries has attracted a lot of interest. Polysaccharides, amino acid-peptides, polyphenols, and carotenoids have biological activities as anti-oxidants, but they also have antibacterial, antineoplastic, diabetes inhibiting, anti-Alzheimer, anti-fibrotic, and neuroprotective characteristics. [1].

Anti-oxidant reservoir in sea

Anti-oxidants are abundant in the marine environment, however many are still untapped. The capacity of anti-oxidants depends on different variables, including the marine species, collection, extraction, etc., and most importantly the environment. For all those factors, variations in composition can be seen in the same biota around the world. Research based evidence tells us that the anti-oxidant molecules are present in several macroorganisms and microorganisms. Sea cucumbers, sea weeds, fishes & invertebrates (soft corals, crabs, sponges, crustaceans) come under macroorganisms. Fungi, bacteria, and microalgae come under microorganism [2].

***Sea cucumbers*:** These are the organisms obtained present in seas and oceans having aspects of cosmetics, foods, pharmaceuticals, etc [3]. Flavonoids and phenolic acids are the main component in this organism having anti-oxidant activity. Some researchers have published their report on Atlantic Sea cucumbers regrading 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) free radical controlling ability (ABTS RSA). It shows that *Holothuriaatra*is having good quantity of anti-oxidant content. For this experiment, Indonesian sea cucumbers were collected. After processing of sea cucumbers, wastes are produced. From these, internal organs were collected and were introduced to high-pressure processing [3]. With the help of DPPH, ABTS assay, FRAP & TAC, it was observed that *Holothuriatubulosa,* found in Tunisia's northern area is having a significant anti-oxidant capacity. Research has shown the anti-oxidant capability of methanolic extract of *Stichopus japonicas;* a cucumber found in Red Sea [2]. Study was conducted among *Holothuriascabra, Holothurialeucospilota, and Stichopuschloronotus*; sea cucumbers of three types. It was seen that *H. leucospilota*was having the maximum content of phenolic substances (TPC). Whereas low TPC was detected with *H.scabra*species. In *S. Chloronotus*; DPPH radical scavenging activity was found high compared to the rest of two and high phenolic content was seen in case of *H. scabra*. Extract with ethanol of *C. frondosa* was found to have good content of anti-oxidant rather than water, methanol and isopropanol extraction where temperature was 60 degrees centigrade. Pressure Liquid Extraction (PLE) was applied in measuring the Oxygen Radical Absorbing Capacity of *C. frondosa*. Studies revealed that TPC is high in dried *Stichopusvariegatus* and it also comes with high DPPH RSA. Husni et al. researched on dried *Apostichopusjaponicus;* found that TPC and TFC are interrelated hence proves phenolic substances has a huge impact on anti-oxidant activity [4].

***Seaweeds*:** Seaweeds are also termed as macroalgae often classified in three types based on their pigmentation; such as – Red seaweeds (rhodophyta), Green seaweeds (chlorophyta) & brown seaweeds (Phaeophyceae). Carbohydrates like carrageenan can be found in Red seaweeds, ulvan in green seaweeds and alginate, fucoidan, laminarin are found in Brown seaweeds. Apart from those; lipids, carotenoids, sterols, acylglycerols and some proteins can be found in seaweeds [5].Research was done on seaweed extracts of dulse, wakame, laminaria, hijiki and arame where arameand kombu were reported to contain maximum anti-oxidant potentiality of 2457.5 TEAC (TroloxEquivalent Anti-oxidant Capacity) and 2641.34 TEAC; dulse was having lowest i.e. 56.31 TEACand there was no anti-oxidant potentiality in laminaria [6]. From several research studies it is observed that the anti-oxidant potentiality is generally high in Brown algae; then red and green form of algae respectively come into the list. Some workers reported that *Polycladiamyrica* is a

notable reservoir of flavonoids, vitamin C, tannins, total anti-oxidant capacity and phenol content [7]. F. afrin et. al reported that highest TPC was found in *B. wrightii* (71.09mg GAE/g); methanolic extract of Porphyria species showed the highest TPC value of 75.61mg QE/g. While methanolic extract of *S. muticum*is reported to contain the highest TAA. The highest ABTS RSA and DPPH was reported to be enormous in *P. tetrastromatica and S. muticum* [8].

***Fishes*:** Fishes are another source of anti-oxidants found in marine sources. High anti-oxidant activity has been found in hydrolysates of a whole *Clupeaharengus* than its byproducts where Pac-linoleic acid model was used. In presence of six enzymes, hydrolysis of two peptides obtained from pipe fish has been done. The enzymes are neutrase, pepsin, alcalase, papain, trypsin and pronase E. These two peptides are found to have greater inhibition on hydroxyl radicals than the other radicals thereby having ability to reduce ROS production [9]. Swim bladder is a good source of collagen which shows anti-oxidant activities. Pepsin solubilized collagen of fish swim bladder scavenges DPPH in a better way than ABTS radicals [10]. Peptides obtained from mucus of *S.lalandi and S.violacea*were reported to have anti-oxidant activity. GAWA and LH, these two peptides obtained from Spanish *Sardinellaaurita, show* a good amount of anti-oxidant activity. Another peptide TCSP obtained from *Gadusmacrocehalus* (pacific cod) was reported to eradicate ROS and shows protecting activity on DNA regarding oxidative damage. *Decapterusmaruadsi*, a fishcontaining two peptides shows a strong anti-oxidant activity [11].

***Invertebrates*:** Sponges, crabs, soft corals, crustaceans etc. come under marine invertebrates. Marine sponges are immobile, colonial species. For food they randomly strain water throughout its system. It hosts a large variety of microorganisms. 35% of a sponge's body is made of it. Those microorganisms also help to produce some secondary metabolites which protects the sponge from infectious agents and any exploiter. Dichloromethane, hexane and ethyl extracts of *Dactylospongiaelegans, haliclona species, Aaptossuberitoides, Stylissamassa* were found to have anti-oxidant activity. A collagen extracted from *Chondrillacaribensis*showed anti-oxidant activity[12]. *Dysedia* speciespredominantly found in Australia, Philippines, coastal region of Red sea and Yap state have reported to show anti-oxidant activity[13]. Notable metal chelating and radical scavenging activity and ferric reducing anti-oxidant power were seen in ethyl acetate extracts of *Neopetrosiaexigua*obtained from waters of Mauritius [14]. *Portunustrituberculatus* is a swimming crab abundantly found in Indian ocean to South east Asia [15]. Protein hydrolysate obtained from *Portunussanguinolentus* (marine crab) shows anti-oxidant activities in combination with trypsin and pepsin. Haemolymph obtained from the legs of a hard shell and soft-shell crab named *Charybdis lucifera* shows FRAP, DPPH -RSA, Deoxyribose radical scavenging activity and H_2O_2-RSA. Hydrolysate obtained from crab shells of *Portunustrituberculatus* shows total anti-oxidant activities, RPA and DPPH-RSA OH-RSA in presence of flavourzyme and protamex modified with fructose. With alkalase, Hydrolysates of *Chionoecetes japonicas* are produced which shows ABTS-RSA, RPA, and SODA [16]. Carotenoids obtained from a bacterial symbiont of a soft coral Sinularia species (obtained from Panjang island of North Java Sea) shows significant amounts of DPPH RSA [17]. Nepthea species are one type of marine soft corals prevalently found in Southeast Sulawesi. Ethyl extracts of Nepthea species and its subfractions are a good source of anti-oxidants [18]. I Sahidin et. alhave researched on a soft coral Lobophytum species. Extraction was done using Ethyl acetate. In fractionation, total 7 fractions were prepared and were named as

A-G where Fraction F was found to have ABTS and DPPH value of 79.30 ± 1.74 and 99.13 ± 2.14 respectively [19].

***Microorganisms*:** Research has shown that a variety of microorganisms, such as microalgae,, fungi, actinomycetes, bacteria (both Gram-positive and Gram-negative), protozoa, archea and yeast, produce natural anti-oxidants. New development in science have found previously unknown bacteria that produce strange secondary metabolites. Upon examining marine microbial resources from Bay of Bengal, for the production of anti-oxidant molecules, researchers found that Kocuria marina CDMP 10 extract effectively lowers DPPH free radicals [20].

Fungi: Fungi are seeing growing utilization as prebiotics. A significant number of divergent and vital natural substances are obtained from fungi. Polysaccharides isolated from different fungi have drawn attention in the recent past. Three secondary metabolites ex-LMSIII, ex-LMSII and ex-LMSI obtained from Cerrena unicolor show anti-oxidant activities. Research shows that *Emericellopsismaritima* and *Trichodermaharzianum* have a significant amount of DPPH RSA [21]. Penicilliumchrysogenum MZ945518 a halotolerant fungus reportedly shows DPPH RSA and it also has an IC50 value of 542.5 µg/ml [21].
A group of researchers demonstrated the protective effect of the extract of the marine fungus Aspergillus puulaauensis TM124-S4 on H2O2-stressed primary human fibroblasts *in vitro* [22]. The extract demonstrates skin protective capabilities through the modulation of cell proliferation, anti-oxidant response, skin hydration, and DNA repair, as revealed by changes in gene transcripts. Additionally, the extracts alter the expression of genes linked to aging and skin pigmentation [22]. Some workers shown that less well-studied sponge-associated endophytic fungus are excellent sources of bioactive chemicals [23]. Nineteen endophytic fungi were discovered to have anti-oxidant, anticancer, and/or anti-inflammatory effects based on related bioassays. Isolation of these fungi was done from three marine sponge species—*Tedaniaanhelans, Myxillaarenaria*, and *Callyspongiafibrosa*—collected from the west and east coasts of the country India using Sabouraud Dextrose Agar Medium. Potential medical benefit is suggested for a number of marine fungus that have been isolated from red, blue, and black sponges, soft corals, and brown, red, and green algae. It has been demonstrated that fungal metabolites, which are produced from *Penicilliumflavigenum* and isolated in hypersaline water, possess anti-oxidant and anti-proliferative properties [24]. Four novel anti-oxidant and anti-inflammatory components were obtained from the deep-sea-derived fungus *Myrothecium* sp. [25].
Microalgae: Microalgae are varied organisms that have the ability to accumulate bioactive metabolites. This property makes them ideal feedstocks for a range of uses, including bio-fertilization and functional meals. Such metabolites frequently combine two or more biological activities, for example, antibacterial, anticancer, and skin-regenerative properties. Some scientists explained the synthesis of microalgal anti-oxidants and their capacity to scavenge free radicals, emphasizing the sterols, vitamins, and phenolic compounds that microalgae produce [26]. Studies also found that for methanol extracts from microalgae, *Dunaliellasalina*, *Tetraselmischuii*, and Isochrysisgalbana, the anti-oxidant potential depended on the total phenol content—assessed by DPPH assay [27]. The well-expressed in vitro anti-oxidant, antibacterial, and anticancer activity of microalgae Nannochloropsisoculata extracts was also reported by some workers [28].

A number of microalgae, including *Schizochytriumlimacinum*, *Ettliacarotinosa*, *Galdieriasulphuraria*, *Neochloristexensis*, *Chlorella minutissima*, *Stichococcusbacillaris*, *Crypthecodiniumcohnii*, and *Chlorella vulgaris*, demonstrated significant cytotoxic potentiality and high free radical scrounging in crude extracts. These findings suggest that the microalgae may find application in new treatment strategies [29].

Marine bacteria: A vast array of physiologically active substances can be found in secondary bacterial metabolites. Researchers reported the screening procedures for the discovery of anti-oxidant metabolites, the detection of bioactive marine microorganisms, and their medicinal uses [30]. Promising antibacterial and anti-oxidant properties of a novel strain of marine Actinobacteria that was isolated from the uncharted sea sediment of Alang, Gulf of Khambhat, Gujarat [31]. In some studies, it was discovered that a Streptomyces species of bacterium exhibited significant anti-oxidant activity when isolated from extracts obtained from marine sediments [32]. Two anti-oxidant chemicals, gramicidin A and gramicidin B, were identified using fractionation and spectroscopic data analysis guided by bioassay. These compounds are known to suppress spore germination and the protein glycine cleavage system (GCS), which is associated to the polyketide type III pathway.

Marine cyanobacteria of the genus *Chroococcidiopsis*sp. were evaluated by Asunción et al. [33] as a valuable supply for the producers of antioxidants; it has now been shown that these bacteria are traveling to Mars. After sulfuric acid was used as the first treatment for Chroococcidiopsis sp. LEGE 06174, DMSO 20%, DMSO 100%, NaOH, PBSO and acetone, combined safe solvent extracts were made. The PBS pre-treatment proved to be the most efficacious in terms of pigment extraction and disintegration of the mucilaginous sheath under investigation. Because PBS pre-treated *Chroococcidiopsis*sp. has a high concentration of scytonemin, phycobiliproteins polysaccharides, and phenolic compounds, the extracts showed a strong potential for anti-oxidants. The highest total carotenoid content was found in methanol–PBS extracts, while the highest concentration of phenolic compounds was found in ethanol–PBS extracts [33]. Catalase, superoxide dismutase, and other useful anti-oxidant chemicals with various biological functions are known to be produced in large quantities by bacteria and microalgae. These substances also include bioactive peptides, carotenoids, and exopolysaccharides.

Anti-oxidant Compounds Derived from Marine Species

Marine organisms are the reservoir of two primary classes of naturally occurring anti-oxidant substances: enzymatic (such as glutathione reductase (GR), glutathione peroxidase (GP), catalase (CAT), superoxide dismutase (SOD), and non-enzymatic (such as various organic chemical and mineral classes). Since anti-oxidant enzymes are the strongest anti-oxidants in cells, they are thought to be the first line of defense against ROS and SOD. Consuming them on a daily basis strengthens the immune system and delays the aging process. The pharmaceutical, food and cosmetics industries are only a few of the businesses that currently use anti-oxidant peptides, polyphenols, amino acids and therpenoids, among others. Taxol, Pneumocandins, astaxanthin, hispinin, and its derivatives were the anti-oxidant compounds formed by marine microorganisms that were the subject of little research [34]. Meanwhile, in some studies it is found that polyphenols, sulfated polysaccharides, carotenoids, and sterols are anti-oxidant substances produced from brown seaweeds [35]. Anti-oxidant peptides [36] and amino-acids [37],

polysachharides [38], terpenes [39], polyphenolic compounds [40], and enzymatic anti-oxidants [41], are present in several marine sources.

Conclusion

Marine-derived anti-oxidants are essential nutrients, providing numerous health benefits. These anti-oxidants exhibit high bioavailability. The health benefits of marine-derived anti-oxidants are significant. These anti-oxidants play a vital role in the prevention of chronic diseases like cancer and cardiovascular ailments. In pharmaceuticals, marine-derived anti-oxidants are being developed into anti-oxidant-rich drugs, offering new treatments for several diseases. They are also widely used in dietary supplements, supporting overall health and wellness. Ongoing cutting-edge research continues to explore the potential of marine-derived anti-oxidants, uncovering new applications and benefits. Forthcoming prospects are quite promising, with enough potential for groundbreaking discoveries in health and environmental sciences.

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Marine Biotoxin Detection in Seafoods: A Review on Bioreceptor Based Biosensors

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Abstract

The ocean is the ultimate crucible of evolution that maintains an intricate equilibrium between the ecological variables of existence and speciation on the planet. It harbours a myriad of life forms ranging from the microscopic planktons to the colossal whales, some of which hold perpetual cultural significance and a rich array of culinary flavours. However, beneath its undulating waves lies a complex marine ecosystem rife with deadly biotoxins originating from oceanic microalgae that frequently contaminate fish or shellfish while they filter-feed on them. The subsequent consumption of such toxin-contaminated seafood can lead to drastic health consequences capable of turning into public health hazards. According, to CDC Yellow Book 2024 there are around 50,000 annual cases of ciguatera fish poisoning globally because the intoxication is underrecognized and underreported. Seafood intoxications are classified as shellfish poisonings and fish poisonings based on the type, nature, pharmacokinetics, clinical symptoms, and source of the toxin. Traditional biochemical methods for marine toxin detection such as ELISA, HPLC, mouse bioassays require extensive laboratory layouts, expensive equipment, and long testing times. Conversely, biosensing technology allows rapid real-time and often on-site biotoxin detection with easier automation, integration, and lower operational costs. Biosensors represent advanced analytical sensors that integrate a biological recognition element with a physicochemical transducer to detect and quantify specific substances (toxins, proteins, etc.) typically at low concentrations. These devices are commonly categorized by their biorecognition components, which can include enzyme-based, nucleic acid-based, or antibody-based sensors. Biosensors are instrumental in advancing food safety, reducing public health risks, and fostering sustainability within the seafood industry.

Keywords: biosensing technology, marine biotoxins, seafood poisonings, bioreceptor-based biosensors

Introduction

The ocean, a vast expanse of sapphire depths, holds an exceptional abundance of seafood, brimming with a diverse array of life forms that captivate the senses and sustain the body. For centuries, seafood has been revered globally as a culinary treasure, offering a delectable variety of tastes and textures that have delighted palates across cultures. Amidst the serene blue waters and the vibrant coral reefs, lies an intricate marine ecosystem swarming with deadly biotoxins produced by marine microalgae. Among the benthic and planktonic species, dinoflagellates a vast and varied group of eukaryotic algae within the marine environment constitute the primary producers of biotoxins that affect aquatic and human health [1].

It is well-established that marine biotoxins can bioaccumulate in the tissues of certain marine organisms, particularly filter-feeding bivalves. These toxins are synthesized de novo by specific photo- or mixotrophic microalgae, rather than by the shellfish themselves, and are subsequently transferred to mollusks through filtering. Mussels, for instance, filter about 20 liters of water per hour, and during harmful algal blooms (HABs), waters may contain millions of algal cells per liter. Human intoxication outbreaks due to marine biotoxins result from the consumption of contaminated shellfish, and the symptoms vary depending on the specific toxic compound involved (Fig. 1) [2].

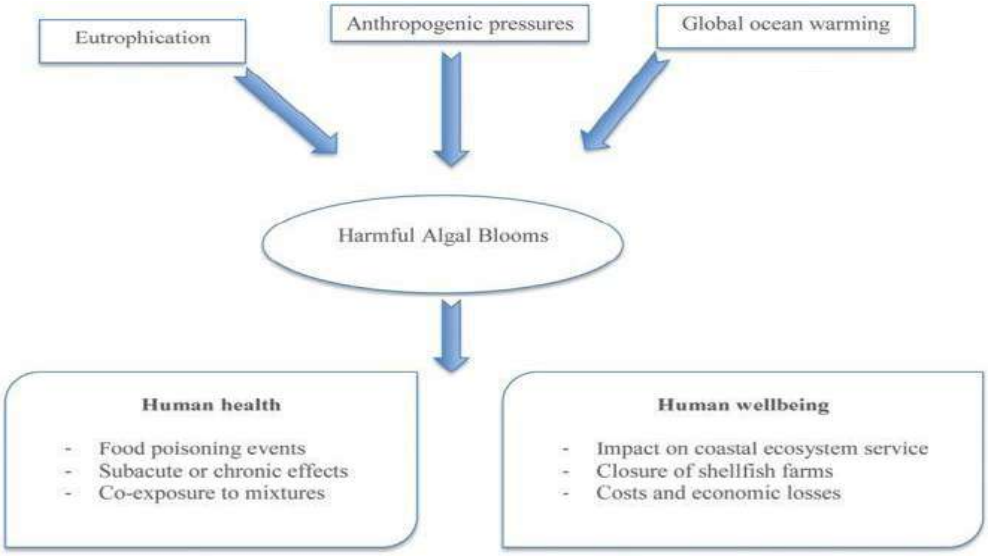

Fig 1: Factors contributing to the emergence of HAB and their effects on human kind.

Marine Biotoxins: Occurrence, Toxicity, Regulatory Limits and Reference Methods - PMC (nih.gov)

Seafood Poisonings

Several seafood poisonings have been linked to toxins produced by HABs including paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP), and ciguatera fish poisoning (CFP) [1]. Certain seafood poisonings are also attributed to the production of toxins by specific fish species such as Puffer Fish Poisoning (PFP)and scombroid fish poisoning that arises from histamine intoxication.

Outbreaks of DSP have been dated back to 1978 in Spain, followed by 5000 cases reported in the year 1981. According to a surveillance data collected between 1973-1992 by the Alaska Division of Public Health, 150 and 1500/1,00,000 persons per year in Kodiak and Old Harbour, respectively, reported cases of Paralytic Shellfish Poisoning. In 1992-1993, the largest documented outbreak of Neurotoxic Shellfish Poisoning occurred in New Zealand with over 186 cases of poisoning spanning over several weeks. Around 39,677 cases of CFP have been reported from 17

PICTs (Pacific Island Countries and Territories) with a mean annual incidence of 194 cases per 1,00,000 people between 1998-2008[3].

Paralytic shellfish poisoning is associated primarily with the proliferation of only a few planktonic species, namely *Alexandrium catenella* (formerly *Gonyaulax catenella*) or *A. tamarense-excavatum* which produce a potent neurotoxin called saxitoxin. This toxin has an intraperitoneal LD_{50} of 8 µg/kg b.w.in mice and exhibits toxicity by binding to voltage-gated sodium channel 1 present in the membranes of neurons and muscle cells, thereby impeding synaptic transmission and causing symptoms of paresthesia, paralysis, and muscular incoordination. Neurotoxic shellfish poisoning is another form of poisoning resulting from the consumption of shellfish (bivalve mollusks) contaminated with toxic phytoplankton species like *Ptychodiscus brevis* or *Karenia brevis* which produce complex polycyclic compounds called brevetoxins, the acute toxicity of which was found to be comparable to that of ciguatoxin, with an intravenous LD_{50} of 0.5 µg/kg in mice. (Fig. 2)

Fig 2: *Karenia brevis*- light micrograph

(Source: Karenia brevis (light micrograph) | Karenia brevis is a sing… | Flickr)

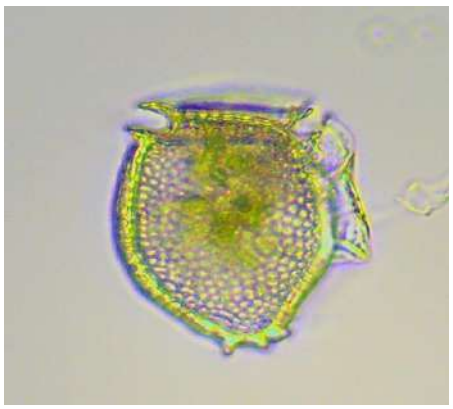

Fig 3: *Dinophysis norvegica*

(Gulf of Maine)

(Source: Diarrhetic Shellfish Poisoning – Harmful Algal Blooms (whoi.edu)

The symptoms of NSP include numbness, tingling in the mouth, extremities, and gastrointestinal discomfort, along with potential reversal of temperature sensitivity. Amnesic shellfish poisoning is a neurotoxic condition triggered by ingesting shellfishes (cockles, mussels, razor clams, scallops, crabs, and mackerel) that have accumulated a heat stable toxin, domoic acid (DA) which is produced by diatomic species of *Pseudo-nitzschia australis, P. multiseries, P. pungens*. DA demonstrates an intravenous LD_{50} of 3.6 mg/kg in mice and causes neuronal depolarization whichmanifests as symptoms of dizziness, headache, disorientation, and short-term memory loss [4]. Diarrhetic shellfish poisoning is associated with ingestion of shellfishes (blue mussels,

scallops, oysters) contaminated with marine dinoflagellates *Prorocentrum* and *Dinophysis* (Fig. 3) which produce lipophilic toxins such as okadaic acid (OA), and dinophysistoxins. OA possesses carcinogenic, genetically toxic, and cytotoxic properties producing gastrointestinal symptoms of nausea, vomiting, fatal diarrhoea, and abdominal pain[5].Ciguatera fish poisoning occurs upon consumption of fishes such as groupers, amberjack, red snappers, sea bass, barracuda, which can accumulate high levels of toxic dinoflagellate species *Gambierdiscus toxicus* that are producers of ciguatoxins. With an intraperitoneal LD_{50} of 0.25 to 4 µg/kg in mice, ciguatoxins cause gastrointestinal symptoms of nausea, vomiting, diarrhoea and neurobiological symptoms of ataxia, hallucinations, and reversal of sensations of heat and cold. Puffer fish poisoning is associated with direct human consumption of fishes like blowfish, swellfish, toadfish, balloon fish, puffers (Fig. 4) which produce non-protein, heat stable toxin called tetrodotoxin in the skin and viscera of their bodies. With a LD_{50} of 8µg/kg in mice, tetrodotoxin causes symptoms of chest muscle paralysis, malaise, hypotension, and gastrointestinal discomfort. Scombroid fish poisoning is a type of ichthyotoxicosis which results from consumption of fish belonging to the *Scombridae* family suchas tuna, mackerel, bonito that are contaminated with dangerous levels of histamine (Fig. 5).

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<tr><td>Fig 4: Pufferfish
(Source:
Pufferfish - Australian Geographic)</td><td>Fig 5: Mackerel
(Source: https://ourmarinespecies.com/c-fishes/mackerel/)</td></tr>
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The symptoms of this poisoning resemble those of acute histamine allergy including, hives, itching, red rashes, hypotension [4]. These poisonings underscore the critical need for highly specific, sensitive, and rapid detection techniques for seafood biotoxins, such as those employed by biosensor technologies, which would contribute to improved food safety, reduced public health risks, and greater sustainability in the seafood industry.

Conventional methods for biotoxin detection (Fig. 6)

The mouse bioassay (MBA) has been the most commonly used technique for screening shellfish tissue. MBA entails intraperitoneally injecting an extract of the suspected shellfish tissue into a mouse and observing the development of symptoms until the animal succumbs. Beyond its significant ethical concerns, this method suffers from inadequate sensitivity and fails to identify the specific toxins present in the sample. While HPLC (High Performance Liquid

Chromatography) methods exhibit superior sensitivity compared to MBA at toxin concentrations that align with current regulatory thresholds, this sensitivity may require enhancement if regulatory limits are reduced. This challenge could potentially be addressed by employing LC techniques. Plate-based ELISA is well-suited for high-throughput analysis and demonstrates exceptional performance characteristics. However, it is not truly rapid, as it does not achieve a turnaround time of less than one hour. Additionally, the assay necessitates multiple reagent additions, wash steps, incubations, skilled personnel, and specialized equipment to obtain accurate results [6].

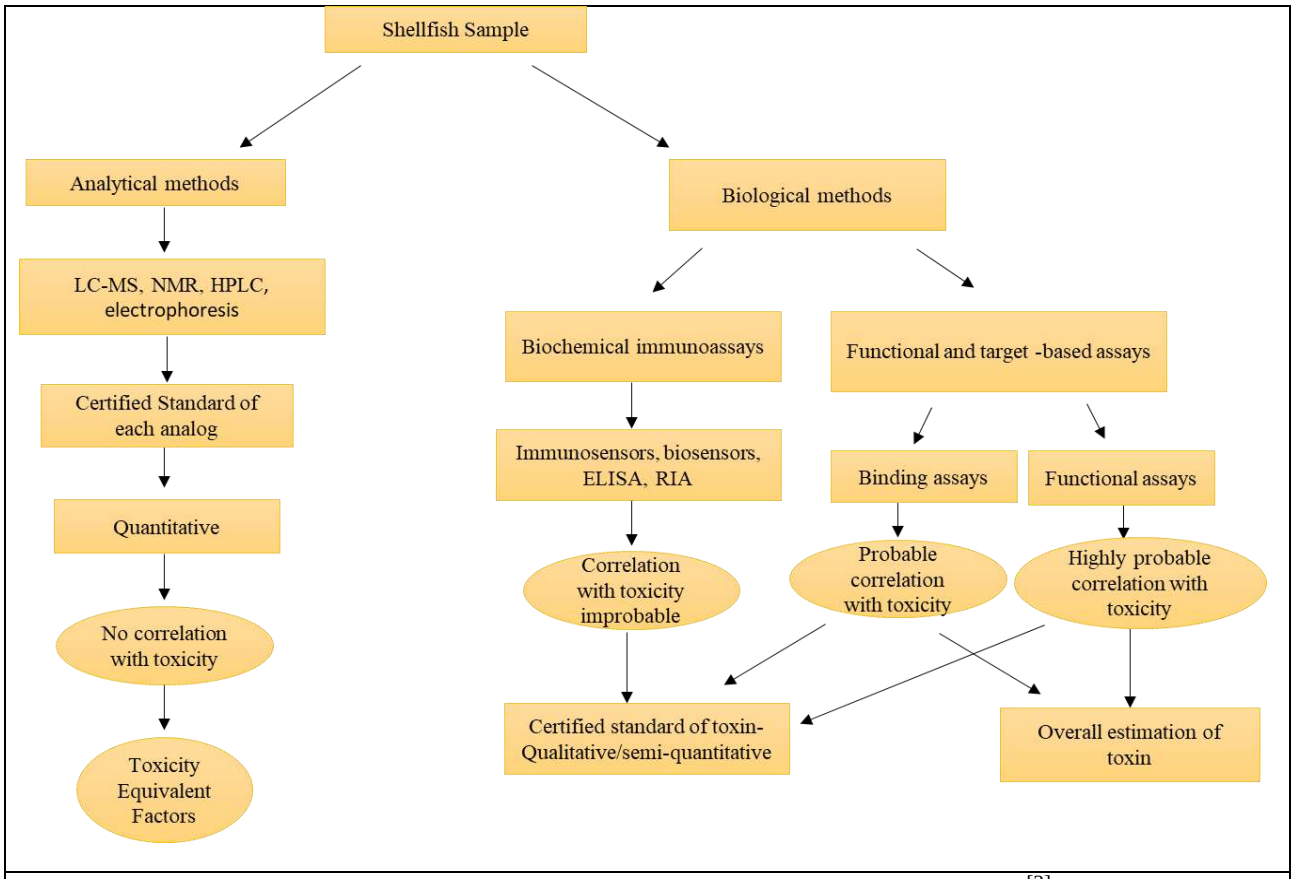

Fig 6: Flowchart presenting marine toxin detection methods. [3]

Nevertheless, the detection methods currently regulated are labor-intensive, costly, and comparatively sluggish. The emerging domain of biosensor diagnostic devices presents an intriguing prospect, capable of yielding robust, user-friendly, cost-efficient, swift, and precise detection methods for marine biotoxins and Harmful Algal Blooms (HABs). Biosensors employ biological recognition elements, such as enzymes, antibodies, nucleic acids, or whole cells, coupled with transducing elements to convert biochemical signals into measurable outputs [7]. Harnessing their inherent specificity and sensitivity shall allow the utilization of biosensors in the food industry and offer numerous advantages over traditional methods, including enhanced speed,portability, cost-effectiveness, and real-time monitoring capabilities.

Design and functionality of biosensors

In recent years, biosensors have emerged as powerful analytical tools revolutionizing the food industry's approach to quality control, safety assessment, and traceability. Moreover, biosensors facilitate real-time monitoring of critical parameters during food processing, storage, and distribution, enabling timely interventions to prevent contamination or deterioration. Coined by Cammann, "biosensor" development traces back to the late 1960s, initiated by pioneers Clarke and Lyons [8]. The operative parts of a biosensing device are given below:

Biological receptor: This component, also referred to as a sensor or detector element, plays a crucial role in sensing or detecting the presence and/or concentration of the target analyte or substance. Upon interaction between the biological receptor and the target analyte, it generates a signal in various forms such as light, heat, pH, charge, or mass change. These components can be classified into two categories: catalytic and are utilized in devices designed for the continuous monitoring of substances at certain concentrations and include enzymes, tissues, and microorganisms, and non-catalytic receptors in contrast, are predominantly employed in biosensor devices measuring analytes like steroids, drugs, and toxins, which typically occur at deficient concentrations in the micro to picomolar range. Receptors in this category include antibodies, antigens, nucleic acids, and others (Fig. 7) [8,9].

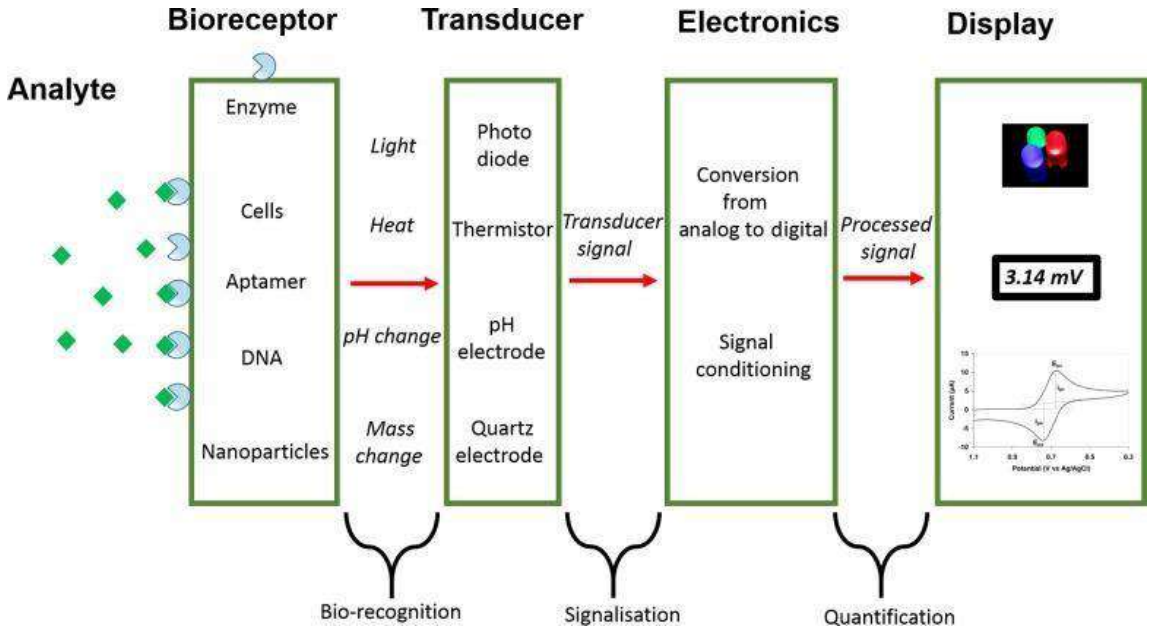

Fig 7: Schematic diagram of a biosensor.[8]

Transducer: The interaction of the bioreceptor with the target analyte potentially alters the physicochemical status of the transducer which plays the role of converting the biochemical signal obtained from the biological receptor, resulting from the interaction between the target analyte and the biological receptor, into a signal that is measurable and quantifiable. This signal can manifest as piezoelectric, optical, or electrochemical output [8].

Signal processing unit: Consequently, the signal processing system boosts the electrical signal and transfers it to a data processor, which generates a measurable output in the form of a digital display, printed output, or color alteration (Fig. 7) [8].

Biosensors are primarily utilized as per the biorecognition component and transduction component. Based on the transduction component, biosensors are broadly categorized as optical, electrochemical, and mass-based biosensors. Based on bioreceptor component, biosensors are classified as enzymatic, antibody-based, and nucleic acid-based biosensors within the context of marine biotoxins serving as the target analytes [9].

Enzymatic Biosensors

Enzyme-based biosensors offer greater specificity compared to cell-based biosensors. These sensors operate on the principle of enzyme-substrate or enzyme-inhibitor interactions. Typically, enzymes are immobilized near the transducing component, through various immobilization techniques which imprison the enzymes on a support matrix or often the transduction surface. Biosensors are designed for high enzyme loading, ensuring that sufficient amount of biocatalyst attaches to the surface, providing the necessary environment to sustain their enzymatic activities. Without adherence to this model, the biosensor would not be viable. Immobilized enzymes can be used repeatedly while retaining their catalytic activity. The primary methods for immobilization include adsorption, covalent bonding, crosslinking, encapsulation, and entrapment [10].

An enzyme-based amperometric biosensor was developed in the year 2019 as a screening tool to detect histamine and histamine-producing bacteria (HPB) in tuna. Samples were collected from eight batches of fresh yellowfin tuna (*Thunnus albacares*) filets purchased from two supermarkets. This biosensor was created by immobilizing histidine decarboxylase (HDC) and horseradish peroxidase on the surface of screen-printed electrodes, using a suitable oxidized mediator like ferrocenium ion. The immobilization was achieved through a cross-linking procedure with glutaraldehyde and bovine serum albumin. The bi-enzyme biosensor system consists of Diamine Oxidase (DAO) from porcine kidney and Peroxidase Type II from horseradish (HRP). HDC immobilized on the bi-enzyme bio-component of the biosensor was then subjected to PCR for amplification of gene encoding for HDC. After statistical evaluation of the linearity of detection of histamine by this biosensor, it was revealed that all 8 lots of tuna were contaminated with HPB particularly *Morganella psychrotolerans* and *Photobacterium phosphoreum.* A histamine biosensor could also be valuable for detecting the activity of histidine decarboxylase that was formed prior to the freezing of tuna. This enzyme remains stable in frozen fish and can potentially be reactivated after thawing. The histamine bi-enzyme biosensor can be used in detecting histamine levels and HPB in histamine rich fishes in the case of scombroid fish poisonings [11].

Antibody based-biosensors

Antibodies, serving as a recognition component, have sparked interest in creating various analytical systems like biosensors for quite some time. All antibodies share a fundamental structural principle composed of paired heavy and light polypeptide chains, categorizing them within the broader chemical class of immunoglobulin. Typically, biosensors utilize immunoglobulin G (IgG), which is abundant in serum and exhibit high specificity for their target antigens, enabling precise detection and discrimination of analytes even at low concentrations. These are relatively stable under a wide range of conditions, and can be engineered and modified to enhance their binding affinity, specificity, and stability, further expanding their utility in

immunosensor development. Scientists have access to two types of antibodies for the development of immunosensors: polyclonal and monoclonal. Polyclonal antibodies consist of a diverse mixture of IgG (s) targeting various epitopes of the antigen, whereas monoclonal antibodies are comprised of a single IgG specifically targeting one epitope [12].

Synthetic antibodies are affinity reagents created entirely in the laboratory, removing the need for animals in their production. This category encompasses recombinant antibodies, nucleic acid aptamers, and non-immunoglobulin protein scaffolds. Utilizing immunosensors based on antigen-antibody reactions offers several advantages including high sensitivity, cost-effectiveness, and reliability in detecting analytes such as residues in food and veterinary products, environmental pollutants, and drugs [13].

The electrochemical immunosensor, a type of biosensor, employs antibodies as capture agents and quantitatively assesses the electrical signal generated by the attachment of antibodies to the target molecule (Fig. 8) [14].

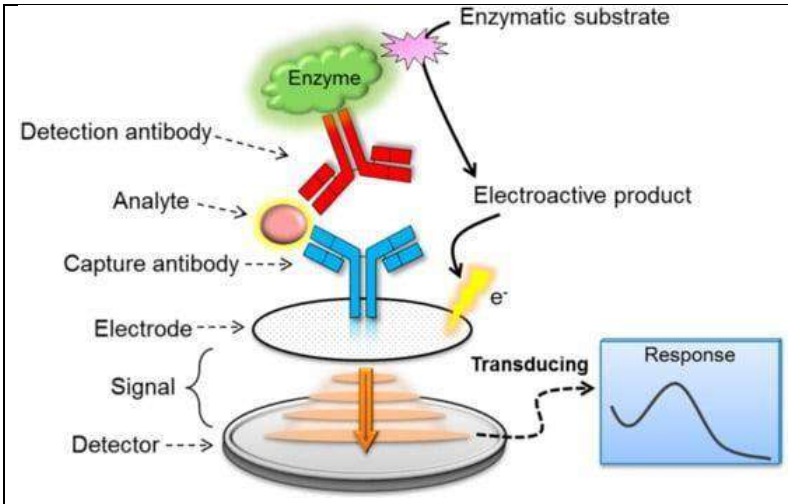

Fig 8: Analytical principle of electrochemical immunosensor.[14]

A study examined the production of domoic acid by natural *Pseudo-nitzschia pungens* populations along the Middle Tyrrhenian coast over a year. Researchers collected 105 phytoplankton samples and employed selective immunosensors as the bioreceptor component and screen-printed electrodes (SPEs) as the transducing component with differential pulse voltammetry for quantitative analysis. Disposable devices monitored domoic acid in algal extracts, with results validated by HPLC-UV method. The findings revealed domoic acid in Italian phytoplankton, particularly in coastal areas, highlighting the risk of toxin contamination in marine and land ecosystems. The polyclonal antibodies react with the epitopes on specific DA antigen molecules and generate a response with a measurable signal. Alkaline phosphatase enzyme conjugates attach to the anti-bodies. A substrate specific to the enzyme is added to the system, and the enzyme-substrate reaction generates a product which produces a signal. The electrochemical transduction system employed DPV to measure current changes in response to applied voltage. The study demonstrated the efficacy of SPE-based immunosensors for annual monitoring of domoic acid, offering a reliable early warning system for algal toxin impacts. This method shows promise for

real-time detection of domoic acid in seafood and monitoring toxin transfer through the food chain [15].

DNA-based biosensors

Due to its exceptional biocompatibility, thermal stability, and versatility in functionalization, DNA has emerged as a compelling material for biosensing applications. Its unique structure enables the detection of various targets, including nucleic acids, proteins, metal ions, and small molecules. Advancements in DNA nanotechnology have facilitated the use of dynamic DNA networks for signal amplification in biosensors. Aptamers are single-stranded oligodeoxynucleotides derived from synthetic DNA or RNA libraries, capable of binding to specific target molecules with high affinity and specificity. Systematic Evolution of Ligands by Exponential Enrichment (SELEX), also known as in vitro selection or in vitro evolution, is a molecular biology technique based on combinatorial chemistry. It is used to generate single- aptamers with high binding affinity, ease of chemical modification, and high sensitivity [16].

Cutting-edge research advancements have been made for various types of DNA-based biosensors encompassing functional DNA strand biosensors, DNA hybridization biosensors, and DNA template biosensors [17].

DNA strand biosensors: These are further divided as Aptamer based biosensors and DNAzyme biosensors-

- Aptamer based biosensors: DNA aptamers exhibit superior adaptability to extreme conditions such as high temperatures, pH extremes, and high ionic strengths. They also enable straightforward modification of functional groups without compromising their biological activity. Moreover, advancements in DNA synthesis technology have significantly lowered manufacturing costs. These advantages collectively promote the extensive use of DNA aptamers in diverse biosensor applications. Graphene oxide (GO) is an inorganic layered material that helps in enhancing the process of immobilization of aptamers on the surface of the sensor via hydrogen bonding and base stacking, without involving any chemical changes [17,18].
- DNAzyme biosensors: Certain DNA strands with efficient catalytic activities, and that are resistant to nuclease degradation are called DNAzyme molecules. Unlike enzymes, these are generally not that sensitive to environmental conditions and can withstand high temperatures. HRP-DNAzymes are frequently utilized as reporting markers in DNAzyme and DNA aptamer biosensors due to their ability to generate strong fluorescent or colorimetric signals without significantly compromising the bioactivity of these sensors [17,18].

DNA hybridization-based biosensors: These biosensors involved ssDNA oriented in certain configurations like DNA Hairpin which bind with complementary target nuclei fragment selectively. Cyclic amplification methods based on hairpins, such as HCR (Hybridization Chain Reaction) and CHA (Catalytic Hairpin Assembly), have been developed to significantly boost biosensing signals. HCR is initiated by a single-stranded nucleic acid, causing DNA hairpins with sticky ends to hybridize alternately, forming an extended double helix [17,18].

DNA Template-based biosensors: Templated DNA biosensors exist as DNA Tile Assembly and DNA Origami arrangements. Application of DNA Tile Assembly involves configuring ssDNA into 3D structures such as tetrahedrons, or biprism. Various DNA tetrahedrons sensors using

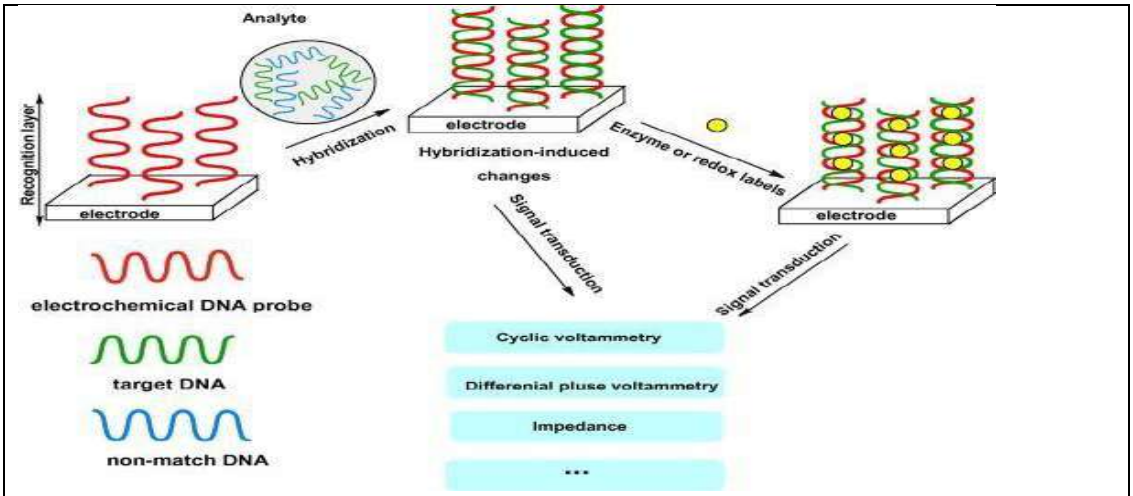

Fig 9: Working principle of an Electrochemical Aptasensor[19].

(Source:https://www.sciencedirect.com/science/article/abs/pii/S016599361200146X)

electrochemical or optical transduction component have been in industrial use. In DNA Origami, the ssDNA is folded into well-defined patterns aiding the target molecule to get anchored at specific or pre-designed sites. Thus, the DNA template proves to be beneficial in organizing materials because of its distinct spatial distributions [17,18].

In a research study conducted in 2022, scientists created an electrochemical aptasensor (Fig. 9) to detect saxitoxin (STX) by utilizing a short anti-STX aptamer integrated with methylene blue (MB) at the 3′-end. The aptamer was immobilized on a gold electrode, and the electro-catalytic properties of MB enabled highly selective and sensitive STX detection. Differential pulse voltammetry (DPV) measured the sensor's performance, showing changes in oxidation current upon STX binding, confirming the sensor's effectiveness. With a low detection limit of 1 nM and a dynamic concentration range from 1 nM to 1 μM, the aptasensor demonstrated enhanced sensitivity. Selectivity was tested against neo-STX and okadaic acid, confirming strong specificity for STX. Shellfish samples spiked with STX at 3, 10, and 30 nM were successfully analyzed using DPV, highlighting the aptasensor's practical potential for detecting saxitoxin in food safety applications [20].

Advancements in marine biotoxin detection

Presently, numerous innovative methods for extraction and purification, such as dispersive liquid–liquid microextraction (DLLME) and solid-phase microextraction (SPME), have made notable progress in the analysis of marine toxins. Similarly, sophisticated detection techniques like two-dimensional (2D) chromatography and high-resolution mass spectrometry (HRMS) have also undergone significant advancements in this field.

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Prevention and Therapeutic Management of Renal Cell Carcinoma

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Abstract

Renal cell carcinoma is prevalent in the developed nations of the world in adults aged above 40 years. Epidemiological studies reveal higher disease rates in males of the Western world although some ethnic groups exhibit a propensity to the cancer. Among the various types, clear cell renal cell carcinoma and papillary carcinoma are most commonly reported. Unmodifiable and modifiable factors predispose a person to carcinoma. Comorbidities such as hepatitis C virus infection, and extreme renal insufficiency pose an increased risk. Lifestyle diseases such as obesity, hypertension, hyperglycemia induce risk but should be appropriately controlled by the change of diet, physical exercise, and abstinence from smoking and drinking. Therapeutic modalities with VEGF inhibitors, mTOR inhibitors, and immune checkpoint inhibitors have formed the mainstay, depending on the carcinoma stage and extent of metastasis. Recent developments in cellular therapy can usher in a new era. Refractory cases may be managed by surgical interventions. The review encompasses various aspects of renal cell carcinoma with a brief overview of its epidemiology, etiology, risk factors, staging and grading, and culminates witha concise description of different preventive and therapeutic approaches in mitigation of renal cell carcinoma.

Keywords: Cellular therapies, immune checkpoint inhibitors, mTOR inhibitors, renal cell carcinoma, VEGF inhibitors

Introduction

Renal cell carcinoma (RCC), alternatively named as renal adenocarcinoma or hypernephroma is an insidious neoplasm. It is ranked as the 7th commonest form of cancer in the Western world. RCCs usually occur in the renal cortex, consisting of the glomerular apparatus, tubular apparatus consisting of renal tubules, and renal ducts for urine collection. RCC is regarded as an immunogenic tumor, as exhibits resistance to conventional chemotherapeutic agents, immunotherapy, and radiation-based approaches, and is marked with a low success rate with immunotherapies [1]. In a few cases only, the “classic triad” of indications of blood in the urine, side pain, and soft masses have been detected in patients with RCC. Early indications of RCC can be manifested as paraneoplastic syndromes (PNS) in 20% of people. Clinical features of PNS must be identified in RCC. In fact, successful management of carcinoma facilitates remission of the symptoms of the PNS [2].

Most RCC cases were detected during magnetic resonance imaging (MRI), computed tomography (CT) scan, or ultrasonography. Fever, loss in appetite, weight loss, anemia, and leucocytosis have also been reported [3]. In approximately 20% of patients, hypercalcemia, polycythemia, Cushing's syndrome, and high blood pressure due to renin hyper-secretion, have also been observed [4].

Renal cell carcinoma is a common form of kidney cancer in adults between 40-75 years, with the highest reports from developed parts of the world and Belarus ranking first. On the other hand, central African countries did not report any case of RCC. The mortality from RCC stood at nearly 1.5lakh deaths worldwide with a number of deaths more in men than in women [4,5]. Gestational RCC occurs rarely in pregnant women. Its diagnosis is challenging and usually diagnosed during the second trimester. The similarity in the type of symptoms with those of pregnancy-associated physiological alterations makes the task difficult [6]. In a study on the prevalence of RCC in young adults in the age bracket of 20-39 years in the US, it was observed that although it was rare, an increasing trend could be identified. The percentage of patients with RCC was independent of socioeconomic status. But ethnicity was found to affect the percentage which might be attributed to differences in risk factors, genetic and environmental factors [7].

Increasing incidences of RCC in young adults and adults in the developed nations of the world emphasizes the need for delving deep into the understanding of its etiology, staging and grading, identification of unmodifiable and modifiable risk factors, prevention strategies, adoption of appropriate therapeutic modalities, and surgical interventions. The present article attempts to provide a comprehensive review of the various aspects of renal cell carcinoma with more focus on prevention and therapeutic approaches for its management.

Types of RCC, Staging, and Grading of RCC

Various types of RCC are clear cell carcinoma, papillary carcinoma, oncocytic and chromophobe carcinoma, collecting duct carcinoma, molecularly defined renal tumors, etc. Among these, the commonest are clear cell and papillary carcinoma [5,8].

RCCs usually are characterized by unique morphological features, rendering easy categorization. Immunohistochemistry studies may or may not be required. However, it becomes difficult to assign a tumor subtype, in different types of renal tumors, as heterogeneous cell populations can be identified such as clear cells, oncocytic cells, or papillary features. The grading of RCC is considered as a prognostic factor. It is done based on the system introduced by Fuhrman *et al* in 1982. Microscopic morphology of a neoplasm stained with hematoxylin and eosin is studied to decide on the grade. The size and shape of the nucleus and the prominence of the nucleolus are carefully observed. But now the technique has been superseded by a validated grading system proposed by the International Society of Urologic Pathologists (ISUP) in 2012 in consultation with WHO for clear cell renal carcinoma(ccRCC) and papillary carcinoma. However, this system of grading is not applicable for chromophobe renal cell carcinoma. In this system, nucleolus and eosinophilicity form the basis of observation at X400 magnification for Grades 1,2, and 3 and at X100 magnification for Grade 4. The nucleoli are visible for Grades 2 and 3. Extreme pleomorphism is a characteristic of Grade 4 RCC. Another grading system is also available for the purpose, which is known as the Rochester Grading panel [9,10].

Pathological tumor stage (Tumor, Nodes, Metastasis: TNM) can serve as a crucial prognostic factor for RCC. The stage can be concluded from macro- and microscopic observations. Some of the features that are normally looked upon are tumor size, extent of invasion into the perinephric

fat, the renal sinus or Gerota's fascia, effect on the renal vein or vena cava, and metastasis of the adrenal gland and lymph node. In the present scenario, the 8th edition of the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) staging system is being followed. Development and advancements in the domain of molecular tests and biomarkers for immunohistochemistry studies will prove valuable in achieving consistent conclusions with respect to diagnosis, grading and staging of RCC [11].

Etiology and Risk Factors

Although the exact cause of RCC is not yet known explicitly, some underlying factors have been recognized as responsible for increasing the propensity for development of RCC. They are age, gender, and ethnicity, all of which are unmodifiable factors. More cases of RCC have been reported in males than in women which may be attributed to workplace stress, sedentary lifestyle, unhealthy dietary practices, wrong habits, and addiction in males. It is interesting to note that although chromophobe RCC and ccRCC have been diagnosed in females, papillary carcinoma is almost unheard of. Another significant unmodifiable cause for RCC is the hereditary factor and the existence of hereditary diseases. Other comorbidities that increase the risk of RCC are chronic renal damage, dialysis, polycystic kidney disease, sickle cell anemia, and stones. Modifiable factors that can be altered by a person voluntarily to avoid RCC are obesity, diabetes mellitus, hypertension, and occupational hazards related to exposure to potential carcinogens like heavy metals like cadmium, asbestos dust, pesticides and herbicides, and volatile organic compounds. Smoking and consumption of alcohol aggravate the risks further. Complications can be avoided favorably by changing sedentary lifestyle and diet [4,5,12,13].

Patients with kidney transplants are susceptible to developing RCC. RCC may develop at any stage during the process. RCC, if detected before transplant, should be immediately addressed and to restrict its recurrence, an essential observation period is recommended. Treatment of RCC in persons receiving kidney is challenging and needs critical adjustment of immunosuppressive and anti-cancer therapeutic modalities. Excision or nephrectomy can cure localized RCC [14].

Hepatitis C virus (HCV) infection, the primary factor of cirrhosis in the US population may be linked with a higher risk of RCC as evident from epidemiologic surveys. Treatment of with direct-acting antiviral agents may prevent the incidence of RCC in HCV patients in future [15].

Preventive Strategies against RCC

Vitamin and Lifestyle (VITAL) study conducted in the U.S. is a landmark study in the area of RCC and has identified the risk factors and subsequent preventive approaches and strategies to avoid the incidences of RCC. Both VITAL study and European studies concluded that life-style diseases, high BMI in women, hypertension, and hyperglycemia carry an increased risk for RCC.Obesity is highly associated with the risk of RCC for BMI $\geq$ 35 kg/m^2. However, thin men with aBMI < 21 kg/m^2 also demonstrated a considerably high risk of RCC. Hypertension enhances the probability of RCC, and its control is necessary Antihypertensive agents such as renin-angiotensinsystem (RAS) inhibitors can prevent metastasis. Although diabetes has been linked to RCC, but definite correlation is yet to be established

Therefore, from above discussion, it is clearly evident that lifestyle changes and modification of dietary habits are essential to leading a healthy life. Smoking addiction and excessive alcohol consumption increase further the risk of developing RCC. In a recent study, Japan Public Health Center-based Prospective Study (JPHC study), it was concluded that heavy smokers (≥40 pack-years) demonstrated a high risk of RCC with hazard ratio of 1.50. There is no well-documented study on the impact of smoking on RCC occurrence in Asians.

Drinking habit with a moderate level of alcohol consumption exerts protection from RCC in contrast to non-alcoholics. The information has conclusive evidence from European prospective observational study by the European Prospective Investigation into Cancer and Nutrition (EPIC).

In terms of food habits, a high intake of red meat > 60g resulted in an increased risk of RCC with a hazard ratio of >1.0 1.19 compared to an intake of approximately 10 g. Intake of fruits and cruciferous vegetables has demonstrated beneficial and preventive effects.

Cafestol palmitate and kahweol palmitate, two key ingredients of coffee have demonstrated anti-proliferative and anti-migratory activities *in vitro* in human renal cancer cells owing to their positive impact on human microvascular endothelial cells. Hence, coffee may be consumed in moderate amounts [1].

Targeted Drugs in Management of RCC

The remission rate in RCC depends on the stage in which the carcinoma is existing, whether metastasis has been initiated or not. If the carcinoma is predominantly localized with partial involvement of blood or lymphatic vessels, a complete cure is possible with high rates of disease-free survival. In the case of localized cancers, surgical removal is the most viable option. Although with RCC, regression may happen on its own, in the absence of any therapeutic intervention, prolonged survival is not guaranteed. However, if metastasis has occurred, chance of survival is meager [16].

The significance of therapeutic intervention with appropriate drugs cannot be overlooked in the management of RCC and appropriate guidelines for clinical practice have been laid down by the European Society for Medical Oncology [17].
ESMO has provided recommendations for adjuvant therapy in the management of local/locoregional ccRCC, first-line, second-line, and third-line systemic treatment of advanced/metastatic ccRCC, and advanced/metastatic papillary RCC.
Before 2004 therapy with cytokines and interferons was the only option available for patients with RCC. In 2005, Modern Age arrived with the introduction of anti-angiogenic VEGF pathway inhibitors and inhibitors of mTORC1, ushering in the era of targeted therapies for RCC. Patient response rate was higher and prolonged disease-free survival was observed with these evolved targeted therapies.

VEGF pathway can be inhibited either by blocking of VEGF receptor thereby preventing VEGF binding to endothelial cells and inhibiting VEGF signaling or by preventing activation of VEGFR by binding of MAbs to free VEGF. Bevacizumab in combination with IFN-α was the first recombinant MAb employed against VEGF. The combination significantly restricted progression and improved survival in patients with metastatic ccRCC. However, in 2022, it was

reported that bevacizumab-atezolizumab, an immune checkpoint inhibitor was inferior to sunitinib alone for tackling metastatic RCC. In ccRCC, tyrosine kinase inhibitor (TKI) mainly halts VEGF signaling by inhibition of VEGFR-2. Among the anti-angiogenic inhibitors, sorafenib was the first drug approved by FDA for clinical applications. Others that came in slowly included sunitinib, pazopanib, cabozantinib axitinib, lenvatinib, and tivozanib of which the first three can be administered orally. They have also been found to be effective against other growth factors, such as PDGFR or c-Kit, which are also upregulated in RCC. Inhibition of VEGF assists in the removal of abnormalities in tumor microvasculature, facilitates infiltration of immune cells, and generates anti-tumor response. Tumor burden can be further reduced by enhanced maturation and activation of dendritic cells [18-20].

In ccRCC, mutations occur in mTORC pathway and the pathway also is hyper-activated, preventing apoptosis, and enabling rapid cancer cell proliferation and angiogenesis. Therefore, mTORC1 inhibitors can be used to inhibit the progression of cancer. In contrast to VEGF inhibitors, mTOR inhibitors can inhibit cell proliferation more effectively than angiogenesis. This class of drugs is represented by temsirolimus and everolimus. They are rapamycin analogs, that form cross-links with FKBP-12, thereby inhibiting the production of the mTORC1 complex. This inhibits cellular proliferation and hampers VEGF signaling by inhibition of hypoxia-inducible factorα(HIFα) and VEGF expression. Therapy with mTOR inhibitors proves beneficial in drug-resistant patients or for those who have not responded successfully to VEGF inhibitors [18,19].

Success with VEGF inhibitors and mTOR inhibitors has been negatively affected by adverse effects of some molecules and also development of drug resistance, and hyposensitivity, both of which can be linked to tumor heterogeneity. Drug resistance is an issue of major concern as it develops within a year of initiation of therapy [19].

With advent of immune-oncology, the domain of immune checkpoint inhibitors(ICI) has developed tremendously. ICI therapy, an advanced form of immunotherapy aims to restore and improve the capability of the patient's immune system against tumors by attacking the immune-cell avoiding tendency of neoplasms. Immune checkpoints are "brake pads" that help in avoiding over-activation of the immune system by negative regulation of T-cell function. This group of molecules has revolutionized RCC therapy and has transformed the Modern Age to Golden Age. Programmed cell death 1 (PD-1), programmed cell death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) are the immune checkpoints that have been targeted by the scientists. Anti-CTLA-4 and anti-PD1/PD-L1 checkpoint inhibitors are the most commonly employed ICIs not only in the treatment of RCC but also in the case of melanoma and other cancers. Anti-CTLA-4 agents permit the native immune system to develop anti-tumor immunity. The success with ICI in management of metastatic RCC can be attributed to the presence of PD-L1 in more than 70% of the tumor biopsies and more than 50% of tumor–infiltrating immune cells and lymphocyte were positive for PD-1. Inhibition of PD-1 or PD-L1 promotes up-regulation of anti-tumor immunity. Nivolumab, pembrolizumab, atezolizumab, avelumab and ipilimumab are commonly employed ICIs used to treat metastatic ccRCC. Therapy with ICI usually constitutes either co-administration of two ICIs or administration of ICI with tyrosine kinase inhibitor combination strategy helps to combat drug resistance. Combination of ICI with TKI, such as axitinib plus pembrolizumab, axitinib plus avelumab has demonstrated better clinical outcomes [18,19].

Emerging Potential Therapeutic Strategies

Emerging therapeutic strategies for the treatment of RCC that have been recently approved and commercialized or are still in different phases of clinical trials are cell-based therapies, novel targeted molecules, Hyper Acute Renal (HAR) immunotherapy, and emerging drugs in combination with classical entities. This development has been necessary to overcome the challenges with treatment of primarily advanced and metastatic RCC.

Cell-based therapy is considered a "living drug" and has met success with blood cancers and satisfactory rates have prompted its exploitation in the management of solid tumors. In the case of RCC, cellular therapies involve transplantation of allogeneic hematopoietic stem cells, T cell receptor gene-modified T cells, chimeric antigen receptor (CAR) T cells with CAIX (Carbonic anhydrase isoform), CAR natural killer (NK) cells, lymphokine-activated killer (LAK) cells, γδ T cells, and dendritic cell vaccination. Apart from CAIX, oncofetal antigen 5T4 and CD70 can be explored as potential targets as they are overexpressed in more than 40% of RCC patients. Tumor cytotoxicity can be improved and the efficiency of CAR-T cells can be enhanced by using the PD-1:28 CSR (chimeric switch receptor). This strategy can be the savior in solid tumors that are not responding to even conventional immunotherapies Approaches, such as CAR macrophages, dendritic cell-cytokine-induced killer cells, and regulatory CAR-T cells are being investigated in the preclinical stages of development [21].

HIF2α inhibitors have demonstrated potential as targeted therapy recently. Inactivation of tumor suppressor of ccRCC blocks the degradation pathway of HIFα. First-generation HIF2a inhibitor MK- 3795 was well tolerated, with satisfactory remission, and restriction of progression in Phase I clinical trial in advanced ccRCC. The second-generation compound, belzutifan, was found to be superior to MK-3795 and thus gained FDA approval and the status of the breakthrough molecule. HAR immunotherapy is an advanced immunotherapy that consists of genetically modified allogeneic kidney cancer cell lines expressing alpha-1,3-galactosyltransferase. As humans are intrinsically immune to the enzyme, HAR immunotherapy offers protection against metastasis. The well-tolerated therapy can be developed as suitable therapeutic option for metastatic RCC [19].

Despite significant advances in interventional strategies for the management of various stages of RCC, active monitoring and/or cytoreductive surgery should be considered in a case-by-case approach. Special metastatic renal cell carcinoma populations with poor remission, metastasis to the brain, and end-organ dysfunction need individualized treatment strategies[20, 21].

Conclusion

It is evident that adoption of preventive measures such as alteration in lifestyle, dietary habits, smoking cessation, and low to moderate alcohol consumption have a positive impact on reducing the risk of renal cell carcinoma. Gradual transition from classical therapy to Modern Age and ultimately to Golden Age has revolutionized the renal cell carcinoma therapy. Therapeutic modalities have employed cytokines and interferons that have been substituted by anti-angiogenic molecules such as VEGF inhibitors, tyrosine kinase inhibitors, mTOR inhibitors and much improved immune checkpoint inhibitor-based combination therapy. Advent of cellular therapies and advanced targeted therapies are going to usher in a new era with unlimited possibilities.

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The Untold Tales: The Study on the Lived Experiences of Casteism

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Abstract

The present study tries to explore the "lived experience" of 3 individuals belonging from different caste systems in India. These individuals belong to the Schedule Tribes, Other Backward Class and General caste categories. In-depth interview was conducted with these participants. Interpretative Phenomenological Analysis proposed by Smith et al was used as the method of analysis and interpretation. Four superordinate themes (social suffering due to casteism , emotional suffering due to casteism, flip side of the effect of casteism , gratitude and sense of connectedness to the roots) 7 subordinate themes emerged from the analysis (systemic oppression in terms of caste, prejudice ,stereotype and discrimination faced by lower castes, identity formation vs role confusion due to caste, distress and emotional imbalance due to caste discrimination , resilience in bringing about change, systemic facilitator as mediating body and gratitude and sense of connectedness to the root).The findings have been discussed in terms of social identity , developmental trajectory and life satisfaction in terms of caste.

Keywords: Castes, Lived experience, Social Identity, Developmental Trajectory, Life Satisfaction

Introduction

India is a very diverse country which celebrates its distinctiveness and uniqueness from other countries in terms of diversity and versatility. This diversity is deeply rooted within the people of the country and it plays a crucial role in how they view themselves and their position in the society within the country. Casteism plays a huge role in Indian society. The roots of the caste system in India can be traced back to many centuries as a social and psychological construct. Caste is a hereditary classification system that assigns people to social roles, positions, power, privilege, influence, occupations, access to wealth, health, and education, among other factors. Caste is ascribed from birth and cannot be changed throughout a person's lifetime [1] People differ due to this classification in numerous aspects, including their social position, power and privileges, access to health and well-being, education, and opportunities for growth as an individual. These factors, thus impacting the overall personality of an individual have an effect on their developmental trajectory and self-concept. The essence of belonging to a particular caste enhances the sense of self-esteem, belongingness and the social status of the individual which have a greater impact on life-satisfaction depending upon the experiences faced by them.

The four fold old varna system in the Indian Caste System classified people on the basis of their occupation and wealth and power. The Savarna consisted of four main endogamous groups -

- ***the Brahmins (scholars and priests)***
- ***the Kshatriyas (warriors and kings)***

● ***the Vaishyas (merchants and traders)***

● ***the Shudras (service classes)***

The people who were not a part of these groups or a fifth group known as 'Avarnas' or the 'Atishudras' are known as Dalits in modern day [2]. The upper castes of the society possessed all the power, privileges, wealth and exerted control over the lower castes by excluding them from society, treating them unfairly, and isolating them on account of their social stigma.

One of the policies to help lower caste people elevate their situation was the introduction of reservation in all the fields of work, education and social services so that they do not fall back to their previous position and are still the same [2].

The earlier caste system was modified, reordered and renamed where the upper sections of the earlier Indian society mainly the Brahmins, Kshatriyas and Vaishyas were categorized as the General Castes. The Shudras and Dalits were categorized into the Schedule Castes, the economically and socially marginalized sections of the indigenous ethnic groups were categorized as the Schedule Tribes and other minority section of the society who were totally excluded in the previous Indian society are categorized into the OBCs - the Other Backward Castes [2]. As a result, the existing caste system is divided into ***the General Caste, Schedule Caste, Schedule Tribe, and the Other Backward Caste.*** And even though the government forbids the caste system and all other forms of discrimination, they nonetheless persist.

- **Caste as a social identity -**

Identity is the idea of "who they are" for a particular person. These ideas are often centered around social categories that people use to determine and organize information about the outside world by labeling themselves as a daughter, student, working professional, etc at the same time. The social categories have the potential to be connected to behavioral guidelines that specify how its members should behave and think. Caste as a social identity plays a crucial role in one's life where they view themselves as members of a group, affirming to the group thereby relishing their sense of belongingness and self-esteem and being a part of the group so that they do not feel left out or a segregated part of the society [3].

- **Social Stigma and Discrimination based on casteism -**

Understanding how caste as a social identity affects people's life, happiness, and satisfaction with their place in society requires an understanding of the idea of stigma. Stigma and stigmatization of the lower caste groups, including SCs, STs, and OBCs, are caused by negative social representations of the lower castes. A person is stigmatized when their social identification, or participation in a particular social category, calls into question their whole humanity or identity; they are disregarded, treated poorly, or seen as having flaws by others.[4]

Caste identification and status are relational processes that form the foundation for social categorization, social interaction, and social differentiation rather than being isolated factors that determine an individual's status in society.

Both stigma and discrimination brought on by caste identity in India leads to societal inequity. It has been observed that job applicants from lower castes do not receive as many opportunities as those from the general caste. However, some opportunities like reserved seats are there for SCs,

STs, OBCs in educational sectors in the forms of quota which again in turn lead to discrimination and may lead to differentiation among people as caste identities.

- **Developmental trajectory, Life-satisfaction and Wellbeing and casteism -**

Developmental trajectory outlines how a particular behavior changes with age. A person's caste identity, a significant component of their identity, affects how they are regarded in society, the experiences they have as they grow up, and the advantages, opportunities, and negative interactions they have with members of the outgroup. Every person, regardless of caste, has diverse experiences that contribute to their varied levels of life satisfaction and well-being and plays a significant role in the mental health of the individual. Mental health is a "state of well-being in which every individual realizes one's potential, can cope with the stressors of life, can work productively as well as can contribute to one's community."(WHO, 2005). Stigma and discrimination against a person because of his or her social identity have an impact on their experiences in life, their emotional health, and their level of life happiness. A person's self-esteem may be challenged if they feel that their in-group is being stigmatized because this will not help them form or maintain a good self-concept based on their social group membership. [4].

Method

Aim of the Study

To explore the subjective experience of the individual belonging to different castes.

Research Design

This study utilized a qualitative research design to analyze the data. Interpretative Phenomenological Analysis (I.P.A.) proposed by Smith (2009) was used as the method of analysis and interpretation. I.P.A. is a qualitative research design which focuses on the lived experiences of the participants and the meaning they attach to them; in this particular research thefour domains analyzed with the help of this design are social suffering due to casteism, emotionalsuffering due to casteism, flip side of the effect of casteism and gratitude and sense of connectedness to the roots.

Materials & Study Design

Study Design

Qualitative exploratory research.

Sampling Technique

Sample was chosen using the purposive sampling technique.

Sample

The Sample was composed of 3 individuals, each of them belonging to different castes (SC , ST , OBC)

Inclusion Criteria

- Participants belonging to specific caste (General, SC, ST, OBC)
- Participants can be of either sex

- Age range: 18-50 Years
- Participants must have completed 8th grade and above.

Exclusion Criteria

- Presence of significant psychiatric illness.
- Presence of significant medical condition.

Tools Used

- ***Information Schedule***

A semi structured interview designed for this study to elicit information pertaining to everyone's Name, and caste and their subjective experiences in their particular caste.

- ***In depth Interview***

It was conducted to enable the participants to tell their daily life stories and to explore their experiences of casteism. The interview lasted about 30-35 minutes with the aim of striving for saturation.

Procedure

The interpretative phenomenological approach was used where the researcher's main goal was to assess participant's perspectives of their experience and interpret their views. Participants were selected through 'purposive sampling' (more specifically homogenous sampling technique') considering the inclusion and exclusion criteria. Data was collected through a semi structured interview of each participant after building a proper rapport. After that those data were transcribed and coded (open code, focus code and axial code) in a proper way. Then the coded data were analyzed and interpreted following triangulation and validation as well as keeping ethical issues in mind.

Results and Analysis

The section summarizes data that emerged from three in-depth individual interviews. The discussion was interpreted using Interpretative Phenomenological Analysis. Data transcript was coded at all levels of analysis and categorized into exploring themes, emerging themes and superordinate themes by four authors of the present study. The Superordinate themes and emerging themes obtained from the Interpretative Phenomenological analysis are described as following:

Emotional suffering due to casteism

- **<u>Identity Formation vs Role Confusion Due To Casteism</u>**

In spite of different kinds of impediments, narratives highlighted their strong desire to create his / her own identity. Two of them are pushing themselves and motivating others also to apply their full potential to be identified as a distinct and positive identity separate from caste stereotypes. Individuals strive to break free from imposed roles, seeking recognition based on their unique qualities rather than conforming to caste-defined expectations. One of them thinks that he is here to chase his dream, to make himself independent and established. The other one participant wants to be self-independent so that she does not have to suffer the same situation like her parents.

"I just knew that I would be not be similar, I would change I would not settle for anything less good than what I deserve that came to me,People pay less attention to lepchas so I want to change that I would love to help to change."

- **Distress and Emotional Dis-balance Due To Casteism**

From the narratives it is observed that more or less every participant has addressed the emotional impact of caste discrimination with respect to society, education, legal reforms which affected them. The participants have reported perpetuating a cycle of poverty and limited social mobility, intensifying feelings of frustration and hopelessness. The social and economic disparities, coupled with discriminatory practices, contribute to a heightened sense of exclusion and psychological distress among individuals.

"baba bike niye dental practice jachhe pash theke uro khobor pash theke keu ekta tont ketedilo, je tui toh okhane jash tui r ki korish, tui loker taaka mere khash. Ei byapar gulo puropuri casteism thekei asto ,"

Social Suffering Due To Casteism

- **Systemic oppression in terms of caste**

From the narrative it could be observed that they felt many kinds of oppression and exploitation by the society and making them feel isolated and even in this progressive society where everyone is developing towards a more uniform society these people were degraded by the society as a whole.The society impacted negatively towards their growth and even when the participants were trying to come out of this oppression, there were many repelling forces against them like one of them experienced that they were never given any kind of priority in the society always felt oppressed by the society due to caste and have always been excluded by the upper caste society. On the other hand, the other individual felt that the other castes have always been promoted and highlighted in all the socio occupational spheres where she feels neglected and not favored by the society and somewhere getting lost in the midst of other castes.

" Amader gram e jekhono siddhantor khtre konodin amader ke priority deowa hotona , jara brahmon ba jara pramanik tara ja bolto tai hobe"

- **Prejudice, stereotype and discrimination faced by lower caste**

From the narratives it is observed that every participant has faced a certain amount of discrimination whether it be social economic status or facial characteristics. Rejected and misjudged by others and find it difficult to share. Their true feelings to others, thinking that achievements and expression will lead to some consequences and judgment.

"so its like they look at me as if I am some kind a spectator, I don't know like whether it my chinki eyes or whether my fair face , I don't know yeah they do stare at me and yeah that staring is like okay , but ching chong chung is not okay,"

Flip Side Of The Effect Of Casteism

- **Resilience to bring about a change**

Narratives contend that despite the bad impacts of casteism, resistance in the face of discrimination may inspire a resolute endeavor to promote social equality and constructive transformation.According to one participant, his parents will stop at nothing to safeguard their offspring and effect change, believing that the only way they could give them a better life was if they broke free from the caste system they had grown up in.One participant has also showcased anger in a way that said, they are fighting for their own thing and make sure that others could understand that they are not solely weak and could bring the change to society. Participants facing adversity may develop a strong desire to challenge and overcome caste based prejudices, fostering a more inclusive society.

Participant 1 -

"baba ma bolechhilo bolei je toderke ekhan theke berote hobe tora baba porashona kore briye jaa. Eta baba maaer e shob theke boro factor r kono factor chhilona sherokom Amar baba ma er khub high ambitions chhilo,"

Participant 2 -

"We have to fight a lot for that what is mine is mine so you know like we came till here making noise. So its like that"

- ***Systemic facilitator as mediating body***

From the narrative it could be observed that the systemic facilitator acting as a mediating body can contribute positively to addressing casteism by implementing structured processes for dialogue education and conflict resolution. One of the participants states that people coming from small villages and minority backgrounds are provided comfort and safety from mediating bodies in order to feel secure and supported in this vicious cycle of casteism.

Participant 1-

"when it comes to the ST from my state we have a lot of advantages because yeah we are you know like small in number minorities so we do have like a lot of advantages compared to the general or OBCs. We have like lot of advantages like scholarships hote hai and we have more preference over like medicals seats like that so that way the government is trying their best to you know like keep us their to in increase are population"

Participant 2 -

"Govt recognise korechhilo bolei amra ato durr aste mane kichhuta holeo parchhi . amar baba ma er khub high ambitions chhilo, ami je OBC r theke je oBC howar jonno je shujog shubidhe gulopeyechhi Govt er theke"

Gratitude and a Sense of Connectedness to the Roots

In some cases, persons who encounter casteism may respond positively by becoming more appreciative of and connected to their cultural roots. One story, out of several, said that the inhabitants in the town have a primitive way of life, are tied to their roots, live in a shared, clustered family structure, and prefer to live their lives in the same way rather than trying new and fancy things.

"we follow very simple lifestyle so you can say that there is not much materialistic you know approach in our community" "people from my community they are more simple you know more rural lifestyle grown people you will find rare like very less people coming out and actually trying out things"

Discussion

This qualitative research is unique in acknowledging the subjective experience and influence of individuals belonging to particular social strata and how this impacted their life and their notion of living with those experiences. And it was found that the participants had many homogeneous experiences in the manner their particular caste impacted them, their identity and their well-being and the various opposing forces of the society that acted against them and became a hindrance in their social growth. From the transcript mainly 4 Superordinate themes and 7 Subordinate themes could be identified.

- ***Social suffering due to casteism***

From the transcript, it is clear that the participants experienced various forms of exploitation and oppression from society, which contributed to their sense of isolation even in a progressive setting where everyone is moving towards uniformity in society. These individuals were treated unfairly as a group simply for being members of a lower caste. This affected not just their development but also their general well-being and sense of fulfillment in life, which contributed to their emotional anguish. Prejudice and discrimination based solely on stereotypes are nothing new in our society or nation, and in this case, the participants had to cope with it with respect to caste. The caste identity has played a major role in debates around social exclusion in India. This is mainly because caste-based violence and prejudice are so widespread.

Studies also indicate that violence and discrimination based on caste cause social exclusion and have an adverse effect on the mental well-being of lower caste members. The research findings indicate that these social behaviors are not merely extreme acts, but rather a pattern of separating lower caste groups from relationships with others in order to generate hazardous societal conditions. These worsen their mental health and highlight social marginalization. [5]

Studies also indicate that women from lower castes, particularly those who identify as SC or ST, encounter additional difficulties in pursuing their jobs and experience heightened social exclusion as a result of the pervasive patriarchal system in our country.

- ***Emotional Suffering due to casteism***

Emotional and social suffering are interconnected. It goes without saying that persistent bias and discrimination will have an effect on a person's mental health as well as their socioeconomic and professional lives. When a person experiences prejudice and discrimination from society during their adolescence, it can lead to anxiety and emotional imbalance in their lives as well as identity formation and role uncertainty.

One of the most important problems in colonial and post-colonial India was a crisis of identity. All ethnic groups aim to claim their identity based on their ethnicity. The Namasudra communities in North Bengal experienced identity issues for a considerable amount of time, just as other communities.[6] Notwithstanding several obstacles, participants in our study expressed a great desire to forge their own identities. Two of them were challenging themselves and inspiring others

to realize all of their potential in order to stand out from caste preconceptions and be recognized as unique, positive identities.

One of them believes he is here to follow his passion and establish himself as an independent individual. In order to avoid having to go through what her parents went through, the other participant wants to be independent. The participants have a strong desire to be independent and self-sufficient.

Furthermore, as previously mentioned, it is evident from the narratives that nearly all of the participants have addressed the psychological effects of caste discrimination in relation to society, education, and legal reforms that have an impact on them. According to the participants, this perpetuates into a pattern of poverty and a lack of social mobility, making people feel more frustrated and hopeless.

- ***Flip side of the effect of casteism***

It can be seen in the study that in spite of the social evils and so much of social, emotional suffering endured by the participants from childhood and even during the tender years of adolescence they never stopped the fight of coming out from those vicious cycle of casteism and fought the social battle with such resilience, strong will-power and leading to the change they wanted to see in their life.

In spite of all the suppression faced by the individuals they claimed that sometimes the society acted as a support system and provided them with the opportunities and privileges that boosted their social, personal and Community growth. It can be said from this that when the society acts as a mediating body it ultimately leads to a more inclusive society where everyone is taken care of and provided with the necessities required by the others to be part of a more just and inclusive society.

- ***Gratitude and sense of connectedness to the roots***

One of the participant's narratives illustrated deep feelings of gratitude and connectedness towards her roots and this was a very unique experience were although the participant suffered many adverse consequences but still felt deeply connected with the roots and emphasized on the simplicity and close knit relationship they have in their community and how they prefer to live in the serene lifestyle in comparison to lavish lifestyles. There were few studies which studied the relationship between gratefulness and well-being and concluded that there is a positive correlation between gratefulness and wellbeing as these elicit some positive emotions that help in framing and building the social and cognitive resources that ultimately helps in social connectedness and presence of meaning in life that effects the well-being an individual, helping them cope with problems in life and resist the obstacles that hamper in their self-development [7].

Conclusion

The research highlights the entrenched nature of casteism, revealing its deep-rooted impact on social structures as well as the emotional and social suffering affecting the mental wellbeing and developmental trajectory of the individuals due to casteism and the prevalent prejudice, stereotype and discrimination faced by people of lower castes. While the emotional and the social suffering faced by people of the lower castes can be generalized to the population due to its presence in other previous researches as well, an interesting unique outcome of this research where participants

showed resilience and strive to come out of that situation and showed gratitude as well in this negative scenario cannot be generalized to the population due to its uniqueness in nature.

Positive findings include increased awareness, grassroots initiatives, and ongoing efforts to address caste-based discrimination, but challenges persist in achieving comprehensive societal change. Despite experiencing significant adversities, challenges and deprivation of emotional attunement, participants expressed a sense of optimism, hope and presence of significant internal and external resources to deal with situations. The particular research on casteism, while valuable for providing in-depth insights into individuals' experiences and perceptions, has its limitations. Casteism, being a complex and context dependent social phenomenon, may vary significantly across different regions and communities. Thus, though this research allows for in-depth exploration, it may limit the generalizability of findings to larger populations on some aspects. Despite these limitations, qualitative research on casteism is crucial for gaining a deep understanding of individuals' lived experiences and perceptions. To address some of these limitations, researchers may use a unique approach in the future to understand the less discovered aspects of casteism namely, gratitude for the government and growing resilience among people belonging to lower castes transforming them into a better individual in a broader population.

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APPENDIX 1

INFORMATION SCHEDULE

- NAME:
- AGE:
- GENDER/SEX:
- CASTE:

APPENDIX 2

INTERVIEW QUESTIONS

1. How can you define your identity in the social context of caste and your experience surrounding it?

2. What can be your preferred future in terms of caste?

3. Do you feel your caste has any impact on your development as a human being, like life satisfaction and wellbeing?

Assessment of Ocular Morbidity Impacting Farmers In The Terai Region Of Nepal

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Abstract

The research effort delves around demographic information and the injury modalities on individuals with farming or agriculture as their main occupation, as well as the prevalence and pattern of ocular morbidity. On account of environmental and occupational eye safety, agriculture—a crucial sector in the Terai region of Nepal causing significant socioeconomic consequences and susceptible to ocular comorbidities, including cataract, which is the leading cause of visual impairment globally, second only to glaucoma in terms of severity. A predesigned questionnaire was employed to collect data on work-related ocular history and socio-demographic characteristics. The most frequent visual morbidity among them was cataract (30%), followed by refractive error (20%). The most common cause of ocular trauma was paddy (18.84%). Most of the farmers (n = 114, 43.85%) reported never wearing PPE. Nuclear sclerosis (22.68%) was the most common type of cataract, and simple myopia (13.85%) constituted the most prevalent kind of refractive error. The ocular diseases identified among farmers were primarily linked to the hazards of the farming environment and prolonged outdoor exposure. It is essential to provide eyecare services, including the distribution of protective eyewear, as part of the general health services offered to farm workers.

Keywords: Ocular Morbidity, cataract, glaucoma, refractive error, Personal protective equipment (PPE).

Introduction

Visual impairment is a widespread issue with profound repercussions, particularly in economically disadvantaged communities across the globe. The challenges posed by impaired vision have a significant impact on societies facing economic hardship.

Due to their frequent exposure to an assortment of environmental and occupational adverse circumstances, farmers are at an elevated risk of inheriting ocular comorbidities. Given that they can be caused by physical trauma from interaction with dust or plant matter, proximity with chemicals (pesticides, fertilizers), and being exposed to ultraviolet (UV) radiation, ocular assaults are an urgent threat. The prospect of corneal abrasions, foreign body incursions, and/or infiltration,

and even more serious injuries like retinal damage or infections, increase while using sharp tools and operating machinery.

In 2020, around 1.1 billion people globally experienced vision loss. This included 43 million individuals who were blind, with a crude prevalence of 0.5%, 295 million with moderate to severe visual impairment, at a theoretical incidence of 3.7%, and 258 million people with minor vision impairment (a crude prevalence of 3.3%) [1]. Cataracts and uncorrected refractive errors are the leading causes of visual impairment worldwide [2]. On the other hand, glaucoma and cataracts account for the majority of blindness cases (45.5% each). Any disorder affecting the eyes, including both visually impaired conditions (VICs) and non-visually impairing conditions (NVICs), is referred to as ocular morbidity.

Due to the high incidence of work-related eye injuries, agriculture is regarded as one of the riskiest professions in the world, right up there with mining, manufacturing, and construction [3]. In Nepal, agriculture plays a central and pivotal role in the country's economy. Approx. 50% of Nepal's population is involved in farming. Work-related eye injuries continue to be a prominent contributor to visual morbidity, carrying substantial socioeconomic consequences. Among the three main geographical regions of Nepal, the Terai region has the most fertile soil, ideal for cultivation, attracting a significant portion of the country's agricultural workforce. Despite the agricultural significance, there are many health problems, particularly concerning the eyes, that affect farmers. To understand the factors contributing to ocular health issues among Terai farmers, we need to look at all the different reasons, like their work and the environment they're in. Spending a lot of time in tough conditions like bright sunlight and dust, using chemicals in farming can be tough on farmers' eyes. Plus, the way they have to work in the fields can also make things harder for them. Also, not having enough healthcare nearby and not knowing enough about eye health make eye problems worse for these communities, keeping them stuck in a cycle of unfair health issues.

While existing literature offers insights into ocular health in agricultural settings globally, we need to look closely at the specific details of how it affects people in Nepal's Terai region. This thesis aims to learn more about how common eye problems are, what causes them, and how factors like money and social status affect them among farmers in Nepal's Terai region [4]. This will help us understand better how farming affects people's health in Nepal. In simple terms, this thesis is a strong call to recognize the eye health problems faced by farmers in Nepal's Terai region. It urges important actions to protect their health better.

The ocular morbidity of patients visiting a satellite clinic in Bhaktapur, Nepal, between March 2007 and February 2008 was published based on a limited number of studies. According to the survey, the most prevalent eye conditions were refractive errors (22.5%), conjunctivitis (14.9%), age-related cataracts (17.5%), and conjunctival degenerations (10.8%) [5, 6].

According to another research, of the 784 patients questioned, 89% were men and 44.6% were in the 31–40 age range. In the manufacturing industry, temporary workers had an increased

risk of unintentional eye injuries (63.2%) compared to permanent workers (52.8%), particularly for those without safety training (67.8%) and those who did not use protective gear (88.3%). The most common diagnosis (58.4%) was foreign bodies on the cornea and conjunctiva, with metallic foreign bodies accounting for the majority (71.5%) [7].

A small number of studies evaluated the usage of ocular protection, eye care services utilization, ocular injuries, and demographic characteristics. They had a clinical examination to determine the state of their eye health. Refractive errors (28.6%), cataracts (20.0%), glaucoma (11.7%), conjunctivitis (13%), pterygium (2.7%), and corneal opacity (2.2%) were the most common ocular disorders found [8].

A related study included 196 individuals with a history of agriculturally associated eye disorders. At a 3:1 ratio, the number of male patients exceeded that of female patients. With 34.69% (68) of the cases, the age group of 31 to 44 years old was the most common. At 53.57% (105) of cases, rice grain damage emerged as the most common cause of eye injury. The most common cause of hospital admission (42.35% (83) was corneal ulceration, which was followed by traumatic hyphema (20.92% (41). In the second week, most patients improved, with 56.13% (110) demonstrating improvement. Moreover, in 76.53% (150) of the cases, the visual result was good [9, 10].

A prospective observational research with 416 patients—mostly males—was carried out. Younger people were more likely to sustain these injuries. Those who engage in agriculture, metalworking, and grinding are more likely to have eye injuries at work. The most common form of eye damage encountered was corneal foreign bodies. Furthermore, a sizable percentage of the patients did not use protective eyewear throughout the events. Roughly 7.2% of the patients had a history of prior occupational eye injury [11].

A related investigation found Thirty-one to forty years old made up fifty percent (62) of the participants, the most common age group. Compared to closed-globe injuries, unilateral ocular injuries were more common. The primary mode of ocular injury was attributed to rice grain, accounting for 32.26% (40) of cases. [9] Corneal ulcer was identified as the most common diagnosis, affecting 20.7% (25) of patients, followed by a ruptured globe at 10.4% (13) [12].

A study that looked back was conducted and enrolled 915 patients total—298 (32.5%) men and 617 (67.5%) women. Refractive error was determined to be the most prevalent ocular morbidity, with a frequency of 26.8%. It was followed by corneal opacities at 2.3%, chalazion/stye at 4%, cataract at 11.8%, pterygium at 6%, entropion/ectropion at 3.9%, keratitis at 3.8%, dry eyes at 2.8%, and conjunctivitis at 20.6% [6].

Research looked at 36 Iowa farmers from the Agricultural Health research whose agricultural activities were connected to eye injuries. The study concentrated on 40 eye injuries connected to farmwork that needed medical advice or treatment. Of the injuries, 11 (27.5%) were caused by grinding or cutting metal, 3 (7.5%) by welding, and 2 (5%), by drilling. The other twenty-four injuries were related to other aspects of farming. Of them, one came from an animal, and the other from exposure to chemicals. A foreign body in the eye was the most frequent kind

of eye damage, occurring in 32 (80%) instances with 20 of those cases containing metallic foreign materials [9].

Of the 1,236 workers in the research, 38.3% (473) reported having sustained an eye injury at work. Moreover, 844 employees, or 68.3% of the workforce surveyed, stated they had never worn safety glasses to work. The usage of personal protection equipment (PPE) was shown to be positively correlated with male sex (P<0.001), prior work-related injuries (P<0.001), and attending school (P=0.016). [6]

Long-term sun exposure, notably in the absence of rightful eye protection, can cause disorders like cataracts. Toxic exposure and chemical burns are additional risks associated with using pesticides without proper safety precautions. In rural agricultural communities, the shortage of utilization of regular eye care and protective eyewear exacerbates these hazards. Farmers may suffer from gradual vision loss in the absence of prompt treatment and proactive therapies, which would severely affect their quality of life and capacity to work. Reducing the frequency of associated comorbidities on behalf of farmers requires increased knowledge and better safety procedures, such as the application of protective apparel and regular eye screening.More research is required to provide thorough safety guidelines about agricultural hazards and how to prevent them in developing nations. This research aims to give a voice to the hardworking farmers in Nepal's Terai region and promote fairer and more equal ways to keep them healthy while they work in agriculture.

Materials & Methods

Methodology

This prospective study proposes to include all consecutive patients with farming or agriculture as occupation, presenting to the outpatient department of Sudarshan Eye Hospital, Gaur, Nepal. The hospital authority has officially issued a No Objection Certificate (NOC) for conducting the research project. Additionally, the research questionnaires have undergone thorough review and validation by the relevant department of the hospital authority.

i. Study design: Cross-sectional. Observational Study (Hospital based)
ii. Population/Participants: Farmers of Rautahat district, Nepal above 20 years of age, attending the outpatient department of Sudarshan Eye Hospital, Gaur, Rautahat
iii. Sampling Method: Convenience sampling
iv. Sample Size calculation: Cross-sectional study conducted by M.K. Shrestha et.al. showed prevalence of 21.2% moderate and severe visual impairment in the terai region of Nepal. (Shrestha, 2021)
    - Margin of error : 5%
    - Confidence level : 95%
    - Population size : 13318705
    - Prevalence rate : 21.2%
    - Sample size : 257
v. Inclusion Criteria:
    - Age> 25 years
    - Working as farmers
    - Consenting to participate in the study
vi. Setting: Sudarshan Eye Hospital, Gaur, Rautahat

vii. Period of study: December 2023- May 2024
viii. Conflict of interest: None

All the patients fulfilling the criteria will be enrolled into the study after proper informed consent. A pre-formed, pre-tested proforma will be used to collect the demographic data, detailed history, ocular examination findings and management. The gathered data will be compiled in Microsoft Excel sheet for statistical analysis.

Ethical approval was granted from Sudarshan Eye Hospital to carry out the study.

Procedure Details

Pretested questionnaires were verbally given to participants in order to collect data on their personal characteristics, employment history (including any eye injuries connected to the job), and overall ocular history.

After informed consent, all subjects underwent complete ophthalmic evaluation. Visual acuity was tested with Snellen's test-type in full illumination at a distance of 6 meters for distance acuity and 40 cm for near acuity. Refraction and Retinoscopy were done to obtain the best-corrected visual acuity and to rule out astigmatism. Slit-lamp biomicroscopy (Haag-Streit model) was used to perform a thorough examination of the eyelids, eyelashes, conjunctiva, cornea, anterior chamber, iris, pupils, and lens. To rule out diseases of the adnexa, anterior segment, and posterior segment, fundoscopy examination was performed using a direct ophthalmoscope. Tromide Plus IP (tropicamide 0.8% w/v+ phenylephrine HCL USP 5.0% w/v) was used for dilated eye examination. The Schiotz tonometer and the Topcon-CT800 non-contact tonometer were used to measure the intraocular pressure.

Dry eye syndrome was diagnosed if patients had complaints of burning sensation, watering and foreign body sensation. For the evaluation, Schirmer’s test I & II and Tear film breakup time (TBUT) were used.

Pterygium was defined as the presence of fibrovascular tissue that extended nasally or temporally from the bulbar conjunctiva onto the cornea.

Lens Opacities Classification System III (LOCS III) was used to grade cataracts [13]. The WHO International Classification of Diseases for Blindness and vision Impairment was used to classify vision impairment (ICD-11) [14.]

Under local anesthetia, minor surgical operations such foreign body removal and surgeries for chalazion, abscess, entropion, and lid lacerations were carried out. Patients requiring more sophisticated procedures or additional investigations for illnesses such as diabetic retinopathy, retinal detachment, and glaucoma were sent to higher centers.

Results and Discussion

Figure 1 and Table 1 present the age distribution of the research participants. The age group of 40–49 years old (n=89, 34.23%) comprised the majority of patients in this study, followed by 50–59 years old (n=86, 33.08%), 30-39 years old (n=38, 14.62%), and 60–69 years old (n=37, 14.23%).

The age groups with the fewest patients were 20–29 years old (1.92%; n = 5) and 70–79 years old (1.92%; n = 5). The patient's age is 48.61 years on average, with a standard deviation of 9.65 years.

Table 1: Distribution of farmers according to age group

Age groups (years)	Frequency	Percentage
20-29	5	1.92%
30-39	38	14.62%
40-49	89	34.23%
50-59	86	33.08%
60-69	37	14.23%
70-79	5	1.92%
Total	**260**	**100.00%**

Source: Researcher's compilation

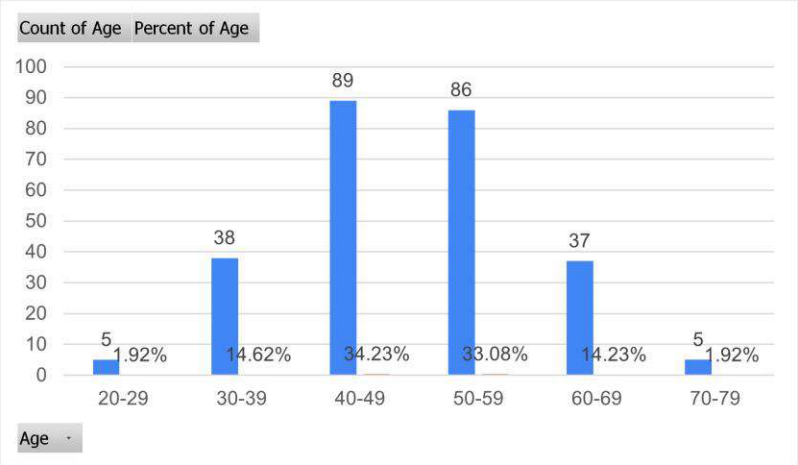

Figure 1: Bar diagram showing distribution of farmers by age group

Source: Researcher's compilation

Out of the 260 instances in this study, 40.38% (n=105) were female cases and 596.26 percent (n=155) were male patients. The ratio of 3:2 did not significantly alter the number of males compared to females. The distribution of genders among my research subjects is displayed in Table 2 and Figure 2.

Table 2: Distribution of farmers according to gender

Gender	Frequency	Percentage
Male	155	59.62%

Female	105	40.38%
Total	**260**	**100.00%**

Source: Researcher's compilation

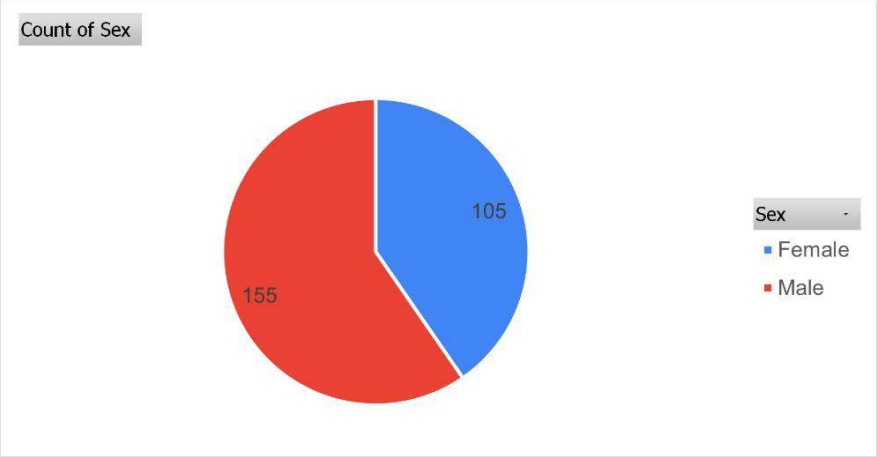

Figure 2: Pie chart showing distribution of farmers according to sex

Source: Researcher's compilation

Table 3 along with Figure 3 show distribution of 260 study subjects according to ethnic groups in Terai region, Nepal. Highest groups of participants 13.08% (n=34) were from Yadav group and least number were from Brahmin group that is 1.15% (n=3).

Table 3: Distribution of farmers according to ethnicity

Ethnicity	Frequency	Percentage
Ansari	26	10.00%
Brahmin	3	1.15%
Harjan	8	3.08%
Kalwar	19	7.31%
Kanu	23	8.85%
Koiri	25	9.62%
Kurmi	5	1.92%
Mansoori	7	2.69%
Nuniya	13	5.00%

Paswan	24	9.23%
Rajput	5	1.92%
Seikh	15	5.77%
Teli	16	6.15%
Tharu	9	3.46%
Yadav	34	13.08%
Others	28	10.77%
Grand total	**260**	**100.00%**

Source: Researcher's compilation

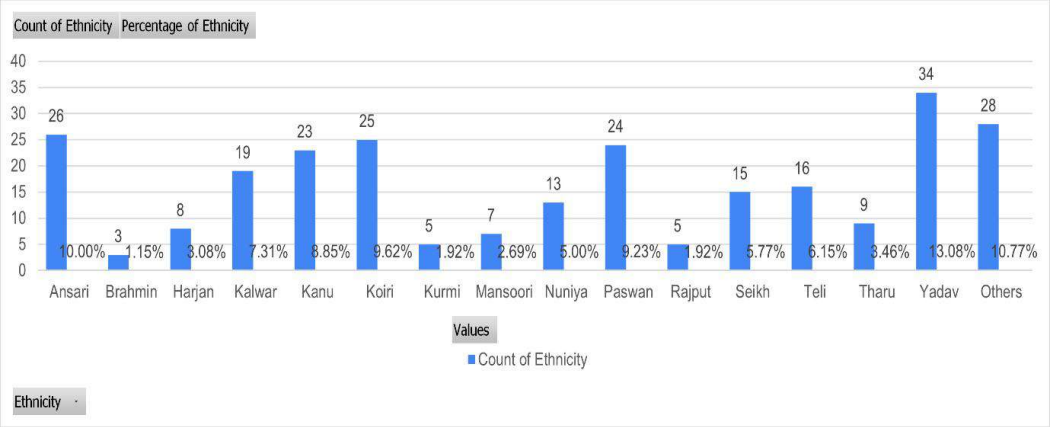

Figure 3: Bar diagram showing distribution of farmers according to ethnicity

Source: Researcher's compilation

Only eighty-two (31.54%) farmers had at least 6 years of primary school education, while one hundred and fifty (57.69%) had not received any formal education. Twenty-seven (10.38%) had secondary level education, and only one patient (0.38%) had higher secondary level education. Among them, the female literacy rate was very poor. Among 105 female farmers, only 18.10% (n = 19) had primary level education, while the majority 81.90% (n = 86) were uneducated.

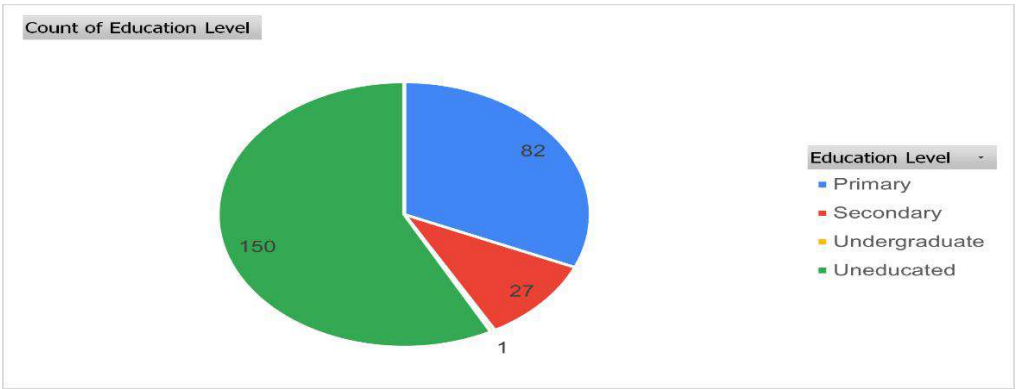

Figure 4: Pie chart showing distribution of farmers according to education level

Source: Researcher's compilation

The farmers were categorized into three groups based on their experience: novice (<10 years), experienced (11-20 years), and veteran (21+ years). Most patients fell under Veteran (43.08%) and Experienced (41.92%). Only 15.00 % fell under Novice category.

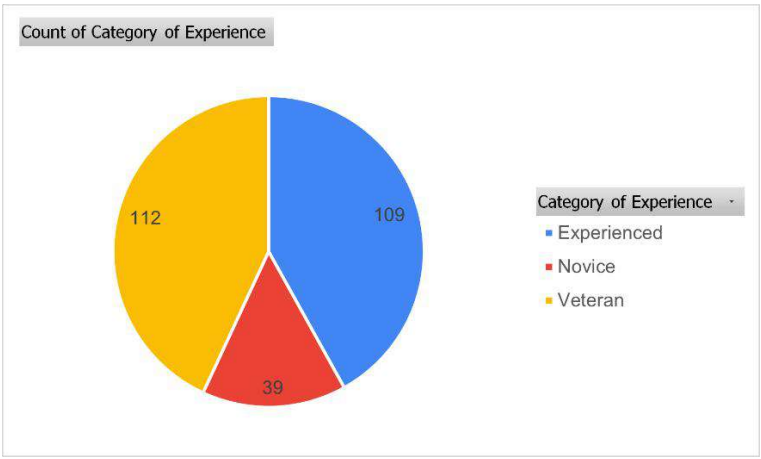

Figure 5: Pie chart showing distribution of farmers according to experience

Source: Researcher's compilation

Based on their weekly involvement in farming, their working hours were divided into three categories: Full-time, part-time and overtime. Most farmers were engaged in full-time farming, n = 179 (68.85%). 45 farmers (17.31%) were engaged in overtime farming, whereas 36 farmers (13.85%) were engaged in part-time farming.

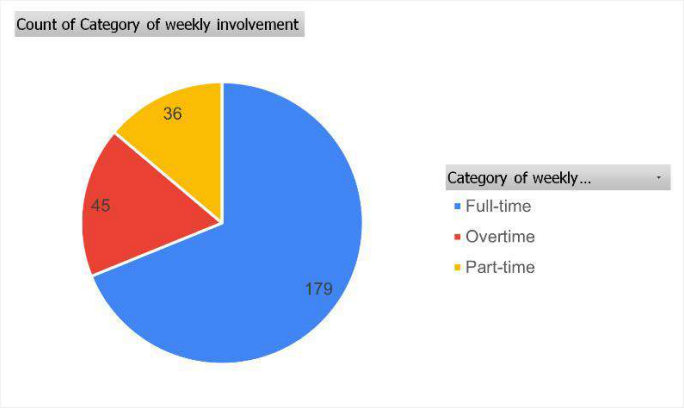

Figure 6: Pie chart showing distribution of farmers according to their weekly involvement in farming

Source: Researcher's compilation

Among 260 farmers, 114 farmers (43.85%) reported never wearing PPE at work, 63(24.23%) farmers reported wearing PPE sometimes, and 83 farmers (31.92%) reported always wearing PPE while working. 224 farmers (86.15%) were frequently exposed to sunlight. 254 farmers (97.69%) were exposed to dust and smoke. 203 farmers (78.08%) were involved in handling agricultural chemicals.

The majority of farmers, n = 111 (42.69%), had normal vision, 54 had monocular blindness (20.77%), 39 (15.00%) had severe visual impairment, 33 (12.09%) had moderate visual impairment, and 23 (8.85%) had mild visual impairment.

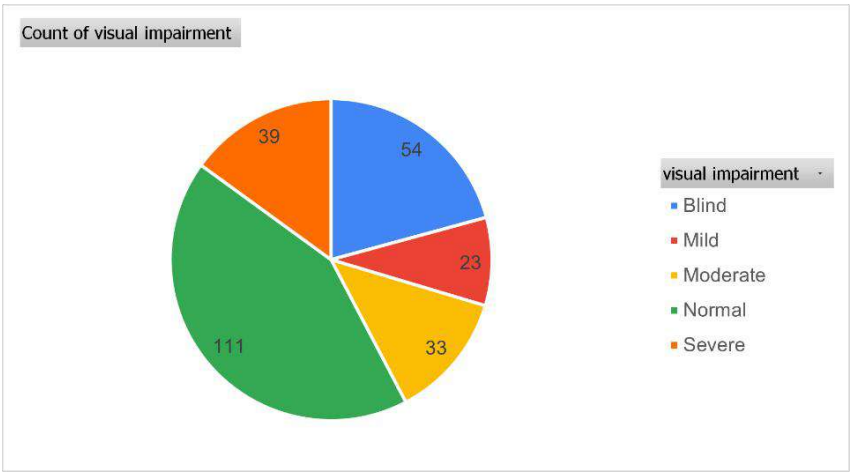

Figure 7: Pie chart showing visual impairment in farmers

Source: Researcher's compilation

Table 4 along with figure 8 shows distribution of 260 study subjects according to clinical symptoms. Majority of participants 61.92% (n = 161) had Difficulty In vision (DIV) as main clinical symptom.

Eighty-nine patients had more than two symptoms, 26 had more than 3 symptoms and 9 had more than 4 symptoms.

Table 4: Distribution of farmers according to clinical symptoms

Clinical symptoms	**Frequency**	**Percentage***
DIV (Difficulty in Vision)	161	61.92%
Loss of vision	28	10.72%
FB sensation	33	12.69%
Pain	68	26.15%
Redness	90	34.62%
Itching	26	10.00%
Burning sensation	11	4.23%
Watering	55	21.15%
Discharge	6	2.31%
Photophobia	31	11.92%
Others	8	3.08%

**Multiple responses*

Source: Researcher's compilation

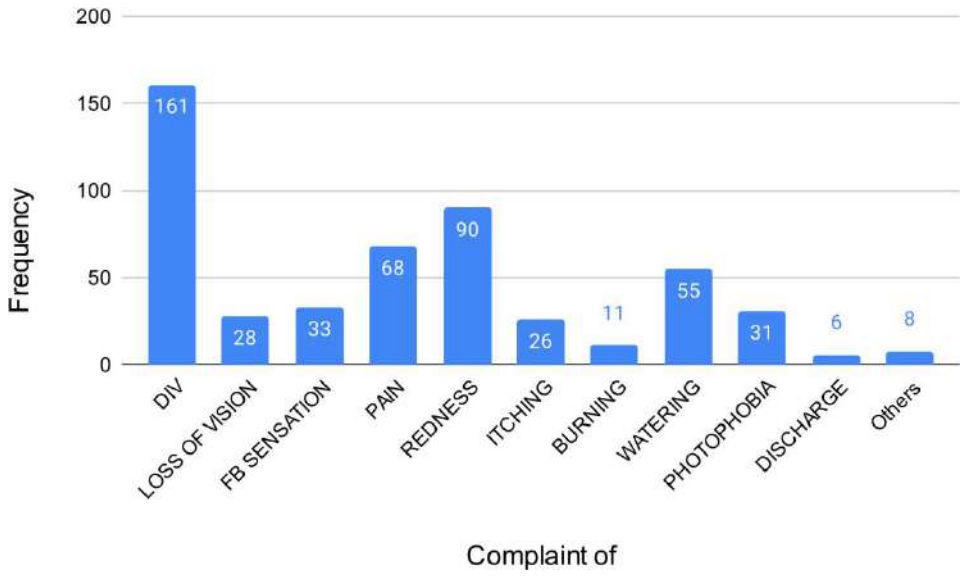

Figure 8: Bar diagram showing distribution according to clinical symptoms

Figure 9 pie chart illustrates the distribution of various systemic health conditions among the participants in the study. Majority of patient (n = 129, 49.62%) have no known systemic health conditions, 50 (19.23%) had Hypertension, 44 (16.92%) were not aware about their health conditions, 30 (13.83%) had Diabetes, 10 (6.15%) had thyroid problems, 5 (1.92%) had Tuberculosis, 4 (1.54%) had arthritis, 2 (0.77%) had asthma and 2 (0.77%) had cardiac problems.

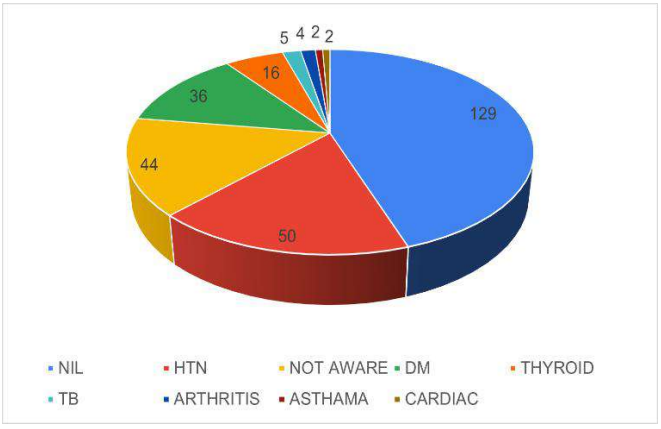

Figure 9: Pie chart showing distribution of systemic illness

Among the 260 patients, 69 reported a history of trauma, with the most common cause being injuries related to paddy (18.84%).

The prevalence of different eye diseases among research participants is shown in Table 5 and Figure 10. It reveals that the most common eye condition is cataracts, which are followed by presbyopia, corneal ulcers with or without hypopyon, and refractive error.

Table 5: Distribution of farmers according to ocular conditions

Diagnosis	Frequency	Percentage*
Ref error	52	20.00%
Corneal ulcer with hypopyon	35	13.46%
Presbyopia	37	14.23%
Cataract	78	30.00%
Allergic conjunctivitis	30	11.54%
Corneal ulcer without hypopyon	36	13.85%
Corneal opacity	8	3.08%
Pseudophakia	19	7.31%
Keratitis	4	1.54%
Pterygium	16	6.15%
Foreign body	5	1.92%
Pinguecula	4	1.54%
Glaucoma suspect	5	1.92%
Dry eyes	17	6.54%
Open globe injury	12	4.62%
Hypertensive retinopathy	2	0.77%
Traumatic hyphaemia	5	1.92%
Chemical injury	7	2.69%
Uveitis	4	1.54%
Dr	25	9.62%

Dacryocystitis	5	1.92%
Retinal disorders**	6	2.31%
Others***	5	1.92%

*Multiple responses **Retinal disorders (1 Retinitis pigmentosa, 2 ARMD, 2 Macular scar and 1 Macular hole) ***Others (2 Corneal abrasion, 1 Scleritis and 2 Ocular tuberculosis)

Source: Researcher's compilation

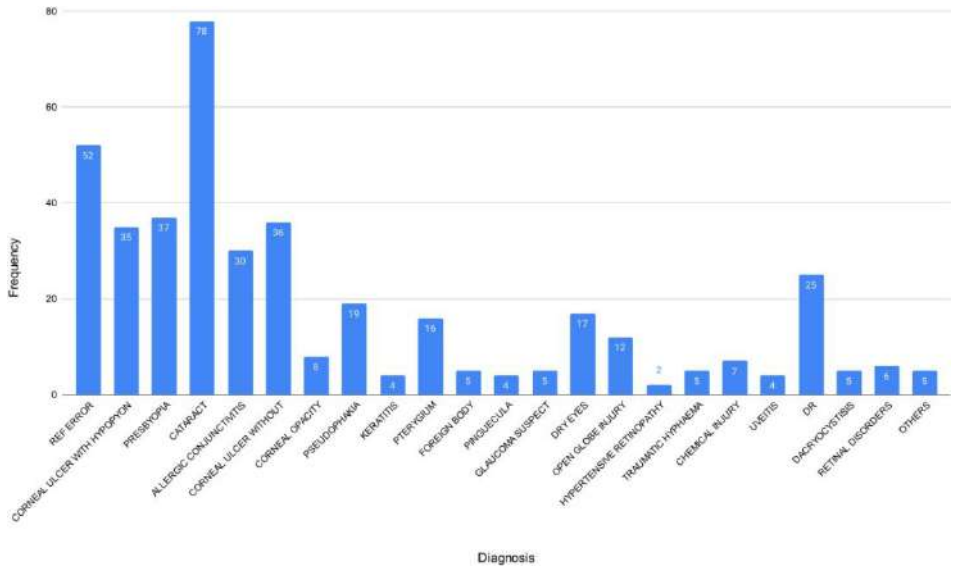

Figure 10: Bar diagram showing distribution of ocular conditions in farmers

Source: Researcher's compilation

Figure 11 indicates that the most common type of cataract among participants was Nuclear Sclerosis (NS) (10.38%), with a notable presence of combined types such as NS+PSC (7.69%) and NS+PSC+CC (4.23%).

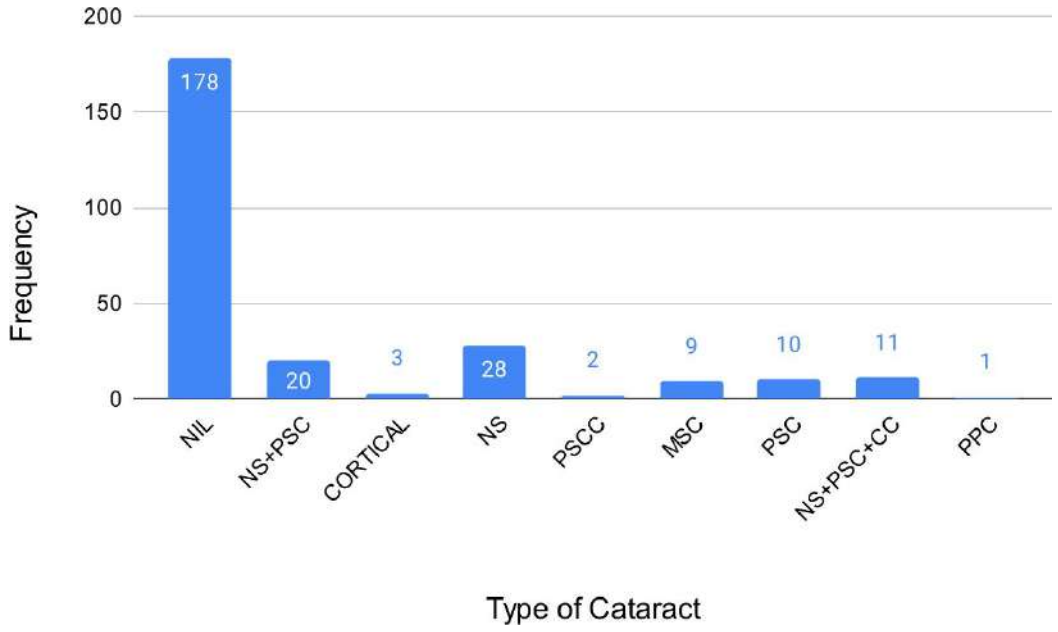

Figure 11: Bar diagram showing distribution of types of cataracts

Source: Researcher's compilation

The most common refractive errors among participants were simple myopia n = 36 (13.85%) and simple hyperopia n = 20 (7.69%), each affecting a notable proportion of the population. The high percentage of participants with no refractive error (69.62%) suggests that a majority of the study population either does not have refractive issues or has undiagnosed or uncorrected refractive errors.

Discussion

Comparing epidemiological research conducted in industrialized and developing countries reveals notable disparities in the incidence and prevalence of ocular illness and worker characteristics. These differences can be ascribed to differences in the location (rural versus urban), cultural norms, and research design. These variations highlight the need for more regional research. The purpose of this study was to evaluate the working conditions, ocular morbidity prevalence, duration, and patterns of use of personal protective equipment (PPE) among farmers in Nepal's Terai area. This is the first research that we are aware of that evaluates ocular morbidity among Nepali farmers.

This study included a nearly equal number of male and female participants, with 60% being male and 40% female. This contrasts sharply with other studies conducted among farmers, which typically show a male preponderance. (Mousumi Banerjee, 2019).

The literacy rate among farmers in this study is relatively low at 42.31%, compared to other similar studies in Nepal. (Limbu, 2018). This disparity may be attributed to the generally low literacy rate in the Rautahat district of Nepal.

The majority of farmers in this study were from the 40-49 (39.25%) and 50-59 (33.08%) age groups. This trend may be due to many younger individuals from the Terai region currently working in Gulf countries, leaving older farmers with limited options and thus compelled to continue farming.

In this study, 114 farmers (43.85%) reported never wearing personal protective equipment (PPE) at work, making them more susceptible to ocular traumas. This and the research "Work Related Ocular Injury: Nepal" were quite comparable. (Limbu, 2018). The low usage of PPE could be attributed to the low literacy rate and lack of awareness about eye safety among the participants.

Personal protective eyewear is highly effective in preventing work-related eye injuries. (Mancini, 2005). The occurrence of ocular traumas may be attributed to insufficient use of PPE.

In this study, 50 (19.23%) had Hypertension, 44 (16.92%) were not aware about their health conditions, and 30 (13.83%) had Diabetes.

This distribution highlights the prevalence of unawareness and chronic conditions such as hypertension and diabetes among the participants, emphasizing the need for improved health awareness and medical support within this population.

Farmers are at risk for work-related eye injuries, some of which can be severe. (Sprince, 2008)

This study's sequence and frequency of eye diseases were as follows: 30% for cataracts, 20% for refractive errors, 13.45% for ulcers without hypopyon, 13.84% for ulcers with hypopyon, 11.53% for allergic conjunctivitis, 9.61% for diabetic retinopathy, 7.30% for pseudophakia, 6.53% for dry eyes, 6.15% for pterygium, and 4.615% for open globe injuries.

The elevated incidence of allergic conjunctivitis observed in this study is likely due to the higher pollen levels characteristic of farming environments compared to industrial settings. Additionally, pterygium was relatively prevalent, which can be attributed to the significant exposure to dust and ultraviolet radiation faced by farmers who work outdoors. These factors are recognized as major risk contributors to the development of pterygium. (Abideen, 2021), (Moran, 1984).

The most common type of cataract was Nuclear Sclerosis (22.68%) and most common type of refractive error was Simple myopia (13.85%). This might be due to 40-49 and 50-59 years being the commonest age groups. The relation between age, type of cataract, and type of refractive error has been found in other studies. (D, 2016)

Summary and Conclusion

The ocular diseases identified among farmers are primarily linked to the hazards of the farming environment and prolonged outdoor exposure. Factors such as dust, chemicals, UV radiation, and physical injuries significantly contribute to these conditions. To mitigate these risks, it is essential to provide comprehensive eyecare services tailored to the needs of farm workers. This includes regular eye examinations, prompt treatment of ocular conditions, and the distribution of protective

eyewear to safeguard against environmental hazards. Integrating these eyecare services into the general health services offered to farm workers is crucial for maintaining their overall health and well-being.

Author Declaration

The study's authors affirm that no conflicts of interest exist and no particular grant from a public, private, or nonprofit organization was received for this research.

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Exploring the Potentiality of Buccal Drug Delivery for the Treatment of Dysphagia

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Abstract

Dysphagia, the impairment of swallowing function, poses a significant challenge in the management of various medical conditions. The condition can lead to severe complications such as aspiration pneumonia, malnutrition, and a decreased quality of life, particularly in vulnerable populations like the elderly and stroke survivors. Recent studies indicate that effective interventions for dysphagia must encompass a multidisciplinary approach, emphasizing not only the diagnosis and treatment but also the prevention of malnutrition associated with this swallowing disorder, which affects a substantial number of individuals across different health care settings. In this context, the exploration of buccal drug delivery as a potential solution for the treatment of dysphagia holds promising implications. Buccal administration, which involves the placement of a drug formulation between the cheek and gum, offers the advantages of bypassing first-pass metabolism, improved drug bioavailability, and the potential for the sustained drug release. Furthermore, advancement in designs of functional oral delivery systems can facilitate the effective release and absorption of therapeutic agents, thereby addressing the unique challenges posed by dysphagia and enhancing treatment outcomes for affected patients. Moreover, the integration of bioinspired oral delivery devices may significantly enhance the effectiveness of buccal formulations, addressing existing challenges related to drug absorption while reducing the adverse effects like flora balance disturbances as well as immunological responses, that are crucial for maintenance of overall health and well-being.

Keywords*:* Dysphagia, Buccal Drug Delivery Systems, Buccal Formulations, Buccal Administration

Introduction

Dysphagia, the impairment of the swallowing process, is a prevalent issue in various neurological conditions, including stroke and head and neck cancer [1,2]. This disorder not only compromises nutritional intake but also has deep effects on quality of life, leading to increased morbidity along with mortality among affected populations. Effective management of dysphagia is crucial for rehabilitation and recovery, making the exploration of the alternative drug delivery systems, like buccal drug delivery, essential to optimizing treatment outcomes and patient comfort in this vulnerable group (Table 1)[3]. Buccal drug delivery offers a non-invasive route that can enhance drug bioavailability while minimizing the risk of aspiration, thereby addressing a critical gap in the management strategies for dysphagia(Table 2)[4]. Moreover, the buccal route can facilitate rapid absorption of medications directly into the systemic circulation, bypassing first-pass metabolism, which is particularly advantageous for patients experiencing difficulty swallowing or those who are at risk of aspiration pneumonia due to dysphagia [5,6]

This review aims to examine the potential of buccal drug delivery in the treatment of dysphagia, highlighting the underlying physiological mechanisms, the current state of research, and the implications for future clinical applications. As dysphagia can significantly hinder the restoration of normality in patients' lives, particularly in recovering stroke survivors, finding effective and

innovative therapeutic approaches is imperative to improve outcomes and enhance their overall well-being[7]. The exploration of buccal drug delivery systems just not only promises enhanced therapeutic efficacy but also offer more user-friendly options for patient who may struggle with traditional oral medications, thus ensuring greater adherence to treatment protocols and ultimately contributing to better rehabilitation outcomes in dysphagia management.

Furthermore, given the complexity of dysphagia and its varying presentations in different populations, a tailored approach leveraging buccal drug delivery could serve as a pivotal strategy in addressing the unique challenges posed by this condition, especially considering the physiological nuances associated with swallowing impairments (Table3)[8,9]. The integration of mucoadhesive characteristics into buccal drug delivery systems further optimizes drug retention and absorption at the buccal mucosa, which is notably less susceptible to enzymatic degradation compared to gastrointestinal tract tissues, offering significant advantages for the administration of labile drugs in dysphagic patients [8,10,11]. This characteristic, coupled with the buccal mucosa's rich vascularization, enhances the potential for effective drug delivery, circumventing many of the complications associated with traditional oral routes, thereby promoting better clinical outcomes in dysphagia management [12].

Table 1: Types of Dysphagia and their Complications

Types of Dysphagia	Description	Complications	References
Oropharyngeal Dysphagia	Oropharyngeal dysphagia, which affects the oral and pharyngeal phases of swallowing, is a common presentation among older adults and can be attributed to various underlying causes, including neurological conditions like stroke, Parkinson's disease, and dementia, all of which can compromise swallowing safety and efficacy, leading to serious complications such as aspiration pneumonia and malnutrition	Aspiration pneumonia, Malnutrition, Dehydration	[13,14,15]
Esophageal Dysphagia	Esophageal dysphagia, which affects the esophageal phase of swallowing, can be caused by structural abnormalities, such as esophageal strictures, or functional disorders, including reflux and esophageal motility issues. These complications not only impair the patient's ability to consume adequate nutrition but also increase the risk of serious health issues like aspiration pneumonia, further complicating the management of dysphagia in affected individuals	Esophageal strictures, Reflux, and Esophageal motility disorders	[16,17]

Aetiology of Dysphagia

There are many different types of causes of dysphagia, and they fall into many categories:

1. Development from the Nerves-One of the important reasons why people have dysphagia is neurological diseases. These disorders impact the muscles and nerves involved in swallowing, which can cause weakening in the muscles and poor coordination[18]. Typical neurological reasons include of:

- Stroke: One of the most frequent causes of dysphagia, especially among the elderly, is stroke.
- Parkinson's disease: It is a progressive neurological diseasewhich affects the swallowing muscles and causes bradykinesia and muscular stiffness.
- Dementia: The capacity to swallow properly may be impacted by cognitive loss brought on by diseases like Alzheimer's disease[19].
- Multiple Systemic Sclerosis (MS): MS can trigger the swallowing muscles to atrophy and become spastic.
- Amyotrophic Lateral Sclerosis (ALS): ALS is characterized by a gradual loss of motor neurons, which impairs the regulation of voluntary muscles, including the swallowing muscles [20].

2. Intrinsic Causes-Dysphagia can also develop from structural problems with esophagus or oropharynx. These might consist of:

- Tumors: Growths that are benign or malignant might impede the flow of food.
- Strictures: Limiting of the esophagus, frequently brought on by GERD's persistent inflammation[21].
- Zenker's Diverticulum: A pouch known as Zenker's Diverticulum can develop in the upper portion of the esophagus, accumulating food particles and making swallowing difficult.
- Osteophytes in the Cervical Spine: The esophagus may be impacted by growths of bone in the cervical spine[22].

Difficulties in Treating Dysphagia

Given the intricacy of dysphagia and the variety of underlying reasons, treating the disease can be difficult. Several of the main obstacles are as follows:

1. Identification-First, identifying dysphagia accurately is a difficulty. Radiologists, gastroenterologists, and speech-language pathologists are frequently involved in the multidisciplinary approach that is needed[23]. Esophageal manometry, Fiberoptic endoscopic evaluation of swallowing (FEES), and video fluoroscopic swallow studies (VFSS) are examples of diagnostic tools. However, there might not be as much access to these specialist diagnostic techniques, especially in settings[24].

2. Addressing Comorbid Conditions-Treatment for dysphagia patients is made more difficult by the prevalence of concomitant illnesses such cancer, diabetes, and neurological problems [25]. Improving dysphagia necessitates managing these underlying problems, which calls for an all-encompassing and well-coordinated treatment strategy [26].

3. Difficulties with Nutrition-Due to their difficulties eating enough calories and fluids, patients with dysphagia are at an elevated risk of malnutrition as well as dehydration. Creating a safe and sufficient diet plan is difficult yet necessary. This frequently entails changing the liquid and food textures, which may make patients less receptive and cause them to consume less[27].

4. Aspiration Risk- For individuals with dysphagia, aspiration is the inhaling of food or fluids into the lungs and poses a high risk of aspiration pneumonia, a potentially fatal illness. Careful evaluation is necessary to prevent aspiration, and in certain cases, using non-traditional feeding techniques such nasogastric or percutaneous endoscopic gastrostomy (PEG) tubes, which can have their own risks and complicationsis necessary[28].

5. Quality of Life and Patient Compliance- The use of thickened liquids or altered food textures, dietary restrictions, and lifestyle modifications are common dysphagia therapies that can have a serious negative effect on a patient's quality of life. One of the biggest challenges in ensuring patient compliance with these adjustments is that the patients might not want to stick with therapies that they feel uncomfortable or socially constrainingarterials[29].

Current Approaches to Treatment

The current approaches to controlling dysphagia centre on symptom management, averting problems, and, where feasible, enhancing swallowing function. Among them are:

1. The Ingestion of Food-The foundation of managing dysphagia is speech-language pathologists' swallowing treatment. The use of safe swallowing procedures, strengthening exercises for the swallowing muscles, and instruction on good swallowing skills are some examples of techniques[30].

2. Adjustments to Diet-One popular strategy to improve safety and ease of swallowing is to modify the texture of meals and beverages. This may entail pureeing meals or thickening liquids to lower the chance of aspiration. These changes, meanwhile, could not be very palatable, which could result in less consumption and even nutritional deficiencies[31].

3. Interventions Using Pharmacology- Medication may be utilized to treat dysphagia in certain situations, especially if it is brought on by disorders like GERD. Acid reflux can be lessened with proton pump inhibitors (PPIs) or H2 blockers, and esophageal motility can be improved with prokinetic drugs. These therapies, however, are condition-specific and donot deal with every dysphagia cause [32].

4. Procedures involving Surgery- When tumors or esophageal strictures are the structural cause of dysphagia, surgical alternatives are taken into consideration. Dilation, stent implantation, and blockage removal are possible procedures. To guarantee proper nourishment in extreme circumstances, feeding tubes may needed to be surgically inserted[33].

Table 2: Various Buccal Drug Delivery Systems with their Advantages and Disadvantages

Delivery Systems	Formulation Description	Advantages	Disadvantages	References
Buccal Films	Thin, flexible, and adhesive films composed of polymers such as chitosan, sodium alginate, and hydroxypropyl methylcellulose that adhere into the buccal mucosa and provide controlled release of drug.	Allows for steady and regulated medication delivery; Simple to use and easy to wear; Flexible and thin, encouraging high patient compliance; Easily removed when needed.	Limited medication loading capacity; Possible rashes or pain at the application site; Potential separation because of the oral cavity's wet environment.	[34,35]
Buccal Tablets	Tablets designed to cleave to the buccal mucosa, often containing mucoadhesive polymers like carbopol, hydroxypropyl methylcellulose, and sodium carboxymethylcellulose	Offers regulated medication release; May be made for unidirectional release; Is simple to make using typical tablet procedures; High patient acceptability because they are accustomed to taking tablets.	May take longer to completely disintegrate; A tablet that moves or separates may result in partial breakdown; Patients may be less compliant if the flavor or mouthfeel is unpleasant; Restricted to medications that show efficacy at modest dosages.	[36,37]
Buccal Patches	Bilayered or multilayered polymeric systems that consist of a drug-containing mucoadhesive layer and a impermeable backing layer to control drug release.	Prevents medication from being broken down by saliva; Offers one-way medication release; Has a longer retention period than movies; Appropriate for medications needing extended exposure.	Possibility of reduced patient compliance as a result of feeling like they have a foreign object in their mouth; Risk of inadvertent detachment; Potential to obstruct speaking or eating; Challenge in maintaining uniform adhesion in various people.	[38]
Buccal Gels	Semi-solid solutions	Simple to use and distribute throughout	Reapplication may be necessary often;	[39,40]

	administered topically to the buccal mucosa. They are designed with bioadhesive polymers to prolong the length of contact and distribute readily across thesurface of mucosa.	the mucosa; Able to disperse medication evenly over a greater surface area; Delivers rapid drug release; Non-invasive and patient-friendly.	Shorter retention duration than films or patches; May be cleaned away by saliva; May result in unequal medication delivery if the gel spreads unevenly.	
Buccal Sprays	Solutions in liquid form that are sprayed straight into the buccal mucosa using a spray instrument. Because the spray covers a broad surface area, the medication is absorbed via the mucosa quickly.	Speedy absorption causes a speedy start of action; Easy to deliver, especially for individuals who have trouble swallowing; Offers a non-invasive injectable substitute; Allows for adjustable dose through a controlled spray.	Controlling the precise dosage administered can be challenging; Variations in spray methods may cause absorption to vary; The product has a short half-life that requires frequent dosages; There is also a chance that the product could be swallowed accidentally, which might limit its effectiveness and raise systemic adverse effects.	[40]
Mucoadhesive Microspheres	Drug-containing, spherical, microscopic particles that stick to the mucosal surface. By gradually disintegrating in the buccal environment, they offer regulated and	Long-term medication release; High surface area for effective drug absorption; Formulation flexibility for targeted delivery to the buccal cavity; Appropriate for a variety of medicines.	The size of the microspheres, that must be sufficiently tiny to prevent irritation; Limits the possibility of irritation or pain from the particles present in the mouth; Uneven dispersion over the mucosal surface is another challenge.	[41,42]

	prolonged release.			
Buccal Buccoadhesive Lozenges	Solid dose forms that stick to the buccal mucosa, like conventional lozenges. As the lozenge dissolves, these offer a gradual release of the medication.	Extended medication release while the lozenge disintegrates; Patients will find this dose form familiar and acceptable; It can even be made with enticing tastes to improve compliance.	A lengthy dissolving period might be painful for some patients; There is a chance that something will be inadvertently swallowed, particularly in young or elderly patients; It might not be appropriate for medications that need to take effect quickly.	[43,44]

Table 3: Prospective Benefits of Buccal Drug Delivery Systems in the Treatment of Disorders Associated to Dysphagia

Healing Domain	Buccal Drug Delivery System	Potential Application in Dysphagia Treatment	References
Neurological Disorders	Mucoadhesive Films/Patches	By supplying steady doses of medicine without swallowing, buccal films or patches that administer dopaminergic pharmaceuticals (such Levodopa) can help treat Parkinson's disease symptoms in individuals who have dysphagia.	[45,46]
Post-Surgical Pain Management	Buccal Patches/Gels	Analgesic or anti-inflammatory drug-delivering buccal patches or gels can help post-operative patients with neck and head surgery-induced dysphagia by relieving discomfort and swelling without oral consumption.	[47,48]

Gastroesophageal Reflux Disease	Buccal Tablets/Films	Proton pump inhibitors (PPIs) and H2 blockers released into the buccal film or tablet form can help control GERD-related esophageal dysphagia by lowering acid exposure and acting locally without requiring swallowing.	[49,50]
Stroke Recovery	Mucoadhesive Patches/Films	By avoiding the necessity for swallowing and lowering the danger of aspiration in individuals with dysphagic stroke, buccal delivery devices that deliver neuroprotective or rehabilitative medications can facilitate stroke recovery.	[51,52]
Nutritional Support	Buccal Lozenges/Films	For dysphagic patients, nutritional assistance in the form of nutraceuticals or appetite enhancers administered by buccal lozenges or films can guarantee sufficient nutrient intake without requiring oral consumption.	[53,54]
Anxiety and Sedation Management	Buccal Sprays/Films	As they are quick to take effect and simple to apply, buccal sprays or films that include sedatives or anxiolytics can assist dysphagic patients—especially those with neurological disorders—manage their anxiety or agitation.	[55,56]
Oral Mucosal Healing	Buccal Gels	Buccal gels that are formulated with wound-healing agents or anti-	[57]

		inflammatories have the potential to alleviate discomfort and accelerate the healing process when used to treat oral mucosal injuries or ulcers that are frequent in individuals with dysphagia.	
Parkinson's Disease	Buccal Films/Tablets	In Parkinson's disease, buccal films or tablets for prolonged release of medicine can offer consistent therapeutic levels, minimizing motor swings and off-periods and enhancing patient compliance.	[58,59]
Management of Xerostomia	Buccal Sprays/Lozenges	For dysphagic patients, xerostomia (dry mouth), which frequently aggravates swallowing difficulties and raises the risk of oral infections, can be managed with buccal sprays or lozenges containing saliva stimulants.	[60,61]

Conclusion

The effective treatment of dysphagia requires a multidisciplinary approach, encompassing swallow therapy, dietary modification, and in some cases, pharmacological interventions. Furthermore, the integration of buccal drug delivery systems offers a promising alternative that could enhance medication adherence and optimize therapeutic outcomes, particularly in populations at heightened risk for complications associated with dysphagia, such as the elderly or stroke survivors. Innovative buccal delivery methods can improve drug absorption while circumventing traditional swallowing difficulties, thereby addressing both the pharmacokinetic challenges and the critical need for timely access to medications that can aid in recovery and enhance quality of life for affected individuals. As the field of buccal drug delivery continues to evolve, the integration of bioinspired design principles and targeted drug release profiles may further expand the utility of this approach in the management of dysphagia, potentially leading to improved patient outcomes and reduced healthcare costs associated with complications like malnutrition and prolonged recovery times. In conclusion, ongoing research and development in smart drug delivery systems hold the potential to revolutionize the treatment landscape for dysphagia by providing controlled, efficient, and patient-friendly routes of administration, thus

addressing barriers related to traditional oral formulation methods and enhancing the overall quality of care for this vulnerable patient population.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tuberculosis among silicosis-affected ramming mass workers in a rural block in North 24 Parganas a cross-section study

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Abstract

Introduction: Silicosis is a progressive occupational disease found in various parts of India. This occurs in workers who are been exposed to silica due to occupation. Silicosis can progress to dyspnoea at rest. This cross sectional study has been done in a rural blocks of North 24 Parganas. The government of West Bengal has recognized this occupational disease in West Bengal and classified some districts as "incident districts" and a "prevalence district". The patients affected by silicosis are prone to tuberculosis and can progress to multi-drug resistant tuberculosis.

***Objective of this study*:** To find out after receiving presumptive treatment, how many patients arestill sputum-positive cases. To find out among these patients are there any evidence of drug resistance case (INH and Rifampicin resistance).

***Methods*:** Structured interview was conducted among silicosis-affected ramming mass workers. Chest X ray, High-resolution CT scan was done to detect radiological evidence of various infections among silicosis patients. Health camps are organized periodically is to decide the quantum of compensation as per laws pf the National Human Right Commission. Silicosis has been identified as a debilitating disease.

***Results*:** A total of 54 patients were enrolled for this study. These patients were proved to be negative after presumptive treatment of tuberculosis by CBNAAT examination. Radiological changes showed signs of chronic infections.

***Discussion*:** Silicosis and drug-resistant tuberculosis may co-exist. Suspected cases received presumptive treatment as per national guidelines. They were found at the time of examination.

Conclusion: No INH and rifampicin-resistant cases were found.

Keywords: Silicosis, dyspnoea, INH and Rifampicin resistance, radiology, presumptive treatment

Introduction

Silicosis is an occupational lung disease caused by inhalation of a crystalline variety of silica [1]. A mineral is so ubiquitous that all of us inhale particles containing it continuously. India has a

large mining industry country with the states of Chhattisgarh, Jharkhand, West Bengal, and Odisha being particularly mineral-rich. workers who work in these mines are at risk of developing silicosis. An ICMR report of 1999 puts the number of workers at high risk of silicosis at 3 million. Out of this 1.7 million are in the mining or quarrying industry 0.6 million are in manufacturing non-metallic products like refractory clay, glass, and mica and 0.7 million workers in the metal industry. These types of workers are at risk [2]. The English word pneumonoultramicroscopicsilicovolcanokoniosis (45 letters), a word signifies that it is a major public health problem among workers affecting workers worldwide [3].

The silica structure makes it insoluble in water and in hydrofluoric acid. Hence crystalline variant is not absorbed and it induces a hypersensitivity reaction.

Ubiquitous generations of silica crystal from stone carving, marble work, polishing of granite et cetera. All are at high-risk jobs for contracting silicosis. Stone quarrying and crushing happens all across India and is mostly carried out by poor people and under informal work settings. Hence, getting accurate information on areas, where silicosis is more prevalanceor the number of people working in jobs that expose them to silica dust, it's very difficult. However, what is evident is that most people affected by silicosis are in economic the and productive age groups [1, 2].

Silicosis is the oldest occupational disease and is caused by silica dust inhalation for long exposure. The duration of exposure could be 10-30 years in chronic silicosis. Acute and accelerated silicosis develops weeks to 5 years after exposure, 3- 10 years after exposure to silica dust. The silica content of artificial stone is greater than 90% and exposures are often intense leading to severe forms of disease Unfortunately, despite improved industrial hygiene standards and stricter occupational exposure limits (OELs), many workers in both developed and developing countries are diagnosed with silicosis, a disease there is currently has only supportive treatment [3-6].

Ramming mass is an industrial term which is required for the manufacture of inner core of blast furnace for smelting iron or preparing insulators in high voltage electricity transmission cables. It is produced from rocks which has a silica content in the range of 98% to 99%. Rocks are grounded to fine dust. Industrial worker who work in this factory work for prolonged period 12-14 hours a day continuously for 6 to 7 months and then take a break and work in occupation like agriculture. But work they do in these 6 to 7 months they inhale dust which becomes fatal and produces a type of hypersensitivity reaction. This has a clinical manifestation like chest pain, dry cough, fever and varying degrees of dyspnoea [7].

Tuberculosis in the background of confirmed cases of silicosis

When we discuss silico tuberculosis, we are referring to a situation in which patients who have silicosis also have tuberculosis.
Evidence of literature puts the risk of development of silico tuberculosis to very high. Review of literature and use of artificial intelligence to assess the risk of tuberculosis among silicosis-affected patients to the range of 2.8 to 39 per cent depending upon multiple factors

Multivariate logistic regression silico-tuberculosis puts the age (years), male sex, sputum positivity, previously treated tuberculosis, and multi-drug resistance tuberculosis were predictors of unfavourable tuberculosis outcome.

Specific conditions where silicosis-affected patients may be at a higher risk are sandblasting, and exposure to crystalline silica, other risk factors are the duration of exposure >30 years, HIV-

positive status, smoking, chronic obstructive pulmonary diseases, The affected silicosis patients who are given anti-tubercular drugs are treated as sputum negative cases.

Mechanism of production of silico tuberculosis- one of the models

One of the proposed mechanisms is as follows. Elevated silica level inhibits alveolar macrophage function, and leads to increased vulnerability to mycobacterial infection. Particles of silica are inhaled, into the alveolar space, engage in interactions with macrophages, and ultimately descend into the phagosome. On the surface of macrophages, a Class A protein scavenger receptor known as the macrophage receptor with a collagenous structure (MARCO) prevents the binding and absorption of opsonized environmental particles like silica, TiO_2, Fe_2O_3, and other nano-sized substrates. According to reports, MARCO is the primary molecule that recognizes and absorbs silica; if this receptor is absent, neither particle uptake nor cell death will occur. Certain receptors that recognize patterns, such Toll-like receptors (TLRs), are impaired [4-7].
The mechanism which is being affected is the down-regulation of Toll-like receptor 2 may increase susceptibility to tuberculosis infection. There may be multiple ways like genetic This phenomenon has been observed in many mine workers who have been affected by silicosis like South African gold miners and Chinese iron miners. Silica particles induce granuloma formation. Silica induces type of granulomatous reaction where bacteria proliferate and there is a release from macrophages. All these immunological reactions may lead to elimination of tuberculosis but mechanism is not well understood but it does not happen.

Why the risk of multi-drug resistance tuberculosis (MDR-TB) in Silicosis?

Emerging evidence suggests a disturbing link between silicosis and an increased susceptibility to multidrug-resistant tuberculosis (MDR-TB).

INH and Rifampicin are the two major drugs used in short-course chemotherapy for tuberculosis. Development of resistance to these two drugs is considered multidrug resistance Tuberculosis. MDR-TB complicates attempts to control and eradicate the illness and represents a danger to world health. Treatment is made more difficult by this resistance, which frequently necessitates longer, more expensive courses of second-line medications with potentially more serious side effects. People who have silicosis are more vulnerable to TB infection because to their lowered lung immunity, which also compromises their immune system's capacity to combat the bacterium. The risk of tuberculosis transmission is further increased by the conditions that are common in workplaces with significant silica dust exposure, such as cramped living quarters and limited access to healthcare.

Treatment is made more difficult by this resistance, which frequently necessitates longer, more expensive courses of second-line medications with potentially more serious side effects. The alarming correlation between silicosis and the increased risk of acquiring MDR-TB has been clarified by a number of research. People who have silicosis are more vulnerable to TB infection because of their lowered lung immunity, which also compromises their immune system’s capacity to combat the bacterium. The risk of tuberculosis transmission is further increased by the conditions that are common in workplaces with significant silica dust exposure, such as cramped living quarters and limited access to healthcare.
A study was carried out among silica dust-exposed employees of a glass business in the northern Indian state of Uttar Pradesh's Firozabad district to evaluate their pulmonary tuberculosis (TB)

condition, with a particular focus on multidrug-resistant TB.The magnitude of the silicosis problem is astounding [8].

When present with the following clinical symptoms and indication to start presumptive treatment

Persistent cough, weight loss, night sweats, and haemoptysis.

Living in an area which is endemic to TB. Alternately conclusively by any means tuberculosis could not be ruled out.

Radiological Evidence: Chest X-ray findings suggestive of TB, such as cavitation or infiltrates

During the initial phase treatment with 4 drug regime followed by 2 drug

Monitoring:during presumptive treatment is regular clinical and radiological follow-up, sputum cultures and drug susceptibility testing when possible, - Liver function tests due to increased risk of hepatotoxicity

Considerations as per clinical guidance in the light of presumptive treatment

- Balance risks of unnecessary treatment against potential benefits
- Consider drug interactions and side effects
- Assess for multi-drug resistant TB in high-risk areas

The risk of complications in sputum-negative silicosis patients who have received presumptive tuberculosis (TB) treatment is an important consideration.

Here's an overview of the potential risks and complications

<u>Misdiagnosis and overtreatment the rationale against (which is overlooked)</u>

Risk of treating for TB when it's not present

Unnecessary exposure to anti-TB drugs and their side effects

Drug-Related Complications:

- Hepatotoxicity [especially with isoniazid and rifampicin}
- Peripheral neuropathy [associated with isoniazid}
- Optic neuritis [related to ethambutol}
- Gastrointestinal disturbances

- Potential development of drug-resistant TB strains if TB is present but not fully eradicated

Risk is masked Progression of Silicosis:

- TB treatment may temporarily improve symptoms, potentially delaying diagnosis and y be worsening silicosis

Interaction with Silicosis Progression:

- Silicosis can continue to progress despite TB treatment
- Ongoing lung damage from silica exposure may complicate recovery

Immune System Effects:

- Altered immune responses due to both silicosis and TB treatment
- Potential increased susceptibility to other infections

Clinical Assessment: Regular follow-ups to monitor symptom resolution and side effects.

- Sputum Smear and Culture: At the end of the initial phase and periodically during the continuation phase.
- Liver Function Tests: Especially important if using INH and Rifampicin, due to potential hepatotoxicity [9].

Rationale for treatment of tuberculosis, specifically presumptive treatment

Sustainable Development Goal 3.3 targets the elimination of tuberculosis (for 2020 and 2025) and targets (2030-2035) for the reduction of TB causes and death. So therefore an increased incidence of Multi-Drug Resistance TB is a cause for concern as it is a hindrance to achieve the SDGs.

Study area: This study has been conducted in one of the rural community development blocks of West Bengal. Factors drives the migration towards this risky profession. [so called push factor and pull factor for migration}. This fact has been acknowledged in DO letter of the Government of West Bengal

As per DO letter of West Bengal cite the following districts like

Purulia

Bankura

Rampurhat Health District

Birbhum

Jhargram

PaschimBardhaman

Above mentioed districts as source district

Basirhat Health District as prevalence district. The study has been conducted in one of the blocks of the prevalence district [10-12].

Objectives of the present study

- To find out among the radiologically confirmed silicosis cases how many have received presumptive treatment for tuberculosis
- Sputum-negative tuberculosis cases after having received presumptive treatment for tuberculosis how manyremained positive [point prevalence of this case}

- To find our INH and Rifampicin resistance among radiologically proven silicosis and who have received presumptive treatment

Methods

A cross-sectional study has been conducted among the residents of rural blocks who have attended health camps in the past 2 years. Health Camps are meant for affected workers to grade the severity of symptoms and provide compensation as per directives of the National Human Rights Commission.

Between the time of May 2023 to April 2024, monthly health cams have been organized

Health records if 54 people were examined and a structured questionnaire was administered to 54 people. Examination for the presence of tuberculosis has been done using microscopic sputum examination and Cartridge Based Nucleic Acid Amplification Test (CBNAAT).

Results

All the 54 patients are male subjects
Study period was last week of March to April 2024
Questionnaire administered and health records were surveyed.
Data analysis of the health records for having received presumptive treatment for tuberculosis have been examined.
Chest X Ray and spirometry were done in all cases and HRCT was done in select cases (physical records were assessed).

Results – All were male affected workers [May 2023- April].
Age range 27-55
Occupational History
History as has been obtained that these workers worked for a period of less than 10 years [range is 2 months to 10 years}. Only 2 worker have worked for more than 10 years. They gave a history of 12 years.

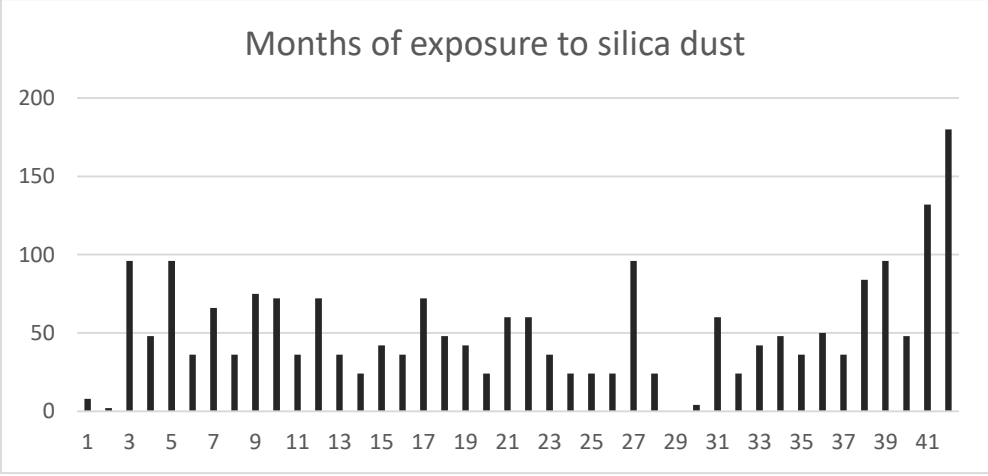


Fig. 1. Work duration is 10 or less in most cases except 2

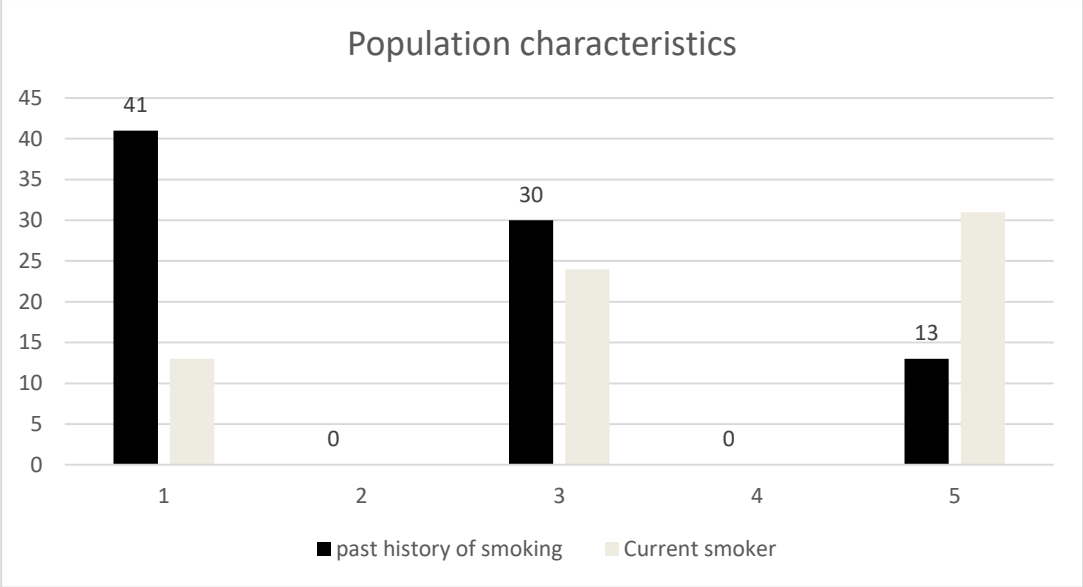
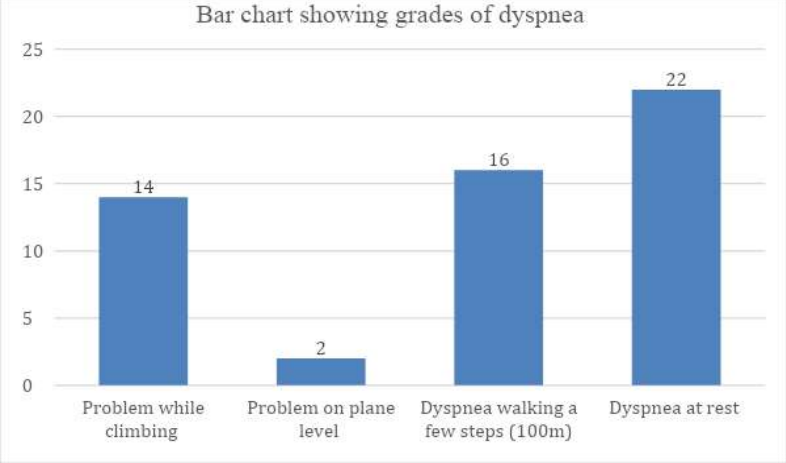


Fig.2. Key – 1. Past History of smoking 2, Complains of cough and 3. Past history of TB



Grades of dyspnoea

Fig. 3. Some chest X Ray findings are given below

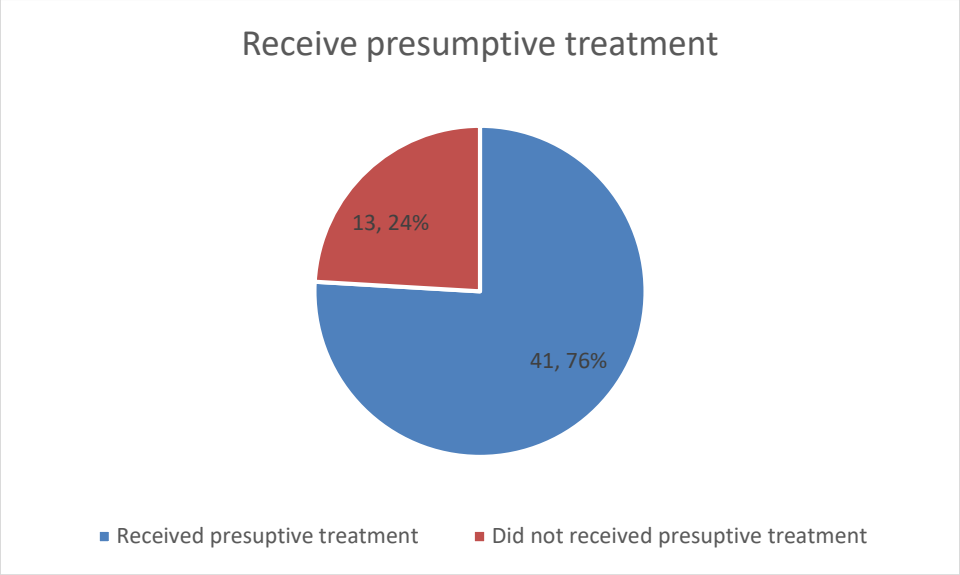


Fig.4. 41 people have presumptive treatment

Some Chest X Ray findings and HRCT findings are recorded in the table below

Case	**X- ray findings**	**HRCT Thorax finding**
Case study 1	Evidence of silicosis	Emphysematous change, ground glass opacities? infective changes
Case study 2	Patchy opacities ? Active Koch,s lesion	Chronic acute on chronic infective changes [CO RADS *
Case study 3	Normal findings	Normal findings
Case study 4	Calcified granuloma in right upper zone Bilateral prominent lung markings Bilateral hila prominent	Shows infective changes
Case study 5	Shows infective changes in the right upper zone	HRCT shows para septal emphysema at upper lobe with multiple fibrosis at right

		middle and lower lobe
Case study 6	Normal study	HRCT shows ground glass opacities lateral and postero basal segment of both lower lobe
Case study 7	Normal study	Fibrotic strands at posterior basal segment there is no bilateral thickening
Case study 8	Normal study	Tiny nodules at bilateral ling fields at both upper, middle lobe and lingular segment with fibrosis at bilateral upper lobe [likely infective changes}
Case study 9		HRCT no abnormality
Case study 10	X ray features shows calcified granuloma at lower zone Bilateral prominent lung marking Bilateral Hila are prominent	Para septal emphysema at both upper lobes with mild fibrosis at right middle and lower lobe
Case study 11	Ground glass opacities	Multifocal areas of consolidation, air space nodules and patchy areas of fibrosis at bilateral lung fields bilaterally infective changes
Case study 12	Post infective changes	multiple thick show fibro cavitary lesions and calcified nodules at bilateral lung fields show infective changes
Case study 13	X-ray features show evidence of silicosis	HRCT of thorax patchy consolidation with ground glass opacity mainly infective changes
Case study 14	X-ray features show evidence of silicosis	tiny nodule at bilateral lung fields more at upper lobe- likely infective changes
Case study 15	X-ray features show evidence of silicosis	HRCT of thorax multiple tiny nodules at bilateral lung fields more at both upper, middle, lower and singular segment with fibrosis at bilateral upper lobe- infective changes

Case study 16	X-ray features show evidence of silicosis	Infective changes
Case study 17	X-ray features show evidence of silicosis	HRCT findings show infective changes
Case study 18	X-ray features show evidence of silicosis	Infective Changes
Case study 19	X-ray features show evidence of silicosis	post-infective changes

X ray features mentioned are highly suggestive of silicosis and background lung infection.

Comments- Ground glass appearance in Chest X Ray highly suggestive of Silicosis.

Spirometry findings are not recordable in all cases

*The Dutch Radiological Society developed CO-RADS based on other efforts for standardisation, such as the Lung Imaging Reporting and Data System or Breast Imaging Reporting and Data System. CO-RADS assesses the suspicion for pulmonary involvement of COVID-19 on a scale from 1 (very low) to 5 (very high). Therefore, this asymptomatic for COVID-19 shows therefore

In this case report X ray plates could not be reprinted as they were deposited with the labour welfare department .

Most cases are acute and accelerated silicosis

After presumptive treatment all cases have become sputum negative for *Mycobacterium tuberculosis*

They are also proved by CBNAAT

Discussion

Silicosis, a lung disease caused by inhaling silica dust, significantly increases the risk of developing tuberculosis (TB). In cases where silicosis patients present with symptoms suggestive of TB, presumptive treatment is often initiated to reduce morbidity and mortality, even before a definitive TB diagnosis is confirmed.

Silicosis and tuberculosis are very common as explained previously once affected by silicosis the patients are said to be susceptible to pulmonary tuberculosis. The lifetime risk of developing pulmonary TB is three to four times more in countries like India and the risk of extra pulmonary tuberculosis can be very high.

Diagnosing silicosis can be intensely challenging

Clinical evaluation: Assess the patient's medical history, occupational exposure, and symptoms like cough, shortness of breath, and chest pain.

In chest X-ray one needs to look for nodular or reticulonodular patterns, pleural thickening, or calcification on such has been stated in the X-ray features.

High-Resolution Computed Tomography (HRCT): More sensitive than X-ray, HRCT can detect early silicosis changes.

Pulmonary Function Tests (PFTs): Measure lung function, including Forced Vital Capacity (FVC). Measuring Spirometry findings can be difficult as authors have observed that this operator-dependent and best of three readings are taken. When lung function deteriorates reading spirometry cannot be obtained. Diffusing Capacity of the Lungs for Carbon Monoxide (DLCO) is not done in health camp settings

Bronchoscopy with biopsy: If necessary, perform bronchoscopy with transbronchial biopsy to rule out other conditions. This is also not done.

Silica exposure assessment: Evaluate the patient's occupational history and silica.

Blood tests: Check for inflammatory markers, such as erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP).

All the cases were tested for Pulmonary Tuberculosis.

Silicosis manifests as a restrictive pattern of lung disease followed by an obstructive pattern

Presumptive anti-TB treatment is given for cases based on radiological and clinical findings. They are treated as sputum-negative tuberculosis

Those who recommend presumptive treatment say the benefit outweighs the risk.

When there is a risk of latent tuberculosis there are is options Tuberculin Skin Test (tuberculin skin test) and IGRA (International Gamma Release Assay).

CB NAAT has a high benefit for early and definite diagnosis of tuberculosis including MDR TB.

The treatment regimen for TB in silicosis patients typically follows the standard TB treatment guidelines, with careful monitoring due to the increased risk of adverse drug reactions.

Problems of silico tuberculosis are

X-Ray plate and Tuberculosis are deposited in the Labor Welfare department and are not available for comparison. Indication of presumptive treatment is high risk cases of tuberculosis specifically. After more than 1 month of presumptive anti-TB treatment all the tests negative for sputum by CBNAAT

The other confirmatory test like the Immunoglobulin Gamma Release Assay test (IGRA) has not been done in the present study.

The problem of doing an IGRA TB test is as follows

It is a blood test where there is a history of exposure to TB and there is a

History of closure to TB cases

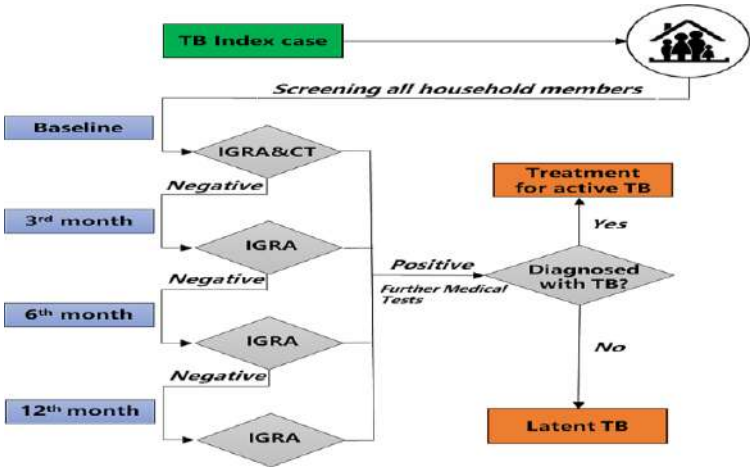
In TB-endemic areas With lung conditions

TB Blood test has been recommended in cases where patients have not been vaccinated for

- BCG (In India BCG vaccination is the norm due to institutional delivery)
- Cough
- Chest Pain
- Fatigue
- Lack of appetite

These are the most common mode of presentation and is an indication start of the presumptive start of anti-TB treatment

As per the National Tuberculosis Elimination Programme, there is necessary screening for close contact for all pulmonary tuberculosis cases as per the following algorithm



Algorithm for the diagnosis of index cases of tuberculosis

[sources: Surveillance of close contact with tuberculosis]

CBNAAT remains a more sensitive test compared with IGRA [9]

Conclusion

Silicosis patients is associated with associated with drug resistance of INH and Rifampicin. In the past 1 ½ hr year the cases of silicosis in the health camps no cases were found after a complete presumptive treatment of tuberculosis remains negative for mycobacterium tuberculosis

Silicosis has developed within less than 10 years of exposure. This signifies that these cases are acute and accelerated silicosis

No information regarding treatment for household contact of silicosis cases are there

Ethical clearance- It was obtained by NSHM Institute Ethics Committee

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Evolution of Hemophilia Treatments: Traditional Remedies to Cutting Edge Gene Therapy

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The disease, hemophilia, traces its origin to 200 BC in Talmudic writings as a bleeding disorder that runs in families. Its genetic connection was confirmed in the 19th century, establishing it as a sex-linked genetic disorder. This led to a classification of the disease as hemophilia A and hemophilia B caused by the deficiency of clotting factors FVIII and FIX, respectively. As the patients in the developed world were identified early and were privileged enough to get better healthcare, the evolutionary journey of prophylactic hemophilia care started quite early. Since the 1990s, it has come a long way from substitution therapy with coagulation factor concentrates to non-replacement therapy with proteins mimicking clotting factors or inhibiting anticoagulant proteins. In 2017, a milestone was achieved when Emicizumab was approved for the first non-replacement therapy for Hemophilia A patients, demonstrating significant prophylactic effect.

The post-2000s saw the introduction of cutting-edge gene therapy as a tool in hemophilia treatment, raising the hope for a sustained cure. In 2022, the FDA approved a gene therapy product, etranacogene dezaparvovec-drlb (trade name: Hemgenix®), for the treatment of hemophilia B. This review aims to provide a comprehensive picture of causes, prevalence, and various treatment modalities, with special emphasis on gene therapy.

Keywords: Hemophilia, Gene therapy, Emicizumab, Hemgenix, clotting factors

Introduction

Hemophilia is a global health issue. It is characterized by an inability to clot the blood due to a deficiency in coagulation Factor VIII(FVIII) (Hemophilia A or classic) or Factor IX (FIX) (Hemophilia B). It is also known as Christmas disease. Apart from the two major types, there is a third type too, known as acquired Haemophilia, which usually occurs as an outcome of immune disorders where autoantibodies are targeted against factors responsible for blood clotting—however, the non-genetic Hemophilia accounts for less than 1% of cases [1].

The mechanism of blood clotting

Haemophilia is a disorder of blood clotting. It is a complicated process involving multiple steps. It starts with a vascular injury that exposes the layers of blood vessels to flowing blood. When platelets come into contact with the damaged sub-endothelial connective tissue, they adhere to the site of injury, initiating a coagulation cascade that converts the plasma protein fibrinogen to insoluble fibrous strand fibrin. Blood cells get trapped in the fibrin network, and

a blood clot is formed, which prevents further bleeding. Two factors in this coagulation cascade, factors VIII and IX, play a crucial role in this event. Factor VIII, a serine protease synthesized in the liver, binds with factor IXa, which in turn activates factor X. A deficiency of factor VIII prevents the formation of complexes and results in hemophilia A. In hemophilia B, the production of factor IX is deficient. This is also known as Christmas disease.

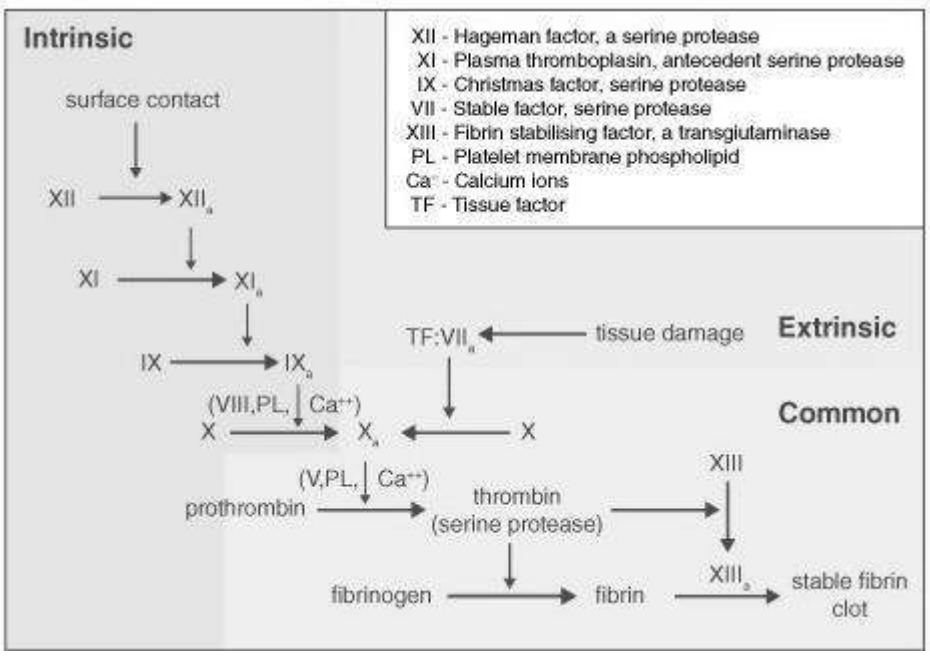

Figure 1: Coagulation cascade

Prevalence of hemophilia

Approximately 1 in 5,000 male births bear the genetic configuration of Hemophilia A. Another 1 in 20,000 male births is affected by hemophilia B. [2] The highest prevalence of the disease is reported in the developing world, which has better diagnostic centers and treatment options. Western Europe and America have the highest prevalence [3]. With increased awareness and reporting, the number of cases in Latin America and Asia has also increased. India is currently the home to the second largest population of hemophilia patients, with an estimated 1,36,000 individuals grappling with hemophilia. Nearly 80 percent of hemophilia cases are undiagnosed in India raising serious questions on the level of awareness. [4]

Brief history of hemophilia

The discovery of hemophilia as a sex-linked inherited disease, predominantly affecting males, was quite early. In the Talmud, the holy Jewish text, a "family disease" was mentioned where male children would bleed to death after circumcision. [5] The Talmudic description dates back to around 200 CE, but the scientific evidence linking hemophilia with genetics was only done in the 19th century when Dr. John Otto described a bleeding disorder affecting males in a Philadelphia medical journal in 1803. Later, Dr Christian Naegeli recognized the hereditary pattern of hemophilia. The name hemophilia was coined by Dr Armand Trousseau in 1876. In

1893, Dr. William Bulloch proposed that hemophilia is inherited through females, and Dr Lionel Penrose confirmed that hemophilia is a sex-linked recessive disorder in 1952.

The impact of the disease on human society

Before the cause of the disease was identified, the outcome of hemophilia was fatal. The afflicted persons were guaranteed to die a slow, premature death. Before the 1960s, the life expectancy of hemophilics was around 20–30 years due to limited treatment options. However, the introduction of clotting factors increased life expectancy to 40–50 years post-1960s. Post-1990, life expectancy increased further due to improved treatment. Finally, there is hope for a final cure with the advent of gene therapy.

Treatment modalities

The evolutionary journey of hemophilia A&B treatment started in 1950 with fresh frozen plasma (FFP)which contained small amounts of factors VIII and IX. This required large volumes and frequent hospitalization, however, these blood products were devoid of the required amount of proteins to stop bleeding.

The introduction of cold insoluble precipitates and lyophilized plasma-derived FVIII and FIX concentrates changed the scenario and made home treatment possible. However, their use was limited by blood-borne viral diseases due to a lack of viral inactivation in the manufacturing process. Fortunately, many blood products have been introduced over the years which improved patient quality of life. However, major improvement came from the availability of safer options for replacing missing clotting factors.

The cloning of FVIII and FIX genes enabled the development of virus-free recombinant FVIII and FIX concentrates which became the basis of prophylactic treatment for hemophilia in the late 1980s [6]. Long-term prophylactic treatment has proven beneficial over episodic treatment and is considered a standard strategy in thwarting life-threatening bleeds and joint damage. [7]

Broadly treatment therapies for hemophilia A&B are categorized as replacement therapies, non-replacement therapies, and Gene therapy

Replacement therapies:

Over the decades(1990s-2010s), advanced technologies have led to the development of unmodified recombinant products with standard half-life(SHL)and clotting factor concentrates with an extended half-life (EHL) which replace the deficient clotting factors.

EHL clotting factors are bioengineered molecules with a half-life (1.3X) times that of SHL FVIII and FIX concentrates. They were developed to improve the pharmacokinetic parameters, resulting in an enhanced half-life, thus reducing the frequency of IV injections and the financial burden on the prophylactic therapy (7,8,9,10).

EHL-recombinant FIX (EHL- rFIX) displays three to five times (3-5) longer half-life than SHL FIX concentrates. However, replacement therapies are limited by recurrent IV infusions which impose a high financial burden on prophylactic treatment.

Nearly 30% of Hemophilia A patients develop alloantibodies against administered factor FVIII and inactivate the procoagulant effect of infused factor VIII (11) Replacement therapy fails to address the challenging situation of patients with neutralizing anti-FVIII or anti-FIX antibodies which leads to frequent uncontrolled bleeding episodes.

Of late, a novel fusion protein BIVV001, a novel recombinant factor VIII (new EHL-rFVIII) has been developed for severe Hemophilia A with four times longer half-lives (15-19 hours) than SHL-FVIII products, which has been in phase III clinical trial till 2021. (12,13) In 2022, efanesoctocog alfa (BIVV001) was granted Breakthrough Therapy designation by the USFDA, and its development and commercialization were undertaken by pharmaceutical giants, Sanofi and Sobi ®. The phase 3 clinical data program results revealed its superiority over prior prophylactic FVIII replacement in terms of sustained protection and tolerance, with no FVIII inhibitor development over once weekly infusion. (14)

Non-replacement therapies

These therapies restore homeostasis by using mimetic products of clotting factors or products inhibiting the anticoagulant pathway. They are administered subcutaneously thus reducing the burden of IV infusion along with catering to the needs of younger patients and those with poor venous access. [13]

I] Mimetic Products

Mimetic products were developed to mimic rather than replace clotting factors This approach displayed significant success. Emcizumab (HEMLIBRA®) was the first approved non-replacement therapy approved for patients with or without inhibitors. It is a humanized bispecific monoclonal antibody (MoAb) with binding sites for factor IX and X and co-factor activity mimicking FVIII. It enhances the generation of thrombin, irrespective of inhibitor status. Being a substitute of FVIII, it is highly effective in Haemophilia A, with a longer half-life and good bioavailability with SC dosing every 1,2, or 4 weeks [15]. It's an effective and safe option for children and adults with Haemophilia A, with /without inhibitors, and is well endorsed by a series of clinical trial results. [16,17]

Nevertheless, its prophylactic use in mild, moderate, and acquired Hemophilia A patients is still under investigation. Current clinical trials are yet to answer the critical questions in situations where Emicizumab fails to fully substitute FVIII in the context of bone metabolism and health.

Newer activated FVIII mimetic bispecific antibodies, BS-027125(Bioverativ, Waltham, USA) and Mim8 (Novo Nordisk, Denmark) developed on similar lines have demonstrated promising potential even higher than Emicizumab in preclinical studies (18).

II] Inhibitors of Anticoagulant Pathways

This mode of therapy inhibits anticoagulant pathways in the absence of FVIIII/FIX and in the presence of inhibitors. Some of the products include:

a] Two monoclonal antibodies (concizumab & marstacimab), with anti-TFPI (tissue factor pathway inhibitors) activity, are currently in phase 3 clinical trial [13,19,20]. TFPI is an

anticoagulant protein that reversibly inhibits activated Factor X, subsequently inhibiting activated Factor VII and Tissue factor(TF).

b] Fitusiran, an investigational small interfering RNA(siRNA), administered through subcutaneous route, is currently in phase 3 trial. Being an inhibitor of antithrombin, it has shown hemostatic efficacy in both hemophilia A and B patients without inhibitors [21].

c] Serpin PC, a subcutaneously administered investigational biologic of the Serpin family of proteins, is a highly specific activated protein C inhibitor that enables more thrombin to be generated. This promising drug candidate for Hemophilia B patients is currently in phase 1/2a trial [13,22].

However, no licensed molecule has been approved yet in this category.

Gene therapy

Gene therapy aims to cure a genetic disease by modifying or replacing disease-causing genes and promoting healthy cellular function. In this therapy, healthy genes are introduced into the body so the deficient proteins leading to the disease can be produced within the body itself. [23].

Recombinant DNA technology and development of viral vectors laid the foundations of gene therapy during the 1970s-80s. The first gene therapy trial in the United States in the 1990s was a pivotal moment in clinical applications. A series of setbacks followed it; however, the efficacy of gene therapy was validated by the approval of over 20 products by 2023[24].

The procedure of inserting the genes into the body is complicated but can be broadly classified into two steps. The first step is the loading of genes into the carriers while the second step is to insert the gene-loaded carriers into a tissue that would provide the genes with an environment for successful expression [25].

For the first, usually, viral vectors like adeno-associated virus (AAAV), lentivirus, and retrovirus are used. These viruses are engineered to carry healthy genes and are introduced into tissues. Viruses infect the cells, deliver their payload, and direct the cell machinery to produce the intended protein [26]. Sometimes, healthy genes, loaded in plasmid DNA, are introduced into the body through special techniques like electroporation, lipofection, or microinjection. With the advancement of targeted drug delivery, dosage forms like liposomes (vesicles made of lipids) are also investigated to encapsulate the therapeutic genes. These vesicles can fuse with cell membranes, and the genetic material gets released inside [27]. Other promising techniques that have generated much attention are gene editing, stem cell transplantation, and ex vivo gene therapy.

The second challenge is successfully introducing vectors into the desired tissue or cells, overcoming biological barriers (e.g., blood-brain barrier, immune responses), and ensuring that the vectors reach the target cells and are taken up efficiently. For this, targeted delivery techniques are used [25,27].

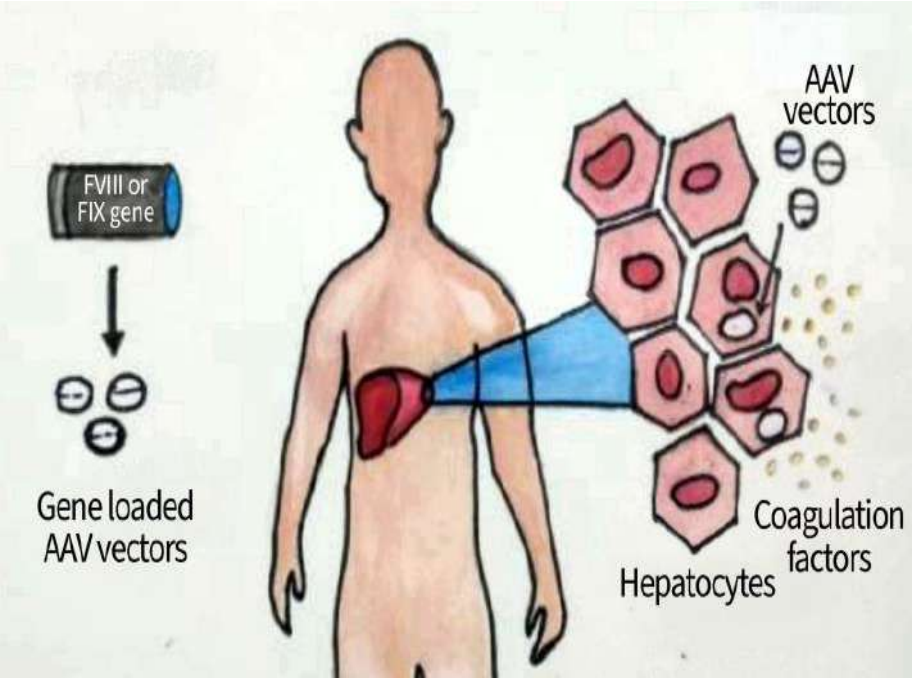

Figure 2: AAV vector-mediated gene therapy in Hemophilia

Gene therapy in hemophilia

In 2022, the FDA approved a gene therapy product called, etranacogene dezaparvovec-drlb (trade name: Hemgenix®) for the treatment of hemophilia B who are on Factor IX prophylactic therapy/have recurrent spontaneous bleeding episodes [28].

The liver produces both the clotting factors VIII and IX in large quantities. Hemgenix is administered as an intravenous infusion through a peripheral antecubital vein in the arm. From there, it reaches the liver via the hepatic artery and portal vein. It is administered via intravenous infusion to reach the liver cells, which normally produce Factor IX, the deficiency of which causes hemophilia B. This treatment has shown promising results in reducing symptoms and improving patients' quality of life. Hemgenix® uses an Adeno-Associated Virus (AAV8) as a vector to carry hF9, the functional Factor IX gene. The advantage of AAV8 is that it is a non-replicating virus and targets hepatocytes efficiently.

So far, the analysis of patients who used Hemgenix® is promising. Approximately 94% of patients remained free from continuous prophylactic treatment three years post-treatment, and 64% of the patients showed a reduction in the mean annualized bleeding rate during 7–36 months. However, cost remains the main deterrent to its widespread use. The price of a single dose of Hemgenix® is $3.5 million [29].

It is quite evident that, though the future seems bright for Hemophilia patients with the advent of gene therapy tools, it might not be affordable for all. Hence, future work involves continued research and development of more affordable and effective treatment options for hemophilia, including gene therapy and other novel approaches. Selection of the best treatment option suiting the needs, financial status, and availability of local treatment remains one of the recurrent challenges for hemophilia management.

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Awareness of Lactose Intolerance among People in the Age-Group 15 to 30 Years

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Abstract

Lactose Intolerance is a disorder in which the consumed lactose through dairy products after reaching into the small intestine is unable to get hydrolysed completely into simple carbohydrate sugars glucose and galactose due to the lack of enough lactase enzyme needed. The unhydrolysed lactose passes further into the intestine and causes symptoms like gas, bloating and diarrhoea, etc. This Lactose Intolerance is of three different types i.e. "Congenital lactase deficiency" which is by birth, "Primary Lactose Intolerance" or adult type lactase deficiency, "Secondary Lactase Deficiency". To identify the lactose intolerance in an individual diagnosis tests are available which are Gold Standard Test, Lactose Tolerant Test (LTT), Lactose Hydrogen Breath Test(HBT), and Quick Lactose Test(QLT). Lactose Intolerance management can be done through low lactose Dietary management with low lactose & alternatives of dairy foods and partially lactose fermented dairy foods, these dietary measures are also known as Personalized Nutrition (PN).The aim of this study is to do a small scale sample study on lactose intolerance awareness among youngsters based on gender. Method used was a descriptive cross section small scale study among youngsters of 15 to 30 years on gender basis in India. The fetching of data was done through pre structured questionnaires with online google form platform. In Demographical Data Age, gender, education, occupation was collected and in study's aim related data Cause of LI, discomfort after dairy consumption, dairy Alternatives required, and more were covered in the questionnaires. Result are as total 75 participant's response were collected out of which 28 were female and 47 were male and all were youngsters from 15 to 30 years of age. 42.7% male and 24% female responded no they are not suffering from LI, but rest of the 44 participants were either not sure and responded "maybe" or totally had no clue about LI and responded "don't know" and rest responded "yes". When asked about any LI symptoms they suffered 17(36.2%) out of 47 males and 11(39.3%) out of 28 females said they suffered the LI symptoms after consuming dairy foods. Conclusion came out through this study that one third individuals are suffering from LI discomfort after dairy consumption and they're not aware of it properly. They also have no proper clue about dairy alternatives, measures & causes, and other factors about LI. Therefore, awareness about lactose intolerance symptoms & alternative consumptions should be increased within youngsters through all possible means.

Keywords: Lactose Intolerance, Congenital lactase deficiency, Primary Lactose Intolerance, SecondaryLactase Deficiency, Personalized Nutrition

Introduction

The simple hexose carbohydrate sugars glucose and galactose binded together in nature are found as “lactose” in milk and milk products. The monosaccharides are hydrolysed by the brush-border enzyme lactase for the absorption of lactose sugar. The small intestine enterocyte’s microvilli contains this enzyme lactase [1].

Lactose intolerance is a disorder in digestion of in which some symptoms are seen such as bloating, diarrhoea, and gas after consuming food or drink that contains lactose such as curd, cheese, etc. Lactose malabsorption is a condition where small intestine is unable to break down all the lactose the individual eats or drinks. digestive symptoms are not experienced by everyone having lactose mal-absorbtion. Only people experienced with the symptoms are lactose intolerant. [2]

Hyperlactation and lactose malabsorption are a bit different as one is determined by estimating lactase enzyme levels in intestinal mucosa, the other one by an indirect assessment of the amount of lactose absorbed from the gut.so the result of incomplete digestion of lactose may be changes in time taken to pass through the wall(intestine) or other condition even with high lactase levels. [3]

The amount of lactose intolerant people Worldwide is said to be nearly 70 percent of the adult population.

Lactose intolerant people may substitute milk & its products with fermented milk products due to low lactose presence like curds due to beta-galactosidase in it or aged cheese which have lactose lower than normal cheeses. Some countries also have pre-digested milk or its products which the lactose intolerant people can eat.[4]

There are 3 different kinds of Lactose Intolerance:

***Congenital lactase deficiency (CLD)*:** is an exceedingly uncommon autosomal recessive illness that causes decreased or absent enzymatic activity since birth.

***Primary lactose intolerance*:** also known as adult-type lactase deficiency

***Secondary lactase deficiency*:** This temporary disorder is caused by intestinal injury and is secondary to a number of illness. [5]

When lactose is unable to get absorbed in the small intestineit passes through the gastrointestinal tract to the colon, it then leads to symptoms of lactose intolerance in the LI patients. Decrease of motility in gastro intestine is seen in some cases leadingto constipation from methane production. Then colonic fermentation of unabsorbed lactose by the bacterial microflora leads to the production of shortchain fatty acids, hydrogen, methane and carbon dioxide, which causes abdominal pain & bloating so the intra-colonic pressure & gut transit time increases. The unabsorbed lactose in the ileum and colon results acidification in the colon &increased osmotic load which leads to large amount of secretion of electrolytes and fluid and a rapid transit time. This results in loose stools & diarrhoea.[6]

Lactose intolerance can be diagnosed by a jejunal biopsy for the in vitro assessment of lactase activity, which is known as the **“gold standard”.**

Lactose Tolerance Test (LTT): This oral test consists the determination of blood glucose levelsthat follows the administration of an oral overload of lactose (25–50 g). [7]

Lactose Hydrogen Breath test (HBT): widely adopted for the detection of an increase in H2 in expired air (25–50 g). [8]

Quick LactoseTest (QLT): This test is based on a colorimetric reaction. [9]

There are several ways through which lactose intolerance can be managed:

***The standard restriction*:** standard restriction of milk and dairy products from the diet is the first and foremost best initiation. [9].

Here standard restriction is said because 12g of lactose i.e 1cup of milk can be consumed by a lactose intolerant individual at once or per meal with minimum or negligible feeling of discomfort [10]

Alternatives of milk and its products: is also a way that can be used as the management of lactose intolerance like soya milks, oat milk, cashew milk, almond milk, soya curds, groundnut curd, soya cheeses, etc.

***Milk products with low lactose content*:** These low lactose diets can also be used other than restricting milk & its products like fermented milk products i.e. curds, buttermilk or aged cheeses,etc.

The most common problem which the LI individuals can face by restricting or completely avoiding dairy foods is that they are deprived of minerals and nutrients that we get from milk and its products.

Important minerals like calcium, protein, magnesium, potassium, zinc, and phosphorous are found in greater amount in dairy products than any other typical food found in the adult diet. Availability and the relative low cost of dairy products makes their consumption more convenient. [11]

So nutrient supplements like calcium,vitamin d, potassium zinc, etccould also be consumed in the form of multi vitamins & mineral tablets or nutraceuticals along with alternatives of milk products

***Drug therapy*:** Enzyme supplementation therapy with lactase from nonhuman sources to hydrolyze lactose is another important approach. Exogen lactase is obtained from Aspergillus oryzae (Lacdigest,Italchimici, Pomezia, Rome, Italy) or from Kluyveromyceslactis (Silact, Sofar, Trezzano, Milano, Italy) are able to break down lactose into glucose and galactose to allow a better absorption [9]

Lactose Intolerance is a disorder which is not known very well among people in developing countries and its causes and symptoms are not paid much attention and goes on unidentified. But it can still cause health issues and certain nutritional lack in the body like lack of calcium & other minerals and if kept ignored may result into weakening of bones and other health issues.

The objectives of this study is:

- To conduct a study on the awareness level among young individual of age group 15 to 30 years

- To collect the data on gender basis comparison about the awareness of Lactose Intolerance

Methodology

To conduct this survey following method was used:

Section of the Area & Sample

These following areas were chosen for conducting the study about Lactose Intolerance among genders of age-group 15 to 30 years and its awareness,

- Bariatu area; METAS Adventist School, Ranchi; Khiddirpur&Mominpur, Kolkata; NSHM Knowledge Campus, Kolkata

***Including criteria*:** Gender male & female, Age-group from 15 to 30 years of age.

***Excluding criteria*:** Particularly younger than 15 years and older than 30 years, Pregnant mother, breast feeding women, and other chronic illness.

Study Design

This was a cross-sectional study conducted from SEPTEMBER to NOVEMBER using online pre structured questionnaires. The questionnairewas distributed through google form format to the different participants.

A total of 75 individuals were made to attend the online questionaire form out of which 28 were female and 47 were male.where12 were secondary School 9^{th}& 10^{th} students among which 9 were male and 3 were female, 10 were Senior secondary students from METAS adventist school out of which 5 were male and 5 were female, 34 were graduation levelindividuals out of which 25 were male and 9 were female, 19 were post graduation level individuals ot of which 8 were male and 11 were female individuals mostlyfrom Masters department NSHM Knowledge Campus in Kolkata.

After data were extracted, it was rechecked, coded and then descriptive analysis & cross tabulations were done through SPSS application.The lactose intolerance questionnaires were a cross-sectional online pre-structured questions using google form.

Demographic Data

The data included in it were as follows; AGE from 15 to 30 years, GENDER (male & female), EDUCATION (i.e. secondary,senior secondary, graduate & post graduate), OCCUPATION (i.e. Employed, Unemployed and Student) of the participants.

Results

Demographical Data

Table 1. Demographical data of the sample (n=75).

DEMOGRAPHICAL CHARACTERISTIC	FREQUENCY	PERCENT (%)
AGE		
15-20y	23	30.7%
21-25y	32	42.7%
26-30y	20	26.7%
GENDER		
M	47	62.7%
F	28	37.3%
EDUCATION		
Secondary School 9^{th} 10^{th}	12	16.0%
Senior Secondary	10	13.3%
Graduation	34	45.3%
Post Graduation	19	25.3%
OCCUPATION		
Employed	26	34.7%
Unemployed	06	8.0%
Student	43	57.3%

The total sample size was 75. Table.1, shows the demographical data of the participants. Among the data about the age group of the sample 30.7% of the total participants n=23 were 15- 20y, 42.7% n=32 were 21- 25y and 26.7% n=20 were 26- 30years of age.

Within the gender data of this sample 62% n=47 were male and 37.3 were female participants.

The data collected on the education of the sample 16% n=12 were secondary school students, 13% n=10 were senior secondary school students, 45.3% n=34 were graduation level and 25.3% n=19 were post graduation level participants.

The data collected on the occupation of the sample 57.3% n=43 were students, 34.7% n=26 were Employed and 8% n=06 were Unemployed.

Lactose Awareness Response

Table 2. Participants aware about lactose intolerance

DEMOGRAPHICAL CHARACTERISTIC	FREQUENCY		PERCENT (%)	
LI AWARE				
YES	62		82.7%	
NO	13		17.3%	
GENDER	YES	NO	Yes	No
MALE	39	8	52%	10.7%
FEMALE	23	5	30.7%	06.6%

In the table 2 above, the demographical data shows the number of participants who know or are aware about lactose intolerance disorder in the survey. Out of the total sample n=75, 82% i.e. n=62 said "YES" they are aware about LI disorder and 12% n=13 said "NO" they are not aware about LI disorder.

Further below in the same table data about gender on LI awareness shows that out of total participants 52% n=39 were male who said "yes" and 10.7% n=8 were male who said "no" while 30.7% n=23 were female who said "yes" and 06.6% n=5 were female who said "no".

Table 3. Participants suffering with lactose intolerance

SUFFERING WITH LI	Gender			
	Male	%	Female	%
yes	04	5.3%	04	5.3%
no	32	42.7%	18	24%
maybe	03	04%	03	04%
don't know	08	10.7%	03	04%

The table 3 above shows the data on gender basis response about participants suffering from lactose intolerance where in this collected data out of total participants 5.3% n=4 male and also 5.3% n=4 female said "yes" they suffer from lactose intolerance while 42.7% n=32 male and 24% n=18 female said "no" they don't suffer from lactose intolerance. 4% n=3 male and 4% n=3 female are not sure and said "maybe" while 10% n=08 male and 04% n=3 female are unaware about LI and said "don't know".

Table 4. Participants feeling discomfort after dairy consumption

		DISCOMFORT AFTER DAIRY CONSUMPTION			
		yes	**no**	**maybe**	**TOTAL**
gender	**Male**	11	33	3	47
	Female	5	20	3	28
Total		16	53	6	75

In the above table 4, it shows response data of the participants on feeling discomfort after consuming dairy food based on gender. Here the collected data shows that out of total participants(n=75), 16 responded “YES” among which 11 were males and 5 were females claimed they feel discomfort after dairy consumption. 53 participants said “NO” among which 33 were males and 20 were females responded they don’t feel any discomfort after dairy consumption, and 6 participants chose “MAYBE” among which 3 were males 3 were females responded they aren't aware and not sure whether they suffer from LI or not.

Table 5. Participants aware about LI causes

		CAUSE OF LACTOSE AWARENESS				
		After GIT disorders/infections	After GIT surgery	after using some drug	don't know	Total
gender	**Male**	9	2	3	33	47
	Female	10	0	0	18	28
Total		19	2	3	51	75

In the table 5, above it is shown through the data based on gender about the awareness level of the participants on the cause of LI. Among the total participants (n=75), 51 participants responded “Don’t Know” about the cause of LI among which 33 were male and 18 were female, 03 participants responded “After using some Drugs” in which 3 were males and none were females, 02 participants responded “After GIT Surgery” out of which 2 were male and none were female, 19 participants responded “After GIT disorder/infections” among which 09 were male and 10 were female participants.

Table 6. Dairy alternatives intake requirement for LI disorder

		Dairy Alternatives Intake Required		Total
		Yes	No	
gender	Male	20	27	47
	Female	18	10	28
Total		38	37	75

The table 6 above shows response data of the participants on gender basis about Dairy alternatives intake requirement for LI patients. Among the total participants (n=75), 38 participants responded "Yes" Dairy Alternatives intake is required by the LI patients out of which 20 were male and 18 were female participants. While 37 participants responded "No" Dairy Alternatives intake is not required where 27 were male and 10 were female.

Table 7. Lactose free dairy alternative nutritional decrease response sample

		Lactose Free Products Nutritional Decrease		Total
		Yes	No	
Gender	Male	23	24	47
	Female	10	18	28
Total		33	42	75

The table 7 above shows data response of the participants sample on basis of gender about Lactose free alternatives nutritional decrease. Here the collected data response shows that out of total participants (n=75), 33 responses were "Yes" Lactose free products have nutritional decrease among which 23 were male and 10 were female participants. 42 responses said "No" Lactose free Alternatives nutritional value doesn't decrease among which 24 were male and 18 were female participants.

Table 8. Lactose intolerance symptoms suffered by participants

		Gender		Total
		Male	Female	
LI Symptoms Suffered	Abdominal Distension/ Gas	6	2	8
	Cramp/ Abdominal Pain	0	1	1
	Constipation	4	0	4
	Nausea/ Indigestion	0	1	1
	Vomitting	2	1	3
	Multiple Symptoms	5	6	11
	None	30	17	47
Total		47	28	75

This cross-sectional study conducted among 15 to 30 years' adult's male and female both gender included in survey, analyzed the level of awareness about lactose intolerance disorder.

Among the total participants (n=75), 8 participants suffered with abdominal distress/ gas where 6 were male and 2 were female, 1 participant felt cramp/ abdominal pain were male and none were female, 4 participants felt constipation out of which 4 were male and none were female, 1 participant felt nausea/Indigestion and none were male, 3 participants felt vomiting out of which 2 were male and 1 were female, 11 participants felt multiple symptoms out of which 5 were male and 6 were female, 47 participants felt none of the symptoms out of which 30 were male and 17 were female.

Table 9. Response on milk allergy and lactose intolerance are same

		Milk Allergy And LI Are Same		
		Yes	No	Total
Gender	Male	17	30	47
	Female	9	19	28
Total		26	49	75

The table 9 above shows the data based on gender of participants' awareness about the difference in milk allergy and Lactose Intolerance. In the data 17 out of 47 males and 09 out of 28 females responded "Yes" milk allergy and LI are same while 30 out of 47 males and 19 out of 28 females responded "N0" milk allergy and LI are not same.

This cross-sectional study conducted among 15 to 30 years' adult's male and female both gender included. In my survey I analyzed the level of awareness about lactose intolerance disorder.

According to the data collected from participants 52% male and 30.7% female claimed they know or are aware about Lactose intolerance disorder but when asked about the cause of LI 33(70.2%) males out of 47 and 18(64.3%) females out of 28 responded they "don't know".

In another data response, 42.7% male & 24% female claimed they are not suffering from LI disorder. When asked about any LI symptoms they suffered 17(36.2%) out of 47 male and 11(39.3%) out of 28 females said they suffered the LI symptoms after consuming dairy foods.

In a response data about nutritional decrease in lactose free products, 23(48.9%) out of 47 male and 10(35.7%) out of 28 female responded "Yes" nutrition decreases while 24(51.1%) out of 47 males and 18(64.3%) out of 28 females responded "No" nutrition doesn't decrease.

In the analysis of data collected about dairy alternatives intake 24(out of 47 male participants and 18 out of 28 female participants responded "No" alternatives of dairy food is not required during Lactose Intolerance

According to the analysis no association (p=0.92) were found between a gender and individuals with LI awareness.

Conclusion

It is concluded through this study that one third individuals are suffering from LI discomfort after dairy consumption and they're not aware of it properly.They also have no proper clue about dairy alternatives, measures & causes, and other factors about LI.About what is Lactose intolerance and around thirdof theparticipants aren't even aware about the symptoms to identify lactose intolerance.

51.1% out of total males and 64.3% out of total female participants did not even know that as compared to dairyfoods alternatives have decreased nutritional value.

Awareness about alternatives consumption is also lower than averageas 56% participants (24 males & 18 females) don't even know about alternatives of dairy products is required for LI suffering individuals.

Therefore, awareness about lactose intolerance symptoms & alternative consumptions should be increased within youngsters through all possible means.

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To Examine Self-esteem, Life Satisfaction, and Interpersonal Relationships with the Spouse of Middle Aged Mothers who are Living with or without Their Children

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Abstract

The current study aims to examine whether self-esteem, life satisfaction, and relationship with spouse differ among mothers who are living with their children currently and mothers who are living without children currently. For the current study, (N=60) consists of 30 mothers who currently live with children and 30 mothers who currently live without their children (age range between 45 to 64). Appropriate statistics were computed. Results showed that mothers who live with their children show higher self-esteem and better relationships with their spouse in comparison to mothers who live without their children. Results also revealed that self-esteem and relationship with spouse are both significant predictors of life satisfaction for mothers who live with their children and relationship with spouse is a significant predictor of life satisfaction in mothers who live without their children. These results were interpreted accordingly.

Keywords: Mother Living with Children, Mothers Living without Children, Self-Esteem, Life Satisfaction, Relationship with Spouse.

Introduction

The bond a person has with their mother is among the most important and defining relationships in their lives. Mothers have a major impact on children's physical and emotional development. They typically take on the role of primary caregiver, giving the child the support, love, and attention they require to thrive. As their children develop and are born, mothers assume new duties, such as nurturing, educator, and caregiver. Mothers encourage their children to grow into strong, self-assured adults by providing them with a sense of protection, comfort, and love [1]. Self-esteem refers to the overall worth of a person, which comprises a person's feelings, perceptions, and attitudes towards him or herself [2].It is a basic expectation in our psychological security and has an extremely viable impact on thought processes, behaviors, and interactions with other people. Self-esteem is the esteem that an individual has for himself, his value, and his ability [3].Mothers who live with their children can exhibit varying levels of self-esteem, which can have implications for both the mothers themselves and their children. Maternal self-esteem plays a crucial role in maternal adaptation, psychological well-being, and parenting behaviors. High self-esteem in mothers has been associated with positive attitudes toward their children, effective parenting even in stressful environments, and better parent-child interactions [4].Satisfaction with life is a person's attitude towards life, based on the standards that they prefer to use to judge the quality of

life. They mean a more general ‘life satisfaction’ that is not tied to some particular sphere of life, such as a job or health. It involves components of the lives, such as personal happiness, social relationships, accomplishment, and personal worth and objective. Numerous factors contribute to life satisfaction, each playing a varying role depending on personal circumstances and values: Health, Relationships, Economic Stability, Personal Achievement, Work-Life Balance, Community, and Environment [5] There are a variety of elements that might impact the amount of life satisfaction experienced by mothers who live with their children. Mothers' contentment with their home life is a key mediator in the connection between their work-life equilibrium and the life happiness of their offspring [6]. Mothers' overall life happiness influences the life contentment of their adult children independently, however, this impact gradually fades [7]. In its turn, life satisfaction seems to be an instance of subjective well-being, stressing the part of it that is deliberately conscious, volitional, and rational, and supposing that people consciously make judgments about their life, regarding it as satisfactory or not. Life satisfaction is crucial for several reasons- Mental Health, Physical Health, Productivity and Success, and Resilience. Interpersonal relationship between a spouse particularly the mother heading their families ages between 45-64 years entails a strong bond, which is an unending, evolving, special, personal, and most importantly non-business-like partnership, which has encompassed several stages in the lives of the two partners involved [8]. Several factors influence the dynamics of the relationship Emotional Connection, Communication, Mutual Respect, Shared Responsibilities, Intimacy, and Conflict Resolution, along with other factors like Empty Nest Syndrome, Career and Retirement, Health Issues, and Parental Care [9]. Lack of family cohesion is another area of concern regarding the importance of a healthy relationship; for people who have strained relationships with their families. Maintaining a healthy relationship with a spouse during this stage is critical for several reasons like Emotional Well-being, Physical Health, and Social Support [10]. Evaluations reveal that self-esteem is a determinant factor in mental health and physical health at large [11]. Children grow older and are more competent and less in need of parenting from their families especially after they have moved out of their childhood homes. The change could call for an alteration of the role one occupies within a social network, which then affects self-esteem. This period usually implicates the time of reflecting the personal or professional development. Positive experiences in the course of achievement can enhance self-confidence levels while the perceived failures in the same process can lower self-confidence [12]All health issues can alter self-image and self-esteem due to aging and other related health changes [13]. This knowledge is useful in attending to the needs of this age group as seen from the biological and psychosocial factors impacting the elderly [14]. Life satisfaction, as a holistic measure of well-being, provides insight into how individuals evaluate their overall life quality[15]. It is impacted by such transitions as children leaving their homes; one experiences joy the other develops mixed feelings [16].There is also evidence that people who enjoy high levels of life satisfaction have plenty of encouraging relationships [17,18].Gaining knowledge about their psychological and relational processes at this developmental phase could be beneficial for both mental health professions, policymaking and social sciences. The present study explores the midlife concept as a time of health and identity transition to promote well-being.

Methodology

The current study aims to examine the self-esteem, life satisfaction, and interpersonal relationship with the spouse of mothers aged between 45 to 64 years who are living with and without their children and whether there exists a correlation between these three variables in the two groups.

Objectives

- To find out if mothers living with children and without children differ concerning
  -Self-esteem

  -Life satisfaction

  -Relationship with spouse

2. To find out if there is any correlation between self-esteem, life satisfaction, and relationship with spouse in

   a. Mothers living with children
   b. Mothers living without children

Hypothesis

Ho1: There is no significant difference in self-esteem, life satisfaction, and relationship with spouses in mothers who are living with their children and mothers who are not living with their children.

Ho2: There is no significant correlation between self-esteem, life satisfaction, and interpersonal relationship with the spouse in mothers living with children.

Ho3: There is no significant correlation between self-esteem, life satisfaction, and interpersonal relationship with the spouse in mothers living without children.

Research Design

This study's research design was quantitative. According to Kerlinger and Lee (2000), "Quantitative research is deductive in nature, and researchers draw conclusions based on direct observations with the main objective to describe cause and effect." Quantitative research's main objective is to quantify the data and make generalizations that can be applied to a larger population. This kind of research seeks to evaluate and classify a problem.

Sampling technique:

Purposive sampling was used for the current study.

Sample:

For the current study, (N = 60) consisted of 30 mothers who live with their children currently and 30 mothers who don't live with their children currently (age range between 45 to 64 years).

Inclusion Criteria

- 45 to 64 years' mothers
- Mothers living with their spouse
- Mothers living with children
- Mothers living without children
- Minimum education level of 12th pass
- Both working and non-working mothers

Exclusion criteria

- Mothers who are separated from their spouse
- Widowed mothers
- Mothers whose children have died
- Mothers whose children are married
- History of significant medical and psychiatric illnesses

Tools used

- **Information Tool**: Demographic questions about the questions we asked at the beginning of the survey, which include their age, family type, residence type, Marital status, number of children, and whether they lived with or without their children and spouse.
- **Rosenberg Self-Esteem Scale:** The Rosenberg Self-Esteem Scale is one of the easily accessiblemeasuring tools intended for assessing the level of self-esteem. Created by Morris Rosenberg in 1965, it is a self-report, ten-item survey in which individuals respond to statements in a four-point, from strongly agree to strongly disagree. The items 1, 2, 4, 6, and 7 the scores are givenas such: Strongly agree = 3, Agree = 2, Disagree = 1, Strongly disagree = 0. For items 3, 5, 8, 9, and 10 (which are reversed in valence): Strongly agree = 0, Agree = 1, Disagree = 2, Strongly disagree = 3. For the Rosenberg Self-Esteem Scale (RSES), Cronbach's alpha coefficient hasbeen reported to range from **0.72 to 0.87**.
- **Satisfaction with Life Scale:** Created by Ed Diener and his colleagues in 1985. A brief self-report survey that has five statements or questions that begin with "strongly disagree" and ends with "strongly agree" (7 - Strongly agree, 6 - Agree, 5 - Slightly agree, 4 - Neither agree nor disagree, 3 - Slightly disagree, 2 - Disagree, 1 - Strongly disagree). The scoring is to be done by summing up the scores on each item. The scores range from 5 to 35 (31 - 35 Extremely satisfied, 26 - 30 Satisfied, 21 - 25 Slightly satisfied, 20 Neutral, 15 - 19 Slightly dissatisfied, 10 - 14 Dissatisfied, 5 - 9 Extremely dissatisfied). Alpha coefficients for the SWLS typically range from **0.80 to 0.90,** suggesting that the items on the scale consistently measure the same underlying construct of life satisfaction.
- **Relationship Assessment Scale**: Constructed by Andrew Christensen and Neil S. Jacobson in 1992. Each of the statements in the survey is usually scored on the respondent's level of agreeableness using a scale that may range from strongly disagree to strongly agree. Scoring is kept continuous. The higher the score, the more satisfied the respondent is with his/her relationship. Items 4 and 7 are reverse-scored. Alpha coefficients ranging from **0.80 to 0.95**

have been reported in various studies, suggesting that the items on the RAS consistently measure the same underlying construct of relationship satisfaction.

Procedure

The participants were selected as per the inclusion and exclusion criteria. After debriefing them regarding the nature of the study, and after making sure all the participants consented to participation in the study, they were administered the above-mentioned scales. The scales were scored as per standard procedures and the data was subjected to appropriate statistical analysis. From the obtained results the appropriate interpretation was drawn and finally, a conclusion was made concerning the findings.

Results

Statistical analysis was done using SPSS16. T-test results in table1 revealed Mothers living with children have higher self-esteem and also share a better relationship with their spouse than mothers who don't live with their children currently. Results also revealed a positive correlation between self- esteem, life satisfaction, and relationships with spouse of mothers who live with their children in table 2 and those mothers who don't in table 3. Regression analysis revealed relationship with spouse is a significant predictor for life satisfaction in mothers living with children in table 4 and mothers living without children in table 5.

Discussion

Mothers who live with their children are generally seen to work with their spouses as a team in taking care of said children, which strengthens bonds between spouses as it focuses on teamwork and motivates them to share a cooperative dynamic for the sake of the children. Cohabiting mothers are on most occasions close to kids and they have the status of high access to social support from other family members and other parents. Thus, friends and other relationships serve as a protection against stress and conflict in a marital connection and contribute to marital happiness [19].

There is no significant difference in life satisfaction between mothers who live with their children and those who do not. Life satisfaction among mothers is influenced by various factors, among which the quality of the spousal relationship is paramount. The correlation between self-esteem, life satisfaction, and relationships with spouses among mothers is a subject of significant interest in psychological and social research. In this study it was found that these three factors are positively correlated, meaning that improvements in one area often correspond to improvements in the others.

The quality of a marital relationship significantly impacts life satisfaction. For mothers, having a reliable partner can alleviate the stresses associated with parenting and household responsibilities, thereby enhancing their overall well-being [20].

Self-esteem influences relationship dynamics, highlighting that individuals with higher self-esteem tend to perceive their partners more positively and report greater relationship satisfaction[21]. The interconnected nature of self-esteem, life satisfaction, and spousal relationships suggests a cyclical reinforcement where each factor positively influences the others.

Mothers with high self-esteem are likely to feel more competent and valued in their roles, which enhances their overall life satisfaction. The relationship with a spouse and self-esteem are not isolated predictors of life satisfaction. Conversely, high self-esteem can lead to more effective communication and conflict resolution within the marriage, further improving the quality of the spousal relationship The relationship with their spouse plays a particularly critical role in determining overall life satisfaction. The spousal relationship provides a fundamental source of emotional and practical support, which is crucial for life satisfaction. In addition to emotional support, practical support from a spouse plays a vital role in life satisfaction [22].

Conclusion

In summary, it is found that mothers living with their children have a better relationship with their spouses and have higher self-esteem due to the feeling of shared responsibility, emotional bonding, sense of fulfillment and achievement. Living with children also significantly impacts maternal self-esteem, with various factors contributing to this relationship. A sense of purpose, social support, emotional attachment, and parenting accomplishments play crucial roles in shaping maternal self-esteem. These factors collectively help maintain stable life satisfaction across different living arrangements. The positive correlations between self-esteem, life satisfaction, and relationships with spouses underscore the importance of a holistic approach to enhancing the well-being of mothers. This interconnectedness highlights the need for comprehensive support systems that address multiple dimensions of maternal well-being.

The quality of the relationship with a spouse and self-esteem are critical predictors of life satisfaction for mothers living with children. The quality of the spousal relationship is also a significant predictor of life satisfaction for mothers living without their children. Emotional support, companionship, practical assistance, and the ability to buffer against stress and adversity are key factors that contribute to this relationship's importance. Understanding and nurturing these aspects can lead to improved life satisfaction and overall well-being for these mothers.

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Result Table

Table 1. Independent sample t-test showing self-esteem, life satisfaction, and relationship withspouse of mothers living with and without children

	T value	Df	significance
Self-esteem	1.948	58	0.05
Life satisfaction	1.512	58	>0.05
Relationship with spouse	2.042	58	<0.05

Table 2. The table shows correlations between self-esteem, life satisfaction, and relationship withspouse for mothers who live with their children

	Self-esteem	Life Satisfaction	Relationship with Spouse
Self-esteem			
Pearson Correlation	1	0.890**	0.582**
Sig (2-tailed)		0.000	0.001
N	30	30	30
Life Satisfaction			
Pearson Correlation	0.890**	1	0.632**
Sig (2-tailed)	0.000		0.000
N	30	30	30
Relationship with Spouse			
Pearson Correlation	0.582**	0.632**	1
Sig (2-tailed)	0.001	0.000	
N	30	30	30

** Correlation is significant at 0.01 level (2 -tailed)

Table 3. Table shows correlations between self-esteem, life satisfaction and relationship with spouse for mothers who live without their children

	Self-esteem	Life Satisfaction	Relationship with Spouse
Self-esteem			
Pearson Correlation	1	0.819**	0.626**
Sig (2-tailed)		0.000	0.000
N	30	30	30
Life Satisfaction			
Pearson Correlation	0.819**	1	0.785**
Sig (2-tailed)	0.000		0.000
N	30	30	30
Relationship with Spouse			
Pearson Correlation	0.626**	0.785**	1
Sig (2-tailed)	0.000	0.000	
N	30	30	30

** Correlation is significant at 0.01 level (2-tailed)

Table 4. Regression table showing predictor of Life Satisfaction (LS) of mothers living with children

Predictors	R square	Beta	Significant level
Relationship with spouse and Self-esteem	0.778	0.447	<0.01

Table 5. Predictor of Life Satisfaction (LS) of mothers living without children using regression

Predictor	R square	Beta	Significant level
Relationship with spouse	0.399	0.632	<0.01

Curcuma longa and *Beta vulgaris* as Colouring Agents in Pharmaceuticals

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Abstract

Natural pigments have been gaining popularity as colouring agents since the last few decades due to several benefits like safety, biocompatibility, biodegradability, etc. However challenges remain with respect to colour stability and pigmentation. Several natural resources have been identified including flowering and fruit bearing plants. *Curcuma longa* and *Beta vulgaris* are one of the most popular resources for extraction of natural colouring pigments from plants. The following account describes them in detail.

Keywords: Natural pigments, *Curcuma longa, Beta vulgaris,* Biocompatible

Introduction

Pigments extracted from natural resources have several advantages with respect to synthetic pigments, lakes and dyes. Synthetic dyes in higher concentrations show toxicity and thus should be avoided in food and pharmaceuticals. Thus the use of natural pigments is on the rise. Several resources have been used specifically the flowering plants, and fruit bearing plants and leaves. The following section gives an account on two of the most popularly used natural resources for extraction of colouring pigments.

Curcumin

Curcumin, extracted from rhizome part of *Curcuma longa*, coloured yellow, is one of the most commonly used natural pigments and is called turmeric. It has been used for centuries in cosmetics, medicine and cuisines as a natural pigment [1].

***Synonyms*:** Turmeric yellow

Biological source*:Curcuma longa* from Zingiberaceae family [2].

***Chemical composition of curcumin*:** Commercial curcumin is typically a mixture of three main curcuminoids, each contributing to its overall properties and colour. The composition of commercial curcumin is usually: curcumin (77%), demethoxy curcumin (18%), and bisdemethoxy curcumin (3%) [3].

***Uses of curcumin*:**

- Curcumin, derived from turmeric, is a popular natural food dye known for its vibrant yellow colour. These pigments serve as alternatives to synthetic dyes such as tartrazine, and used cuisines for colouring and flavouring purposes [4].

- Curcumin is utilized as a natural dye in the textile sector. It adds a bright yellow colour to fabrics and is commonly used in eco-friendly and sustainable clothes [5].
- Curcumin can be used in natural hair dyes to achieve a yellow or golden tint, often in combination with other herbal ingredients.
- In the cosmetic industry, curcumin is used to add colour to various products such as creams, lotions, and face masks. Its natural origin makes it a preferred choice for herbal and natural cosmetics [6].
- Curcumin is sometimes used to colour pharmaceutical products, including capsules and syrups, due to its non-toxic nature and health benefits.

Studies based on curcumin extraction and use as natural pigment

Kim H. J.et al. (2013) extracted curcumin dye from *Curcuma longa* L for use as a sensitizer in dye-sensitized solar cells (DSSCs). Curcumin, a natural colour was isolated from *Curcuma longa L.* using a simple extraction method. It was extracted from commercially obtained turmeric powder derived from the ground rhizome of Curcuma longa L. The sample was dissolved in ethanol, filtered to remove solid residues, and used directly as a dye without further purification.The dye was utilized as a sensitiser in a dye-sensitized solar cell (DSSC) and its properties were investigated.Acetic, nitric, and hydrochloric acids were used to stabilize the dye and improve conversion efficiency. The acetic acid-treated curcumin dye had a current conversion efficiency of 0.60%, which was twice as high as the nitric and hydrochloric acid-treated dyes. Acetic acid increases the conversion efficiency of curcumin colour slightly, while nitric and hydrochloric acids reduce it [7].

Nandiyanto, A. B. D. et al. (2017) aimed to show a simple method for extraction ofcurcumin pigment from Indonesian turmeric and analyzed its infrared spectra and thermal decomposition properties.This procedure involved rinsing the turmeric, dissolving it in an ethanol solution, and placed in a rotary evaporator to increase the concentration of curcumin.The outcome demonstrated that the current approach is successful in separating the curcumin component from Indonesian local turmeric. This method can be utilized for home industrial applications as it is very simple process. Furthermore, understanding of curcumin's thermal degradation characteristics is useful in choosing an approach when curcumin is subjected to a thermal-related process [8].

Rouhani, S. H. et al (2009) used ultrasonic-assisted extraction technique to extract the Curcuma Longa L rhizomes.Ultrasound-assisted extraction (UAE) was found to be a more efficient approach for extracting curcuminoids from turmeric plant rhizomes compared to traditional methods. The turmeric samples were subjected to indirect sonication in an ultrasonic bath and the results were compared with those from conventional extraction methods. It was discovered that the UAE method yielded approximately three times greater than that of the conventional technique.The impact of pH, extraction time,and solvent composition on the yield of curcuminoids extracted from turmeric was investigated using the Taguchi experimental design.The optimal conditions were identified as a solvent composition of 70:30 ethanol/water (V), pH of 3, and an extraction period of 15 minutes. High-performance liquid chromatography (HPLC) was used to analyze the extracts [9].

Liu, Y. et al (2019) evaluated natural deep eutectic solvents (NADESs) composed of organic acids and sugars for their ability to extract curcuminoids, which are natural pigments.In

comparison with standard extraction solvents, higher extraction yields were obtained under optimum conditions (50 °C temperature, solid-to-liquid ratio of 0.1/10 g/mL, and an extraction time of 30 minutes) using a solvent with a 1:1 ratio of citric acid to glucose and 15% water (CGH).Furthermore, the antioxidant activity and stability of the target curcuminoids were investigated in various solvents. It was found that solid phase extraction was an effective method for recycling the natural pigments from the extracts [10].

Arango-Ruiz, L. et al. (2018) developed a turmeric-based dye extract formulation using supercritical antisolvent (SAS) technology with various encapsulating polymers to enhance the solubility and aqueous stability of curcumin. The resulting dye formulation from SAS comprises a blend of Eudragit® L100 and Pluronic® 127, with tween 20 as a surfactant.HPLC was employed to characterize and quantify the curcumin extracts and encapsulation products.The dye's characteristics were analyzed using XRD, DSC, SEM, oxygen radical absorbance capacity (ORAC), zeta potential, and particle size to assess antioxidant activity. Theformulation comprised a concentration of curcumin of 4.45µg/mL with an average particle diameter of 5667.4 nm and a zeta potential of 11.21 mV. At pH 4, highest solubility and aqueous stability of the dye were observed. Colour comparison indicated that a 200 µg/mL curcuminoid-based dye formulation is approximately equivalent to a tartrazine solution of 30 µg/mL concentration [4].

Abdeldaiem, M. H. (2013) focused on extracting turmeric extraction to obtain yellow pigment and converting it into a liquid form to produce oil-soluble and aqueous-soluble and yellow pigment colours. The oil-soluble yellow pigment's preservative and antioxidant effectiveness was tested in soybean oil after accelerated oxidation at 65°C for 7 days, in comparison to soybean oil devoid of any antioxidants. Sensory evaluation was conducted to determine the optimal ratio of yellow pigment which was water-soluble for blending with fillet samples of chicken breast. The findings indicated that adding oil-soluble yellow pigment of 0.2% significantly enhanced the oxidative stability of soybean oil. The sensory evaluation revealed that a 3% ratio of water-soluble yellow pigment enhanced the colour, flavour, and appearance of the chicken breast fillets. The study concluded that yellow pigment from the extracts of turmeric rhizomes exhibits potent antimicrobial and antioxidant properties. These pigments can also be used as natural food colouring agent and preservatives [11].

Almeida, H. H. et al (2018) compared various natural curcumin (E100) formulations and incorporated into yogurts.Curcumin (E100) is a natural colourant provides bioactivity in addition to colour, making it a good substitute for several artificial colourants. Since it is a hydrophobic colourant, it must be modified or made compatible with aqueous media in order to increase stability and allow it to be used in hydrophilic food matrices. In this study, several curcumin formulations—curcumin powder (PC), curcumin nanoencapsulated (NC), and curcumin water-dispersible (DC)—were tested as yogurt colourants. The results of all the tests indicated that PC had the highest bioactivity which may suggest that DC and NC decrease curcumin's short-term accessibility. The tested curcumin formulations produced yogurts with varying appearances, particularly in terms of colour parameters, as well as small alterations in nutritional composition, including free sugars, and fatty acid profile. Water-compatible formulations such as DC and NC were found to be more advantageous than hydrophobic ones (PC) due to their broader application in industry [12].

Zhou, Y. et al (2016) synthesized a novel water-soluble curcumin by adding a water-soluble UV absorber to the curcumin molecule and used for dyeing and treating silk fabric. This modified curcumin exhibited favorable solubility in water and increased sensitivity to the pH of the dye

bath compared to normal curcumin. Additionally, modified curcumin-dyed silk fabric demonstrated excellent UV protection, good colour fastness, and high antibacterial activity compared to silk dyed with curcumin, although its antioxidant capability was slightly inferior [5].

Boonroeng, S. et al. (2015) aimed to explore the possibility of modifying curcumin for use in mordant-free textile dyeing.In this study, curcumin underwent chemical modification in an aqueous system with glycidyltrimethylammonium chloride. The resulting modified compound, CurGTMAC, underwent structural analysis via mass spectrometry and was evaluated for its absorption characteristics. The findings revealed a significant shift in absorbance to the UV region, with a sufficiently high extinction coefficient to act as a natural dye with UV protection characteristics. When cotton fabrics were treated with sodium hypophosphite and citric acid and then CurGTMAC was applied, the fabrics achieved effective UV protection and lasting durability during home laundering. This outcome is likely due to the ionic interaction between the cations of CurGTMAC and citrate groups of cellulose [13].

Wang, Y. et al. (2009) investigated the microencapsulation of curcumin pigments using a method of spray-drying. Curcumin microcapsules were made using a spray-drying technique with gelatine and porous starch.Findings indicated that the best parameters were a core-to-wall material ratio of 1/30, 2 hours of embedding time, 70 °C temperature, 190 °C of inlet gas temperature, 70 mL/min of feed flow rate, and a drying air flow of 70 m^3/h. Under these conditions, the microcapsules demonstrated high encapsulation efficiency. Microencapsulation of curcumin significantly enhanced its stability against pH, light, and heat, while also significantly improving its solubility [14].

Wu, Y. et al. (2024)attempted to improve the hair dyeing capabilities of a natural colourant (curcumin) by prelinking thiol groups, a process known as "click dyeing," and evaluated its impact on dyeing performance and restorative properties. It was shown by the results that click dyeing greatly enhanced the colouring capacity and colour fastness of curcumin, with a colour retention rate of 80% after seven shampoo washes.Fluorescence labeling tests showed that curcumin underwent a thiol-Michael click reaction with the added thiol groups. Following the click dyeing process, the mechanical properties of the hair fibers were restored due to chemical bond formation during the click reaction, leading to a 9.81% rise in tensile strength when compared to the bleached hair prior to dyeing. The improvement is due to the reformation of keratin structure through click dyeing, allowing the properties to revert to their original form. Moreover, the research confirmed that curcumin can withstand photoaging via xenon arc lamp radiation and electron spin resonance (ESR) spectroscopy [15].

Betalain

Scientific name*:Beta vulgaris*

Family: Amaranthaceae

***Synonyms*: Beet**

Beet is generally obtained from Beta vulgaris commonly found in areas of Europe, Asia and Mediterranean regions. It has been used traditionally in medicines in many countries for long. The colouring pigments present are betalains which are nitrogeneous and readily soluble in aqueous medium. Betalains are derived from betalamic acid and are of mainly two types:

betacyanins and betaxanthins which are red and yellow coloured pigments, respectively. They have been used as colouring pigments in food products [16].

Studies based on extraction of Beta vulgaris

Hernández-Aguirre,*et al* used red and violet betalain from beetroot (Beta vulgaris) extracted waste and stabilised using deep eutectic solvents (DES) made with magnesium chloride hexahydrate [MgCl2·6H2O] and urea [U] in the ratios of 1:1 and 2:1. Similar to eutectic mixtures, the synthesised DES [MgCl2 ·6H2O] [U] exhibited low values of conductivity and melting points, range of viscosity, liquid state of matter and thermally stable. In response, betalain DES extracts (2:1) shown compatibility with betalain extracts in terms of betalain content when it came to the extraction and recovery of betalain from beetroot wastes. Degradation studies were used to examine the stability of betalain; the exposure settings were 12 hours of visible light, atmospheric air molecule oxygen, and 20–27 ◦C room temperature for 40 days. The betalain degradation kinetic curves for the water samples showed a first-order model, which showed that the violet hue of betalain derived from beetroot waste changed over the course of five to seven days. But when betalain from DES extracts were stored in amber jars for 340 days and exposed to light for 150 days, they achieved a 75% stability compared to the original beetroot extracts [17].

The process focused on extracting bioactives from peels of beetroot for potential use cuisines. Lazăr, S. *et al.* used spectrophotometry to evaluate the effect of extraction on betalains and polyphenolic compounds with conventional solvents, adjusting variables such as ethanol and citric acid concentrations, time of extraction and temperature. A Central Composite Design (CCD) helped optimize these parameters, using a quadratic model to analyse their effects. Results showed that the betalain content ranged from 0.29 to 1.44 mg/g DW, while polyphenolic yield varied between 1.64 and 2.74 mg/g DW. The optimal extraction conditions for maximizing these compounds were temperature of 52.52°C, time of extraction of 49.9 min,ethanol concentration of 50% and 1.5% citric acid [18].

Kaba, B.. *et al.* used deep eutectic solvents (DESs) as green alternatives for extracting betalains from red beetroot. Researchers tested 10 different DESs made from choline chloride combined with sugars, organic acids, various alcohols, comparing their effectiveness to traditional solvents. They found that the properties of DESs, including electrical conductivity, pH, viscosity, and density, were influenced by the specific combination of hydrogen bonddonors (HBD) and acceptors (HBA). Among the DESs, choline chloride and glucose (ChCl:Glu) yielded the highest levels of betalains, including betacyanin and betaxanthin. For optimization, they adjusted temperature, water content and the molar ratio, with central composite design. The optimum conditions were determined to be a 1:0.75 molar ratio of ChCl:Glu, 30°C and 30.83% water content, resulting in the highest betalain content: 1192.17 mg/kg for total betalains, 738.83 mg/kg for betacyanin, and 453.34 mg/kg for betaxanthin. Stability tests showed that betalains were more stable in the dark than in light, and bio accessibility analysis revealed values of 44.67% for betacyanin, 75.02% for betaxanthin, and 56.21% for total betalains [19].

Zin, M. *et al.* investigated the impact of various process variables on the extraction of colouredpigments from beetroot peel. By employing a central composite design, different extraction conditions were tested. The researchers measured the concentrations of the colour compounds using a spectrophotometer. The optimal extraction conditions identified were an time

of extraction of 1 hour,0.8 w/v of solvent ratio and temperature of 20°C. Under these conditions, the highest yields were achieved: betaxanthin concentration of 952.5 mg/l and betacyanin concentration of 1361 mg/l. The study concluded that the used method is simple, cost effective and for obtaining high yields of colour compounds from beetroot peel [20].

Singh, A., et al. evaluated the effectiveness of extraction of peels of beetroot by microwave. The process started initially with drying in hot air oven and microwave extraction, chosen for its improved absorbance in the 0.2-0.4 range. This method was effective in opening the vacuole pores in beetroot powder, facilitating pigment release. Using the Box-Behnken method for optimization, the study determined optimal conditions for two solvents. For Solvent A, the ideal conditions were 224.61 MW of microwave power, pH of 5.20, , and extraction time of 57.06 seconds, resulting in concentration of betanin of 229.264 mg/L. For Solvent B, the best conditions were a pH of 4.74, 384.25 MW of microwave power and 74.91 seconds of extraction time, achieving a concentration of betanin of 472.113 mg/L. The extraction process had an order of 1.42 and 0.00126 of rate constant value. FTIR analysis confirmed that key functional groups, including H-bond, N-H bend, O–H, C-C and C-H stretch, remained unchanged, indicating the extraction did not alter the chemical structure of the pigments [21].

Permana, L., *et al* optimized the extraction of betalains from dragon fruit peels, which are often discarded but rich in phytochemicals. Using water as an eco-friendly solvent, the study employed the Box-Behnken design to examine how various factors—pH (2.0–6.0), temperature (30–60°C), extraction time (10–60 minutes), and solid-to-liquid ratio (1:15-1:40)affect the extraction process. The research found that the solid-to-liquid ratio had the most significant impact on betacyanin extraction, the primary betalain component. Optimal extraction conditions were identified as a pH of 3.6, a temperature of 30°C, an extraction time of 10 minutes, and a solid-to-liquid ratio of 1:15, resulting in a betacyanin concentration of 72.37 mg/L. The study also observed that the extracted betalain colour, measured by CIELAB parameters, changed with pH variations, likely due to the degradation of betacyanin into betalamic acid [22].

Koubaier, H. B. H., *et al.*compared the betalain content, phenolic composition, and antioxidant activity of different parts of beetroot, specifically stem and root parts. The results showed that the extract obtained from beetroot roots had the highest betalain content, with 53 ± 4 mg of betanin equivalent and 46 ± 3 mg of vulgaxanthin I equivalent per gram of extract. In contrast, the stems had a greater phenolic amount, ranging from 2.0 ± 0.4 to 14.6 ± 0.5 mg of gallic acid equivalent per gram of extract. Using LC-MS analysis, various betalains including betanin, vulgaxanthin I and isobetanin; and phenolics including chlorogenic acid, gallic acid, etc. were identified in both roots and stems. The antioxidant effect of betalain extracts was higher in the root extracts compared to the stems [23].

This study addresses the issue of waste from beetroot stems and leaves, which are often underutilized and primarily used as cattle feed. To tackle this problem, Rosa, M. E., *et al.* developed a novel integrated process using thermos reversible aqueous biphasic systems(ABS) with quaternary ammonium-based ionic liquids (ILs) and polypropylene glycol (PPG). This process enables the simultaneous extraction and isolation from the stems and leaves of beetroot of two types of pigments including betalain and chlorophyll respectively. The extraction was conducted with an aqueous solution consisting of PPG and IL and phases were separated by temperature modification. This method allowed for the effective separation of betalains and chlorophylls into different phases. Optimization using a central composite design

determined that the best extraction conditions were at 20°C, 70 minutes, and a solid-to-liquid ratio of 0.12. Under these conditions, the maximum yields were: betalain of 6.67 wt.% and chlorophylls of 1.82 wt%. The study found that aqueous IL solutions were more effective in stabilizing betalains compared to the traditional solvents. Among the ILs tested, [N21(2OH)(2OH)]Br demonstrated the highest performance, achieving 92% efficiency for chlorophylls and 95% for betalains. The pigments were subsequently recovered with high efficiency using resins, with 96% recovery for betalains and 98% recovered for chlorophylls. Additionally, the ILs showed low to negligible toxicity, with [N21(2OH)(2OH)]Br being environmentally safe [24].

Conclusion

The review describes the sources of two of the most common plant sources of colouring pigments. Curcumin and betalain have found several applications in pharma and food industries. However the stability of the pigments poses challenge. Thus several extraction methods have been evaluated for improved yields and stable pigment products. Thus, future research on their extraction process and use is prospective.

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Weeping Behind the Walls: Lived Experience of an Individual Who Have Lost One of Their Parent

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Abstract

Losing a parent is one of the hardest losses we experience. For many, the loss of a parent comes too early. Losing a parent as a teenager has lasting effects. This research examines the literature review and proposes a study for further investigation of this important healthcare matter. The present study tries to explore the 'lived experience of 3 adolescents who lost one of their parents and now established themselves as a strong individual. In depth interview was conducted with these participants. Interpretative phenomenological Analysis (I.P.A) was used as a method of analysis and interpretation. 5 superordinate theme (**Coping, Catharsis, Defense, Affective Turmoil, Past Traumatic Growth**) (**We shall overcome by hook or crook: Use of coping as a part of dealing with separation, Inner unease: Ego Catharsis with the separated person, The unconscious rules use of defence as a part of dealing, Life is a roller coaster ride: Affective turmoil during the transitional period, Rise like a phoenix post-traumatic growth after separation**) and 10 Subordinate themes (**Transition phase of coping, Experience hypo arousal symptom in response to arousal, Adapting coping to deal with the situation, Self-regulation in dealing with different situations, Nurturance and succorance from separated person, Emotional gratification before separation, Rationalization as defence, Introjection as defence, Showcasing resilience in dealing with difficult situation, Family as support system**). Findings have been discussed in terms of coping, defence, resilience and life adjustment.

Overall, when a teenager loses a parent, it is important for the teenager to seek out help in order to improve their mental health. It is also important for other family members, such as the living parent, to understand some of the needs that these teenagers require to help improve their mental health after this traumatic experience.

Keywords: Coping, catharsis, defense, affective turmoil, post traumatic growth, death, losing of parent, adapting, resilience

Introduction

Losing a parent is one of the hardest losses one can experience. For many, the loss of a parent comes too early. The death of a parent is a highly stressful life event for bereaved children as well and has lasting effects. Early parental loss is associated with negative outcomes including anxiety; depression; prolonged grief reactions; negative effects on sense of self; increased risk for suicide, substance abuse, and eating problems; difficulty with executive function; reduced quality of life; and changes to how survivors approach adult relationships.

The adolescent period refers to individuals between the ages of 10 and 19 years and is characterized as a time of intense change, in biological, psychological, and social development. [1] It is well established that losing a parent in this formative stage of life may negatively affect an individual.

Some of the domains that can affect during the stage if losing parents include:

Attachment style: Attachment theory focuses on relationships and bonds (particularly long-term) between people, including those between a parent and child and between romantic partners. The attachment styles are as follows Secure attachment, Anxious preoccupied attachment style, avoidant attachment and disorganized attachment styles. [2]

Change of attachment style after loss of one of the parents: The loss of a loved one through death is an event that triggers activation of the attachment system, giving rise to emotional and behavioural responses that serve to relieve distress through seeking proximity to others. [3]

Death of a parent: Whether an individual serves as a caregiver for an aging parent or not, parental loss can be a difficult and life-changing event that can impact one's mental and physical health.

Psychological adjustment to parental loss: Psychological adjustment is defined as normative, age-appropriate behaviour and functioning that follows a course of positive functioning into adulthood. Psychological adjustment includes behavioural functioning, emotional functioning, social functioning, and quality of life. [4]

Coping skills: are those thoughts and actions an individual uses to respond to events that may cause them distress. These skills are conscious strategies an individual puts in place to manage emotions such as anger, anxiety, fear, or sadness.

Method

Aim

The aim of the present study was to explore the subjective experience of "an individual who has lost one of their parents".

Understanding interpretative phenomenological analysis

Interpretative Phenomenological Analysis (IPA) is an approach to qualitative research which is concerned with exploring and understanding the lived experience of a specified phenomenon (in our case; 'the experience of an individual losing one their parents') (Smith, 2004). IPA was introduced by Smith in 1996 as a means of analysing data but it has evolved to be a methodology in its own right. To be more precise, IPA involves the detailed examination of participants'. [5]

- Life-worlds
- Their experiences of a particular phenomenon
- How they have made sense of these experiences [6]
- The meanings they attach to them

The other distinctive feature of IPA is the concept of 'double hermeneutic' [5]. Smith and Osborn (2003) used the term 'double hermeneutic' to emphasize that two layers of interpretation are imbued in IPA viz.

1. the first is the participant's meaning-making (interpreting their own experience),
2. The second is the researcher's sense-making (interpreting the participant's account).
3. Thus there is an inevitable circularity in the process involving questioning, uncovering meaning, and further questioning); this circular process of understanding a phenomenon is called the "hermeneutic circle".

Operational definition of key constructs

Definition of coping strategies – an action, a series of actions, or a thought process used in meeting a stressful or unpleasant situation or in modifying one's reaction to such a situation.

Definition of life adjustment – adjustment is the process of adapting to changes in a person's physical, social, and occupational environment. The adjustment process begins when a need is felt and ends when it is satisfied.

Definition of defense mechanism- defence mechanism is an unconscious psychological operation that functions to protect a person from anxiety-producing thoughts and feelings related to internal conflicts and outer stressors.

Definition of resilience- Resilience is the process and outcome of successfully adapting to difficult or challenging life experiences, especially through mental, emotional, and behavioral flexibility and adjustment to external and internal demands. [7]

Definition of hope- hope is a positive cognitive state based on a sense of successful goal-directed determination and planning to meet these goals. In other words, hope is like a snap-shot of a person's current goal-directed thinking, highlighting the motivated pursuit of goals and the expectation that those goals can be achieved. [8]

Definition of loss- loss is a reduction in resources, whether tangible or intangible, in which a person has a significant emotional investment.

Definition of death- Traditionally, death is defined as **the absence of a heartbeat**. Death is when circulatory and respiratory functions or brain functions cease irreversibly. [9]

Selection criteria for participants

Inclusion criteria	Exclusion criteria
• Age- 20-27 years • Educational qualification- above class 10^{th} • Sex- either sex • Loss of one parent during the period of adolescence	• Participant having significant psychiatric illness • Presence of any chronic physical illness

Sampling

Purposive sampling was used for selection of participants.

Definition of Purposive sampling

According to the aim, need and objective of the study participants were selected through purposive sampling technique where the researcher used the expertise to choose specific participants that helped to conduct the study. In this particular study, the researcher uses one specific subtype of purposive sampling that is "Homogeneous sampling" which aims to reduce variation, simplify the analysis and describe a particular subgroup in depth. The final sample consisted of 3 participants

- Participants 1- J.S.Y
- Participants 2- S.D.S

- Participants 3- S.D

Tools Used

Information Schedule: A semi structured interview designed for this study to elicit information pertaining to each individual's Name, age, schooling, education level, details of family origin, quality of relationship with existing authoritative figures, quality of peer relationships, hobbies, etc.

In Depth Interview: was conducted to enable the participants to tell their daily lifestyle, life stories and to explore their experiences of losing one of the parents. The interviews for each participant were conducted till "saturation" was achieved. Typically, each interview lasted for about 20 minutes to 35 minutes. The interview was conducted keeping in mind the certain attachment issues, psycho-social issues and personal issues which might be pertinent for the "lived experience" of losing a parent.

- Life transition before and after loss of a parent
- How they perceive loss and separation in their life and how they are perceiving it now
- How is their overall experience losing a parent
- Their attachment style with the existing parent or any other

Procedure

The specific qualitative research was conducted "to explore the lived experience of an individual who has lost one of their parents". So here the purpose of the study was "to explore how participants make sense of their experiences". The Interpretative phenomenological approach was used where the researcher's main goal was to assess participant's perspectives of their experience and interpret their views. Participants were selected through 'purposive sampling' (more specifically 'homogenous sampling technique') considering the inclusion and exclusion criteria. Data was collected through a semi structured interview of each participant after building a proper rapport.

The data was transcribed by the researcher and the data was simultaneously coded (open code, focus code and axial code) and interpreted by two researchers. Then the coded data were analysed and interpreted.

Ethical consideration

The data were kept strictly confidential and the identity of the participants was never disclosed even to the co-researchers. Since the participants would be discussing life experiences which are extremely personal and might evoke unpleasant emotions at times, participants were told that they can opt out of the research at any point even if they had given prior consent. This was done to ensure that the well-being of the participants is not affected.

Results and Analysis

The section summarizes data that emerged from three in-depth individual interviews. The discussion was interpreted using Interpretative Phenomenological Analysis. Data transcript was coded at all the levels of analysis and categorized into exploring themes, emerging themes and superordinate themes. The Superordinate themes and emerging themes obtained from the Interpretative Phenomenological analysis are described as following:

We shall overcome by hocks or by crooks: Use of coping as part of dealing with separation

Transitional phases of coping with situation

The study reveals a major theme of 'use of coping as part of dealing with separation', which includes four sub-themes. Two participants highlighted their own transitional phases with their situations. The narrative of one of the participants are reflected below:-

"tarpor ami gelam giye amra taxi te gelam maa bolche je oi ki bole rokto change korte hobe smiling- abr rokto chorate hobe toh jehetu maa nurse maa jane maa rastay bolchilo e nei ma kadchilo ami ashay chilam shotti bodhoy roktoi change korte hobe oi jonnoi dakche giye dekhi baba r nei"

Experiencing hypo arousal symptom in response to separation

Two of the participants were experiencing hypo-arousal symptoms in response to separation. They became emotionally numb in the time of distressing situations. The narratives of one of the participants are reflected below:-

"Ami oivabe je khub khusi ba khub dukho je baba nei ei hocchena oi hocchenate mon kichu feel korina…."

Adapting coping to deal with the situation

Apart from both of them, there was another participant who was developing adaptive coping to deal with the situation. The narratives of the participant are reflected below:-

"….ami jehetu onek activities kortam I kept myself involved ami onek onek hockey kheltam, onek music kortam….music helped a lot"

Self regulation in dealing with difficult situation

A participant was also trying to cope by developing self regulating thoughts to deal with this kind of difficult situation on his own. The narrative of the participant are reflected below:-

"Eina je ami baba hoye jabo but kotota bhalo baba hobo setake amake ek honde khte hobe"

"Amr future a ei manush na thakle Amar kichu hobena to karon ki ami akta oi jinish perye eshechi….ami nijeke nijer moto chaliyenite parbo".

The inner unease: Ego catharsis with the separated person

Nurturance and succorance from separated person

The study reveals a major theme of 'ego cathexis with the separated person', which includes two sub- themes. It was found that all the three participants were having a strong bond with their close ones with whom they had spent their good times. For them the separated person was their primary caregiver who used to give them full support and love. The narratives of the participants are reflected below:-

"….babar sathe beshi valo relation chilo amar prothom cycle o baba kine diachilo….ami babar sathei thaktam besir bhag somoy….baba sobsomoy e support korechilo amay sob kichute…."

"...jodi maa ektu bokloto didar kache dida ektu boklo toh maaer kache erom kortam r eromi bapartchilo r onek I was loved"

Emotional gratification before separation

For them the separated person was their primary caregiver who used to give them full support and love. They were highly emotionally gratified before separation which highlights the phenomenon of ego catharsis. The narratives of the participant are reflected below:-

"….I was very pampered and loved ...khubi ami khub lovable chilam puro family tepuro family te center of attention only child tai like maa r didarbeshi close chilam ami tai. R khub valoi legeche mane amr childhood ta was best"

"onek free chilam mane free as a bird mane jaki chukortepari mane I didn't have a routine…."

<u>The unconscious rules: Use of defense as part of dealing</u>

Rationalization as defense

The study reveals a major theme of 'use of defense as part of dealing', which includes two sub-themes. One participant rationalizes and feels to accept the fact of his father's death. The participant conveys that no further incident can impact him or make him sad as he instantly forgets the fact which would make him sad. The narratives of the participant are reflected below:-

"ami maa k bolchina je maa support koreni ba maaer kichu ongsho grohonkor eni ekjon maa khubi busy chilo taka income korte karon ghor o chalate hobe r maaer salary babar theke joteshto valochilo"

"ba acceptance chole eshechilo ba ami oi trauma ta k feel korar thik oi sthitite chilamna je amr mone ache ami ekdubar e kedechi….I am quite normal and that's about it even amion nokono trauma face korle ba onno kono emni separate face korle amrakta time erpor r mone thakena what I was sad about"

Introjection as defense

Participants have also used introjections with his father's personality as he desires to associate with someone who is more responsible than him like his father. This can be clearly depicted that these two defenses are the key highlights found in this thematic study. The narratives of the participant are reflected below:-

"….I wanted someone who's more responsible than me who can take up more responsibility than me that's all I want. I am not giving that place to someone else but it's just that tiny bit of him like jemon responsibility ami feel kortam je babar chilo je like an umbrella…."

<u>Life is a roller-coaster ride: Affective turmoil during transitional period</u>

According to the narrative reflected by one of the participants it was difficult to believe the shocking fact about her mother's death after 2-4 years as her grandmother kept the fact untold to keep her stress free. This turmoil during her transitional period affected her emotions. The narratives of the participants are reflected below:-

"maake dekhlam mone hoyni maa ache bole(smiling) mane monei hoyni biswas e korte parini like around 2-4 years toh etai jantam na je maa mara gache bole karon dida oi feel ta konodin hote ditona haain tohamr kono feel e hoyni mane ami mantei parini maa k dekhe"

"….jockon dida mara jay I hugged her but maake ami bhoyte touch korini bhoyt epaliye gechi okhan theke r berye biswas e korini I was crying but I couldn't believe that was my mom and that's how she looked"

Rise like a phoenix: Post traumatic growth after separation

Showcasing resilience in dealing with difficult situation

Here participants had showcased their resilience in dealing with difficult situations like one person realized that everyone has to die one day, so basically for him it's useless to retain death related thoughts in our mind while another participant also avoids disturbing situations by recalling good memories. The narratives of the participants are reflected below:-

"….I always felt that these things are parts and passes of life death is the only truth shey amio ekna ek din morbo amra shepasher jara ache morbeto hotath eke amy ber korar jonno ekta positive thought amar lagena ami ei factor ta bujechi je death is inevitable"

"….life jockon onek problems hoy toh onek difficulties hoy baki chuonek baje hoy jay to ckonamichestakorivalo memories guloimonekorte r khusihote…."

Family as support system

On the other hand, one participant got enormous support and love from his family after his separation. It can be depicted that these factors play a crucial role in post-traumatic growth.

"External help obossoi peyechi bari theke. Dada, jhetima, baba, bondhu-bandhob sobai bolbona je keu khub kharap bhabe dekhe chesobai khub oi je bollam je khub maya diye agle rakhar chesta koreche….Toh help peyechi help paini ekdomeina"

Discussion

This study is unique in conceptualizing the "lived" experience of those individuals who lost one of the parental figures who are currently staying at orphanages in Kolkata. As they lost their parents, they usually face some personal and social issues. It is very interesting that all the participants share some similarities in the manner they describe their lived experiences. Mainly 11 sub-ordinate themes or axial coding and 5 super-ordinate themes could be identified from the transcript.

- **We shall overcome by hocks or by crooks: Use of coping as a part of dealing with separation**

From the narratives it is clearly understood that all of them have used some or the other conscious state of problem solving to deal with the period of separation. Qualitative analysis explored two themes of coping strategies, emotion-focused and problem-focused in their lived experiences.

One of the participants, who is male and lost his father, has shown emotionally shut down and numbness as coping. He may have a more problem solving approach view on separation and tends to emotionally constrain from almost every emotional situation of life. Hence he prepared gradually to bear the absence of his father by accepting the change in his life.

Another male participant who has lost his mother showed similar emotional numbness as coping. He has shown a gradual and systematic coping strategy throughout his life, where he postulated from experiencing hypo arousal symptoms in emotion to gradually adapting to the separation and

to be self-dependent and lastly it is understood that he has achieved the confidence. Hence there is a dearth of research reflecting upon the gradual process of healing in the context of loss.

On the other hand, the present research found an interesting domain where only the women participant who lost her mother showed adaptive coping to deal with the difficult situation. According to the role-constraint hypothesis, how a person responds to stress depends on their social role. Women are socialized into using more passive, emotion-focused coping strategies, whereas men are socialized into using more active, problem-focused coping strategies. According these studies, women tend to use more adaptive coping strategies than men. Interventions for women may focus on increasing the use of adaptive strategies such as praying and talking to friends and family. [10]

- **Down the memory lane: emphasis on the past before separation**

All three of the participants had one thing similar and they emphasised on this domain, they submerged at this very stage where we were connected with their inner voice of 'woh din bhi kya din they' with joyful faces and eyes filled with memories.

They showed their resilience through connecting with them only through portraying the happy memories, joyful situations, caring nature of their deceased parents and the love they felt from them. However, all the participants did not just stop and look back at the memory but used those memories to grow in the future and to shine more in life.

- **The unconscious rules: Use of defense as a part of dealing**

The two defenses we could find from the study were rationalization and introjection. The definition of both is mentioned below

- **Rationalisation**- Rationalization is a defense mechanism in which people justify difficult or unacceptable feelings with seemingly logical reasons and explanations.
- **Introjection**- Introjection also known as identification is the unconscious adoption of the thoughts or personality traits of others.

He rationalised the absence of his mother during the developmental years because his mother was the bread earner of their family. Although he pointed out that he was close with his father in the previous transitional period.

With the participant's verbatim it is clear that, despite the emotional turmoil he faced with his father, he has used certain qualities of his father as the defense 'introjection' in his romantic relationship that the woman he looks for needs to have more responsibility than him.

- **Life is a roller-coaster ride: Affective turmoil during transitional period**

Both of the participants have shown a sense of strong regret for some of the past actions they could not complete and feel emotionally chained to that particular situation. Sense of worry, feeling emotional vacuum, sense of helplessness in dealing with the difficult situation these all are domains or focus coding which depict their emotional waxes and waves that can to some extent prove this statement.

- **Rise like a phoenix: Post traumatic growth after separation**

Posttraumatic growth refers to positive psychological change resulting from a struggle with traumatic or highly challenging life circumstances. [11]

The commonality between all the three participants at this domain made this study successful. The verbatim were clear indicators of the post traumatic growth they experienced. By accepting the harsh reality the participants have articulated that life keeps going like the stream of a river.

Conclusion

This study contributes to the understanding of the lived experience of an individual who has lost one of their parents. Specially highlighting the importance of coping strategies, resilience, life adjustment after loss of a parent and defense used by them. It is clear from this study that parental death has an important impact on adults' psychological and physical well-being and that this impact varies by gender. Additional research attention to this topic is important to help further illuminate the many ways in which parent-child relationships continue to be an important factor in determining well-being for both parents and adult children across the adult life course. Findings suggest that though they have been devastated after loss, yet they have shown immense resilience which may be reflected in their growth process after the traumatic incident.

Future Implication

In the future we can apply the findings from the literature review to a clinical setting and incorporate such important situational trauma and grief to understand their unique journey. so doctors, nurses, psychologists, and anyone else who may be in contact with a teenager who lost, or is about to lose a parent, can give advice and direction to that grieving teenager. It is also beneficial to tell the living parent what the teenager needs, so they can be there for them as well.

Limitation

Since this study has been qualitatively studied hence few limitations of qualitative research may be there. Participants' perception of death may differ from one another hence we cannot generalize the findings here.

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APPENDIX 1

1. Can you disclose about your experiences in the phase of transition that is before and after the demise of your parents?
2. Any significant memories associated with the deceased person?

   a) When you imagine these memories, what kind of experiences do you feel right now?
   b) How do you deal with emotions?

3. What Does your preferred future look like?

The questions mentioned here are just for guidance, apart from these questions we have used probe questions during the interview.

Effect of Polycystic Ovary Syndrome on Pregnancy and Fetal Health and its Intervention

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Abstract

Polycystic ovary syndrome is a common gynecological condition in women of childbearing age, marked by metabolic disorders, irregular menstrual cycles, anovulation, reduced fertility rates, underdeveloped fetuses, and risk of perinatal mortality. The effects may be manifested even in lactating mothers and can alter the metabolic profile of the offspring in their later years. Appropriate interventional strategies should be adopted to improve the chances of conception, and complication-free pregnancies and ensure maternal and fetal well-being. The approaches comprise hormonal therapy with female reproductive hormones, treatment with ovulation inducers (e.g. metformin, clomiphene citrate), assisted reproductive technologies, surgical interventions, and lifestyle modifications. However, the benefits should outweigh the risks of any such strategy as risks of ovarian hyperstimulation syndrome and multiple pregnancies are very frequent. The ultimate objective of a successful intervention should be to ensure improved fertilization rate, and clinical outcome of pregnancy, enhancing the chances of live births, without potential adverse effects. The review article underscores the merits and demerits of the commonly employed strategies in improving metabolic profile and pregnancy outcomes in women with Polycystic ovary syndrome.

Keywords: Assisted reproductive technology, hormonal therapy, multiple pregnancies, ovarian hyperstimulation syndrome, ovulation inducers, polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a hormonal disorder of immense significance to females of reproductive age. Hormonal imbalances, ovarian dysfunction, and metabolic disturbances occur, resulting in irregular menstrual cycles, infertility, elevated androgen levels, hirsutism, acne, insulin resistance, dyslipidemia, and obesity. The specific etiology of PCOS is still not known, however it is probably due to hereditary and environmental factors. PCOS manifests in each individual differently, ranging from mild symptoms to severe ones. It can also elevate the risks of comorbidities such as Type 2 diabetes mellitus, gestational diabetes, cardiovascular disorders, depression, and anxiety [1-4].

A complete medical history, physical examination, and regular monitoring of hormone levels in blood are required for early diagnosis of PCOS. Pelvic ultrasonography should be conducted periodically in susceptible women to visualize the state of ovaries and detect the development and progression of cysts [5-7].

Although PCOS can be difficult to manage, early detection and treatment can reduce the risk of long-term health-related issues. Adoption of appropriate interventional strategies enables successful conception [5,6].The treatment of PCOS mainly focuses on the management of symptoms with medicines, surgical interventions, or by changing lifestyles to restore menstrual cycles, minimize metabolic disorders, overcome fertility challenges, facilitate conception, avoid risks during pregnancy, preterm birth, and lower risks of affecting fetal health, which may even lead to perinatal morbidity [6-8]. Not only are fetuses and newborns exposed to risks of adverse effects in women with PCOS, but mothers should also be made aware of the potential risk of breastfeeding immediately after delivery and during the lactation period. Hormonal imbalances, metabolic disorders may be responsible for improper development of mammary glands, delayed lactogenesis, and insufficient milk supply, alteration in the nutritional composition of breast milk. Breastfeeding efficiency may be affected as women may fail to lose their weight gain during pregnancy [9-11].

Furthermore, ongoing research into the underlying causes of PCOS continues to prosper our understanding and improve treatment options for women affected by PCOS.The present review targets to delve into the consequences of PCOS on chances of conception, during pregnancy, fetal well-being, and post-pregnancy in lactating mothers, and in offsprings in their later years. It also suggests various interventional strategies that can be employed for pre-pregnancy monitoring, increasing the likelihood of conception depending on the status of the endocrinological and metabolic systems of the individual, culminating in personalized approaches, and focussing on the potentials and challenges of the strategies. No article has provided a brief, comprehensive review of the various aspects of PCOS and its management opportunities.

Interventional strategies for minimizing complications in pre-, during, and post-pregnancy in women with PCOS

Pre-conception screening

Since PCOS and metabolic, hormonal, ovarian functions and fertilization rates are linked, and the risk of irregular ovulation or anovulation is of frequent occurrence, pre-conception screening allows physicians to identify the risk of occurrence of these imbalances and disturbances before conception. This improves the woman's overall health status and increases the chances of complication-free pregnancies [12,13]. The occurrence of multiple pregnancies can be detected early by USG monitoring [14,15].

PCOS may adversely affect fetal growth and development owing to placental insufficiency and metabolic dysfunction. The effects may be manifested as stunted or restricted fetal growth, smaller biparietal diameters and femur lengths, and lower birth weight. Female fetuses carry the risk of virilization, leading to post-birth reproductive and metabolic repercussions. Offsprings born to mothers with PCOS are susceptible to irregularities in their metabolic profiles, enhanced risk of obesity, and the risk of insulin resistance in their later years. Since not all fetuses or offspring are negatively affected by their mothers' health condition, early identification, timely intervention, and individualized management plans are key in ensuring fetal well-being. Collaboration and coordination between various medical professionals with interdisciplinary knowledge and expertise are essential in optimizing maternal and fetal health outcomes [16-18].

The advent of newborn anthropometry helps in monitoring fetal growth and measurement by periodic measurement of infant physical characteristics like the circumference of the head, length, and weight [19].

Therapeutic recommendations for conception and uncomplicated pregnancy in women with PCOS

Effective holistic interventional strategies for ensuring conception and complication-free pregnancy can ameliorate hormonal and metabolic disturbances, improve ovulation, and fertility, and facilitate easy conception, healthy pregnancy, and ensure the well-being of the fetus, and offspring. Therapeutic interventions emphasize managing hormonal imbalances, and PCOS-related complications affecting various systems, and improving ovulation, menstrual cycle, and fertility.

Hormonal therapy

Supraphysiological estradiol

Estradiol, a form of estrogen, plays an important role in PCOS management, as it works in tandem with other female hormones and exerts influence on the reproductive system. Supraphysiological or above-normal estradiol levels can attenuate or exaggerate PCOS, which has been represented in Table 1 [20-22]. Outcomes of supraphysiological estradiol levels can vary depending on age,genetic predispositions, and overall health constitution. Hormonal treatments should be highly tailor-made and personalized, [23-25] designed and monitored by a reproductive endocrinologist or gynecologist to meet the unmet clinical needs of females with PCOS.

Table No. 1. Consequences of supraphysiological estrogen therapy in PCOS

POSITIVE IMPACT	NEGATIVE IMPACT
• Regulates menstrual cycles • Enhances scope of fertility in affected women with irregular ovulation • Lowers hyperandrogenism symptoms like acne and hirsutism	• Aggravates insulin resistance resulting in type 2 diabetes and cardiovascular complications • Stimulates endometrial proliferation due to insufficient progesterone • Increased levels of estradiol results in anxiety, mood swings, or depression in some women

Most frequent events in PCOS, menstrual irregularities and hyperandrogenism symptoms, can be managed with combined hormonal contraceptive (CHCs) preparations, containing estrogen and progestogen. Estrogen exhibits antiandrogenic activity by promoting the hepatic formation of sex hormone-binding globulin that brings down the levels of circulating testosterone. Progestogen suppresses the secretion of luteinizing hormone. Progestogens may also counteract androgens' effects on their receptor. But CHCs should be prescribed cautiously in PCOS especially taking into consideration age, metabolic disorders, and cardiovascular and endothelial diseases[26]. On the other hand, estrogen-progestin combination can regularize menstrual cycle, reduce androgen excess, achieve effective contraception, and offer protection

against endometrial cancer. The risk of arterial and venous thrombosis in smokers with PCOS, development of insulin resistance, and diabetes in obese women necessitate the adoption of an individualized interventional strategy for PCOS management with hormonal contraceptive pills. Before prescribing hormonal contraceptives, physicians should identify patient-specific risk factors for maximization of benefits and minimization of adverse effects [27].In women with metabolic dysfunctions, obesity, or moderate insulin sensitivity with no requirement for metformin, the vaginal contraceptive ring is preferred over oral ethinylestradiol/drospirenone. In addition, a combination of CHC and myoinositol may improve endocrine and metabolic profile. However, the option of PCOS therapy with CHC needs to be explored further for the determination of optimal duration and specifying effects of such agents on long-term metabolic outcomes [28].

Gonadotropin therapy becomes the option for those women with PCOS who did not show favorable responses with clomiphene citrate or aromatase inhibitors. However, they carry a risk of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies and should be prescribed by experienced physicians[29].

Clomiphene citrate

Clomiphene citrate, a non-steroidal, selective estrogen receptor modulator (SERM) triggers ovulation in cases of anovulation, as observed in PCOS. It stimulates pulsatile secretion of gonadotrophin-releasing hormone (GnRH) by the hypothalamus [30], and anterior pituitaryin producing follicle-stimulating hormone (FSH) and luteinizing hormone (LH), thereby inducing the final maturation of follicles. An imbalance in the pro- and anti-inflammatory cytokine levels leads to chronic sub-clinical low-grade inflammation. Interleukin-10 is responsible for maintaining the fetomaternal unit by virtue of its pleiotropic effects. Clomiphene citrate enhances nitric oxide levels, and anti-inflammatory cytokine, interleukin-10, and lowers matrix metalloproteinase-9 levels in women with PCOS [31].It exerts beneficial effect not only on clinical pregnancy outcomes, especially in combination with L-carnitine, but also yields high-quality embryos in women with PCOS. However, it is to be noted that although the ovulation rate is significantly high, the pregnancy rate is comparatively low. Some of the adverse effects reported with the use of the molecule are drug resistance, OHSS, and mood changes, negatively influencing overall well-being [32].Women who are resistant to clomiphene citrate canbe treated with metformin to increase ovulation rate, and pregnancy outcomes but no pronouncedeffect on the rate of live birth [29-33].The anti-estrogenic effect can alter endometrium and cervical mucus, where endometrial proliferation is suppressed, thus hindering implantation of the embryo. Other side effects experienced by the users include hot flashes, nauseating tendencies, tenderness of the mammary glands, dizziness, and blurred vision [33].

Letrozole

Letrozole is a highly selective non-steroidal third-generation aromatase inhibitor, inhibiting the conversion of androgen to estrogen. Ovulation is induced by increased secretion of FSH from the pituitary gland, followed by an increased sensitivity of follicles towards FSH. It can be employed in women who do not respond to clomiphene citrate forinduction of ovulation. The risk of adverse effects and multiple pregnancies is less [29].

Metformin

Administration of metformin, a popular anti-diabetic drug before and during pregnancy is known to manage PCOS and gestational diabetes mellitus (GDM) owing to its potential benefits on maternal and fetal health. In women with PCOS, metformin enhances sensitivity to insulin. Metformin reduces hepatic glucose synthesis, reduces insulin-resistance in peripheral tissues, lowers fasting blood glucose levels, controls glycemic index, reduces the risk of type 2 diabetes mellitus, [34,35]lowers levels of androgen in serum, and free-androgen index[36], reduces the levels of LH, enhances the levels of SHBG (sex hormone-binding globulin), ultimately leading to lowering of irregularities in menstrual cycles, which in turn regularizes ovulation, thereby increasing the chances of conception[37,38].In contrast to clomiphene citrate, metformin does not suffer from inducing the risk of OHSS during the restoration of ovulation[39]. Metformin is even recommended for the remediation of PCOS-induced infertility in women without diabetes or glucose intolerance. It also improves the total lipid profile, reduces the risk of cardiovascular diseases[35],and facilitates weight control, a prerequisite to increasing the likelihood of pregnancy in complicated cases [40].However, exposure to metformin may lead to various adverse effects during and post-pregnancy which are illustrated in Table 2.

Table No. 2. Pregnancy and post-pregnancyadverse outcomes of metformin use in women with PCOS[41-43]

During pregnancy	Post-pregnancy
• Risk of hypoglycemia in case of treatment with insulin and other diabetic medications • Risk of delayed fetal growth • Risk of lactic acidosis due to changes in kidney function and metabolism • Gastrointestinal side effects like diarrhea, nausea and vomiting. • Risk of miscarriage in first trimester	• A higher dose can expose the infant to the medication during breastfeeding • Metformin discontinuation can exacerbate insulin resistance or glucose intolerance • Discontinuation of metformin can cause reoccurrence of PCOS symptoms. • Discontinuing metformin may negatively affect long-term metabolic health, especially in women at higher risk of type 2 diabetes.

Interventional and assisted strategies in PCOS management

In-vitro fertilization (IVF)

In-vitro fertilization is employed in the management of anovulatory PCOS when all other options with monotherapy or combination therapy have proved futile. The rate of success with a single treatment cycle of IVF is quite high in young women with superior reproductive health. Still, there are several risks associated with IVF which include OHSS, multiple pregnancies, and rapid endometrial maturation. Moreover, it may not be affordable and accessible for most of the women. Strategies like employing short antagonist cycles and freeze-all cycles can mitigate the risk of OHSS.

Although metformin lowers the risk of OHSS, its use with IVF/ICSI-ET (Intra-cytoplasmic sperm injection – embryo transfer) in women with BMI<26 is not advisable as only few studies demonstratedits positive relation to increasing the probabilities of overall clinical pregnancy rate or live birth rate in women with PCOS [44-46].On a positive note, metformin treatment for ovulation stimulation in PCOS does not lead to multiple pregnancies as it promotes mono-follicle development[29-33].

In vitro maturation (IVM)

Retrieval of the immature oocytes and their *in vitro* maturation (IVM) is likely to prove beneficial in women opting for pregnancy but are having PCOS. Its success rate is comparatively low but is suitable for women who are extremely sensitive to ovulation induction with gonadotropin therapy and can exhibit OHSS [47].

Intracytoplasmic sperm injection (ICSI)

Oocytes obtained from polycystic ovary possess abnormal zona pellucida which is the main cause of lower fertility rate in women with PCOS. Adoption of ICSI leads to improved fertilization rate and higher quality of embryos. In this technique, oocytes are artificially inseminated, enhancing the chances of fertilization, followed by the selection of best quality embryos which are implanted in the uterus. The sequence of events increases the likelihood of pregnancy outcomes[33].

Bariatric surgery

Bariatric surgery exhibits promising potential in obese women with PCOS. Weight loss enables amelioration of the key symptoms of menstrual irregularity. Removal of excess fat from the body cures metabolic disorders e.g. type 2 diabetes mellitus, gestational diabetes, hypertension, and dyslipidemia, and reduces the risk of pre-eclampsia, and overweight babies. However, bariatric surgery can lead to nutritional deficiencies, as nutrients may be poorly absorbed. Moreover, the gestational period is shortened, and smaller offspring may be produced with an enhanced risk of perinatal death. So obese women opting for bariatric surgery should be informed of the benefits and risks of the technique [29].

Laparoscopic ovarian diathermy or ovarian drilling

Laparoscopic ovarian diathermy is a safe and effective surgical option for anovulatory women with PCOS, who are not responding to clomiphene citrate and are trying to conceive. Its efficacy is comparable to that of gonadotropins, with long-term effects but lacks the risk of OHSS and multiple pregnancies. Moreover, it increases sensitivity to other ovulation induction agents. Laparoscopic ovarian diathermy is suggested in cases of women resistant to any conventional therapeutic strategy [48,49].

Lifestyle interventions in PCOS management

Regular physical exercise brings about improvement in cardiorespiratory fitness, menstrual regularity, ovulation rates, reduces BMI, and mood swings, and leads to better overall well-being. Thus, physical exercise may be considered anon-pharmacological approach for PCOS management. However, there is no conclusive evidence of the link of the extent and duration of physical exercise and PCOS [50]. Although lifestyle intervention can reduce the free androgen index, its effect on glucose tolerance is yet to be explored. Similarly, its influence on ensuring live birth, and risk of miscarriages has not been studied [51].

Guidance for lactating mothers with PCOS

To encounter lactation and breastfeeding challenges, women with PCOS are advised to seek professional help that can offer nutritional advice, and specific guidance and train them with appropriate breastfeeding techniques and management. Lactation can be improved by the administration of hormonal contraceptives. Therefore, women with PCOS should know about alternative contraceptive options. In a patent, the benefit of using infant formula with human milk peptides) has been suggested to be beneficial for lactating mothers with PCOS [52].

Conclusion

Metabolic disorders, hormonal imbalances, and improper lifestyle may lead to the development of polycystic ovary syndrome in women of childbearing age. The condition produces a negative impact on pre-conception, affects the fetal growth and development, may result in miscarriages and if left untreated, can cause perinatal mortality. Management of PCOS is thus essential to ensure a safe pregnancy, development of high-quality embryos, and healthy offspring at the end. Therapeutic interventions with hormonal contraceptives, drugs like metformin. clomiphene citrate, surgical options should be decided with utmost care and impact so as not to affect adversely pregnancy, and fetus, and to reduce the risk of OHSS and multiple pregnancies.

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Rapid-Assessment of Hypertension among Young Adults (18-40 years) residing in an Urban Community under Kolkata Metropolitan Area

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Abstract

Hypertension (HTN), in the present scenario, is not limited to the elderly alone; it is percolating to the young adult population. The objective was to assess hypertension in young adults (aged 18 to 40 years), residing in an urban community under the Kolkata Metropolitan Area. 184 young adults (men 47.8%) and (females 52.2%) in adults (18-40 years) were selected by systematic random sampling method. Study was conducted for two months, from May to June 2024, and received approval from the NSHM Institutional Ethics Committee. The researchers found that the percentageof males with normal blood pressure was 48.9%, 36.4% had elevated blood pressure, and 6.8% and 7.9% were identified to have Grade 1 and2 Level HTN respectively. Among women,51% of the study population had normal blood pressure, 30.2% had elevated blood pressure, and 10.5% had Grade 1 and 9.3% had Grade 2 hypertension respectively. The researchers conclude that more than 50% of the study population is in various stages of hypertension (Elevated, Grade 1 and Grade2). Needless, to mention, there is an immediate need for public health intervention to assess the risk factors of the problem and counsel the people for regular medication and preferred non- pharmacological management of the disease.

Keywords: Young adults, hypertension, risk factors, intervention

Introduction

About 1.28 billion adults in the age group, 30–79 years globally are estimated to have high blood pressure levels with **two-thirds** of them residing in low- and middle-income nations. 46% of the populace does not know that they are affected by the disease. The prevalence of HTN is higher and nearly constant across income levels and all-inclusive socio-economic groups, and researchers identify that the number of cases surges with age, with sixty percent of the population above the age of 60 years, being majorly affected [1]. The disease in current scenario, is not limited to the elderly alone; it is percolating to the young adult population and has arisen as a grim concern for health practitioners across the globe. Assuming from one study that was conducted in India, the prevalence of the disease is nearly 7% among young adults (20–39 years of age), it interprets that approximately 2.7 crores of young adults could be suffering from hypertension [3]. It is well-researched that raised blood pressure levels during adulthood can cause vascular impairment that would require medical attention and risk towards mortality [2, 4].

One of the widely accepted definitions of hypertension (HTN) is *"an increased systolic blood pressure (SBP) value (≥130 mm Hg) and/or increase in diastolic blood pressure (DBP) value (≥80 mm Hg)"*

It is classified based on blood pressure levels and causes, aiding in its management and treatment. It is divided into primary (essential) hypertension and secondary hypertension, with blood pressure levels further categorising the condition's severity:

Primary (Essential) Hypertension

Nearly accounting for 90-95% of cases, does not have an identifiable cause, and is influenced by-

- Genetic predisposition: Family history.
- Dietary factors: High salt, low potassium, calcium, and magnesium intake.
- Physical inactivity: Lack of exercise.
- Obesity: Excess body weight.
- Alcohol consumption: Excessive drinking.
- Chronic stress.

Secondary Hypertension

It accounts for 5-10% of cases, caused by identifiable conditions like:

- Kidney disease namely Glomerulonephritis, Chronic and polycystic kidney disease.
- Endocrine conditions like Hyperaldosteronism, Cushing's syndrome, and hyperthyroidism.
- Vascular disorders like Aortic Coarctation
- Sleep apnea: Obstructive sleep apnea [1].

Classification by Blood Pressure Level

The American College of Cardiology (ACC) and the American Heart Association (AHA) provide the following guidelines/categories based on systolic and diastolic blood pressure values [5]. Thisis illustrated in Table 1 given below-

Table 1. Classification by Blood Pressure level

Classification of Blood Pressure	Systolic	Diastolic
Normal blood pressure	<120 mm Hg	<80 mm Hg.
Elevated blood pressure	120-129 mm Hg	<80 mm Hg
Hypertension Grade 1	130-139 mm Hg	80-89 mm Hg
Hypertension Grade 2	≥140 mm Hg	≥90 mm Hg
Hypertensive crisis	>180 mm Hg	>120 mm Hg

(Source: Whelton et al, 2017)

Hypertension (HTN) Prevalence in India

Hypertension is following an upward trend. National Family Health Survey (NFHS-4, 2015-16), found that 18.1% of adults aged 15–49 had hypertension. 11%of women and 14.8% of men in the same age group have hypertension. 30%women and 43% males are in the status of pre-hypertensive. NFHS-5 (conducted in 2019-21) findings reveal that 21.3% of women and 24% of

men above the age of fifteen years have hypertension. The findings suggest that HTN prevalence is higher among men aged 15 years, 39%of women and 49%of men aged 15 years and above areata pre-hypertensive level. For both women and men, the prevalence of hypertension increases sharply with age (refer to Figure 1). About 25% of females and males aged 40-49 years have hypertension. One in eight females and nearly one in five males have been diagnosed with hypertension, at 30- 39 years of age [6].

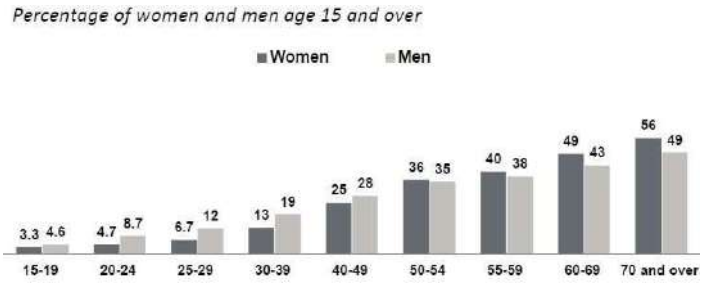

Fig 1. Hypertension Prevalence in Indian Population, Age and Sex, NFHS-5

Epidemiological studies in the country on hypertension in the last two decades have identified that Grade 2 HTN prevalence (diagnosed by systolic BP ⩾140 mm Hg and/or diastolic BP ⩾90 mm Hg) in the urban locality has stabilized (in between 25%–30%) however, it has amplified from 15% to 25% in rural areas [7]. Another study involving 1,267,786 individuals, aged15-49 years, showed that the nationwide predominance of hypertension was 18.3%, which has risen from the previous NFHS-4 survey. Men (21.6%) were identified to have a much greater prevalencein comparison to women (14.8%) [8].

Burden of hypertension in West Bengal

Hypertension significantly increases the burden of heart-related diseases, stressing our healthcare systems. Strengthening primary care and integrating hypertension management is crucial. The prevalence of HTN in West Bengal, as per a report published by the DOHFW, WB is 22% and hence about one crore of the adult population is estimated to suffer from hypertension. HTN is a silent destroyer, and efforts should be driven to control it with an uninterrupted medicine-based pharmacological intervention and lifestyle modification, which still is a major challenge [9].

Methodology

***Study design*:** This was a community-based, cross-sectional study. It was conducted among adult residents of Ward No. 115. Study received approval from the Institutional Ethics Committee of NSHM Knowledge Campus; vide approval no NSHMKOL/IEC/3/2024/PR-02.

***Sample size*:** The sample size was calculated for this study using the formula $n = z^2 pq/d^2$, where n represents the sample size, z is the statistic corresponding to the level of confidence, p denotes the expected prevalence, and d is the precision. At a 95% level of confidence, and a taking prevalence of 22% (mentioned in the earlier section) in this study, the sample size is calculated as 264. However, owing to the inaccessibility of citizens living in high-rise apartments and the paucityof time, the researchers could obtain data from 184 residents. The residents were selected by systematic random sampling.

***Study duration*:** Two months, May to June 2024.

***Study tools*:** Participants were surveyed by using a pre-designed and tested semi-structured questionnaire. The BP was measured for the participants (aged 18-40 years only) with the help of *Omron Upper Arm Blood Pressure Monitor* (HEM- 712IJ). The BP reading for every respondent was taken thrice, with a gap of five minutes between the readings. Participants whose meansystolic blood pressure (SBP) was 120-129mm Hg and/or whose mean diastolic blood pressure (DBP) was >80 mmHg were considered to have elevated BP and at risk of developing HTN. Data analysis was performed using MS Excel and SPSS. Verbal informed consent (IC) was acquired from all participants after a clear explanation about the purpose of the study. Assurance was provided regarding their anonymity and the confidentiality of all information that would be collected in the study.

Results and Discussion

Table 2 and Figure 2 depict the age-wise distribution of respondents.

33.7% respondents were in the age category18-25 years, 39.1% belonged to the 25-35 years age category, and the remaining (27.2%) were from age category36- 40 years.

Table 2. Age-wise distribution of Respondents

Age-wise distribution (years)	Number	Total (%)
18-25	62	33.7
25-35	72	39.1
36-40	50	27.2

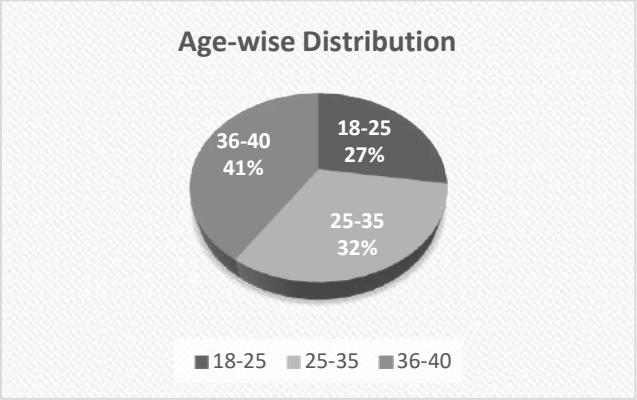

Fig 2. Age-wise distribution of Respondents

Table 3 and Figure 3 depict that 47.8% of participants were males and the remaining (52.2%) were females. 30.7% of males belong to the age category of 18-25 years, 43.2% were in the age category of 25-35 years, and 26.1% were over 35 years of age. Of the 96 women, 36.5% were in the age group of 18-25 years, 35.4% were in the age category of 25-35 years, and 28.1% were in the category of 35-40 years.

Table 3. Age and Sex-wise Distribution of Respondents

Age-wise distribution	18-25	25-36	>35	Total %
Male (N_m=88)	27 (30.7)	38 (43.2)	23 (26.1)	47.8
Female (N_f= 96)	35 (36.5)	34 (35.4)	27 (28.1)	52.2
Total Study Population	62 (33.7)	72 (39.1)	50 (27.2)	100

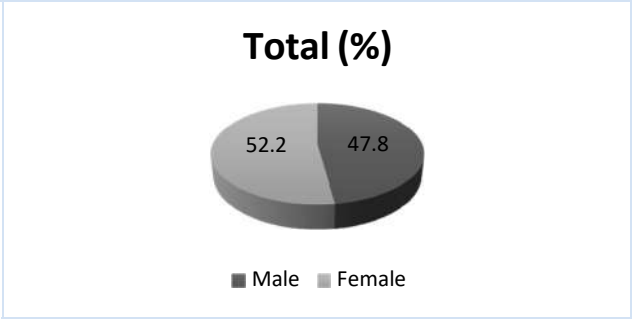

Fig 3. Sex-wise distribution of Respondents

Table 4. Sex-wise Distribution of Blood pressure in Respondents

Sex wise distribution	*Normotensive* Systolic BP (<120mm Hg) Diastolic BP (<80 mmHg)	*Elevated* Systolic BP (120-129mm Hg) Diastolic BP (<80 mm Hg)	*Grade 1 hypertension* Systolic BP(130-139 mm Hg) Diastolic (80-89mm Hg)	*Grade 2 hypertension* Systolic BP (≥140 mm Hg) Diastolic BP (≥ 90 mm Hg
Male	43 (48.9)	32 (36.4)	6 (6.8)	7 (7.9)
Female	49 (51.0)	29 (30.2)	10 (10.5)	8 (9.3)
Total	92 (50%)	61 (33.1%)	15(8.2)	16 (8.7)

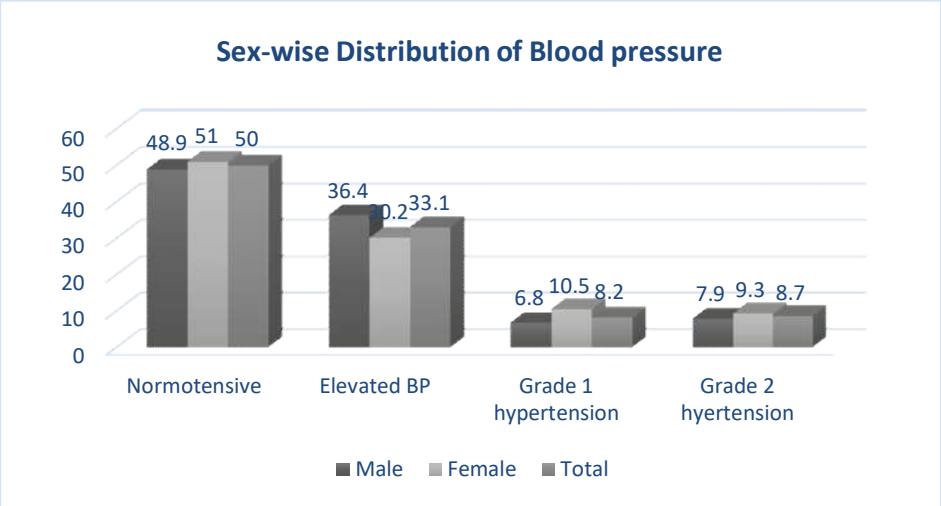

Fig 4. Sex-wise distribution of blood pressure in respondents

Percentage of males having normal blood pressure in our study population was established to be 48.9%, 36.4% had elevated blood pressure, while 6.8% and 7.9% of the male respondents were identified to have Grade 1 and Grade 2 levels of hypertension correspondingly. Among the female respondents, more than half of respondents (51%) in our sample population had normal blood pressure, 30.2% had elevated BP, and 10.5% and 9.3% had Grade 1 and Grade 2 hypertension respectively (refer to Table 4 and Figure 4).

The overall age-wise blood pressure distribution in the study population across the different age groups is illustrated in Figure 5.

Age * Blood Pressure Crosstabulation

			Blood Pressure				
			Normal Blood Pressure: Systolic <120 mm Hg and Diastolic <80 mm Hg.	Elevated Blood Pressure: Systolic 120-129 mm Hg and Diastolic <80 mm Hg	Hypertension Stage 1: Systolic 130-139 mm Hg or Diastolic 80-89 mm Hg	Hypertension Stage 2: Systolic ≥140 mm Hg or Diastolic ≥90 mm Hg.	Total
Age	18-25	Count	45	17	0	0	62
		% within Age	72.6%	27.4%	0.0%	0.0%	100.0%
	25-35	Count	36	23	7	6	72
		% within Age	50.0%	31.9%	9.7%	8.3%	100.0%
	above 35	Count	11	21	8	10	50
		% within Age	22.0%	42.0%	16.0%	20.0%	100.0%
Total		Count	92	61	15	16	184
		% within Age	50.0%	33.2%	8.2%	8.7%	100.0%

Fig 5. Age-wise Blood Pressure distribution across Age groups

Hypertension, traditionally seen in older adults, is increasingly affecting young adults and the middle-aged. A review study identified key risk factors of HTN in young adults, aged 18-50 years as unhealthy eating, lack of exercise, excessive salt and alcohol intake apart from smoking, obesity, genetics and stress factors. The impacts are significant, leading to long-term health risks such as cardiovascular disease and stroke, reduced quality of life (QoL), and substantial economic burdens. Addressing this requires increased awareness, lifestyle modifications, regular monitoring, and effective management strategies [10]. The study recommends the following interventions for managing hypertension in young adults-

- Lifestyle Modification: Encourage regular exercise, a balanced diet, and limit alcohol and tobacco use
- Regular Monitoring: Conduct routine blood pressure checks, especially for those with risk factors.
- Public Health Education and Awareness about hypertension risks and healthy living
- Stress Management: Use techniques like mindfulness and counselling to reduce stress.\
- Pharmacological Intervention: Use medication, if lifestyle changes are insufficient, tailored to individual need

Conclusion

In this study, it was found that more than 50% of the young adult population in our study area were in various stages of hypertension (Elevated, Grade 1 and Grade 2). Needless, to mention, therefore there is an immediate need for health intervention to assess and identify the risk factors and counselthe people for regular medication and preferred non-pharmacological management of the disease. Due to time constraints, the study was limited to a small sample and hence it cannot be generalizedto the whole population ofthe Kolkata Metropolitan Area (KMA), however, it could definitely pave out ideas for future research and health-related interventions. Adoption of a typical treatment practice for managing uncomplicated patients suffering from hypertension is a noble step towardsHTN prevention.

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Friendship Quality, Coping & Stress- A Correlational Study

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Abstract

This study explores the relationships between friendship quality, stress, and coping in emerging adults aged 18-25 years. Using a purposive sample of 42 participants, the research employs the Perceived Stress Scale, a coping questionnaire, and the McGill Friendship Questionnaire to assess these variables. The findings reveal a significant negative correlation between friendship quality and stress ($r = -0.812$, $p < 0.01$), indicating that higher friendship quality is associated with lower stress levels. Additionally, a significant positive correlation exists between friendship quality and coping ($r = 0.512$, $p < 0.01$), suggesting that better friendship quality enhances adaptive coping mechanisms. These results underscore the crucial role of high-quality friendships in mitigating stress and promoting effective coping in young adults, highlighting the importance of supportive social relationships for mental well-being during this critical developmental stage.

Keywords: Friendship quality, Stress, Coping, Emerging adulthood, Social support, Psychology.

Introduction

Stress is a common and inevitable aspect of human life, defined as the body's physiological response to challenging or threatening situations. It involves a complex interplay of psychological, emotional, and physical reactions. While a manageable level of stress can be adaptive, chronic or excessive stress can have harmful effects on mental and physical health, such as anxiety, depression, and cardiovascular issues [1,2].

Coping refers to the cognitive, emotional, and behavioral strategies individuals use to handle stressors and challenges in life [3]. These strategies can be broadly categorized into problem-focused coping, which involves directly addressing the source of stress, and emotion-focused coping, which involves managing one's emotional response to the stressor [4]. The effectiveness of coping strategies varies depending on the nature of the stressor and individual differences, with some strategies promoting resilience and others potentially leading to maladaptation [5].

Friendship is a key component of human social interaction, characterized by mutual affection, trust, and companionship [6]. High-quality friendships provide emotional support, a sense of belonging, and contribute to personal growth and mental health. They help reduce stress and enhance psychological well-being by offering safe spaces for self-expression, empathy, and emotional validation [7].

The quality of friendships can significantly impact one's ability to cope with stress, with higher friendship quality linked to lower levels of stress and better mental health outcomes. Understanding the interplay between friendship quality, coping mechanisms, and stress is crucial for promoting well-being and resilience. This study aims to explore the relationship between these variables, emphasizing the role of supportive friendships in managing stress and enhancing overall psychological health.

Method

Objectives of the Study

1. To explore the relationship between friendship quality and stress in emerging adults.
2. To examine the relationship between friendship quality and coping strategies in emerging adults.

Hypotheses

H01: There is no significant relationship between friendship quality and stress in emerging adults.
H02: There is no significant relationship between friendship quality and coping in emerging adults.

Research Design

This study employed a correlational design using a quantitative research paradigm.

Selection Criteria for Participants

Inclusion Criteria

1. Age: Participants aged between 18-25 years.
2. Educational Qualification: Participants with at least a 12th-grade qualified or higher education.
3. Friendship: Participants should have at least one friend.

Sample

The study utilized purposive sampling to select the participants. A total of 42 emerging adults, aged between 18-25 years, were recruited for the study. Among them 20 were males & 22 were females. The mean age was 20 years with a standard deviation of 2.11 years.

Tools Used

- **Sociodemographic Datasheet:** A sociodemographic datasheet was used to gather information on age, gender, educational level, occupation, marital status, and ethnicity. This sheet was essential for understanding the social & demographic background of the participants.
- **Perceived Stress Scale (PSS-10):** PSS-10 is a widely used 10-item questionnaire designed to assess the levels of perceived stress. [8]. The items measure the degree to which situations in one's life are appraised as stressful. Responses are scored by reversing the answers to the four

positively stated items and summing them to get the total score. The PSS-10 has demonstrated internal consistency, with Cronbach's alpha ranging from 0.71 to 0.91, and test-retest reliability above 0.70.

- **Coping Scale:** This 13-item scale, assesses cognitive, emotional, and behavioral coping methods. [9, 10]. Responses are scored on a scale, with higher scores indicating better coping mechanisms. The scale shows strong reliability, with internal consistency coefficients of 0.88 to 0.91, and validity evidenced by significant correlations with measures of regulatory strengths and well-being.
- **McGill Friendship Questionnaire:** This 15-item questionnaire assesses the quality and strength of friendships. The scale ranges from -4 to 4, with higher scores indicating stronger and more positive friendship qualities. The questionnaire captures various dimensions of friendship to provide a comprehensive assessment of interpersonal bonds. [11]

Procedure

Participants meeting the inclusion criteria were recruited for the correlational study. Rapport was established, and participants were informed about the study's purpose and the reason for their selection. Informed consent was obtained, with participants assured of confidentiality and the voluntary nature of their participation. Data collection was conducted in both online and offline mode to maximize participation. Participants were provided with assistance as needed while completing the sociodemographic datasheet, Perceived Stress Scale, Coping Scale, and McGill Friendship Questionnaire. After data collection, responses were scored and analyzed to determine the relationships among the study variables.

Statistical Analysis

Pearson product-moment correlation coefficient was used to assess the relationships between friendship quality, stress, and coping mechanisms in emerging adults.

Results

Table 1 presents the correlation matrix for friendship quality, stress, and coping, including the associated p-values.

Variables	**Stress**	**Coping**
Friendship Quality	-0.812**	0.512**

Note: Correlation is significant at the 0.01 level (2-tailed test).

The findings indicate a significant negative correlation between friendship quality and stress ($r = -0.812$, $p < 0.01$). This suggests that higher friendship quality is associated with lower stress levels in young adults. Conversely, there is a significant positive correlation between friendship quality and coping ($r = 0.512$, $p < 0.01$), indicating that better friendship quality is related to more effective coping strategies.

Discussion

This study aimed to explore the relationships between friendship quality, stress, and coping among young adults aged 18-25 years. Our analysis sought to determine whether friendship quality correlates with stress and coping mechanisms, hypothesizing no significant relationships between these variables.

Friendship Quality and Coping

Our findings revealed a significant positive relationship between friendship quality and coping ($r = 0.512$, $p < 0.01$). This indicates that higher quality friendships are associated with more effective coping mechanisms in young adults. This result aligns with previous research demonstrating that high-quality friendships contribute to better coping strategies and mental well-being. For instance, Smith et al. [12, 13] observed that adolescents with high-quality friendships exhibited more adaptive coping strategies and better mental health outcomes. Similarly, Jones et al. [14] found that young adults with supportive friendships reported lower perceived stress and enhanced stress resilience, with adaptive coping serving as a mediator in this relationship. These findings underscore the crucial role of high-quality friendships in providing emotional support, reducing feelings of loneliness, and fostering a sense of belonging, all of which facilitate better coping and stress management.

Friendship Quality and Stress

Additionally, our study identified a significant negative relationship between friendship quality and stress ($r = -0.812$, $p < 0.01$). This suggests that higher quality friendships are linked to lower stress levels. This finding supports the notion that supportive friendships play a protective role in mitigating stress. Berndt et al. [15] found that adolescents with high-quality friendships experienced lower levels of anxiety and depression, attributing this to the emotional support and sense of belonging provided by these relationships. Parker et al. [16] also highlighted that children with high-quality friendships demonstrated better social skills and resilience to stress. These results emphasize the importance of supportive and positive friendships in reducing stress and enhancing overall mental health.

Conclusion

Thus it can be concluded that:

I. there is a significant negative correlation between friendship quality & stress

II. there is a significant positive correlation between friendship quality & coping

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The role and underlying mechanisms of various nutraceuticals in the management of neurodegenerative diseases

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Abstract

A degenerative process involving several immune cells, neurodegeneration is defined by the progressive loss of the structure and function of neurons. It is the main cause of neurodegenerative disorders, which include dementia and a number of other symptoms. Age-related disorders: It is projected that 81.1 million individuals worldwide would be affected by these diseases by 2040.Although the mechanisms underlying neurodegenerative illnesses are intricate and multifaceted, there aren't many broad shared pathways, according to mounting data. These common mechanisms include inflammation, oxidative stress, intracellular Ca2+ excess, and dysfunctional mitochondria. Numerous routes frequently coexist, which restricts the advantages of treatment interventions. Because of their many benefits, nutraceuticals have become more and more significant in recent years. It is thought that these dietary-based strategies target several pathways in a more gradual and physiological fashion, avoiding serious side effects. The evidence that is now available is compelling in favour of the theory that nutraceuticals have the ability to slow down the ongoing degeneration of neurons in addition to preventing the initiation of neuronal damage. In this piece, we go over the common pathways that contribute to the pathophysiology of neurotoxicity caused by toxins and neurodegenerative diseases.

Keywords: Nutraceuticals, Herbal therapeutics, Neurodegenerative diseases, Mitochondria, Oxidative stress, Inflammation

Introduction

The phrase "neurodegenerative diseases/disorders" (NDDs) describes a wide range of debilitating, long-term illnesses that impact the central or peripheral nervous systems (PNS and CNS). The neurodegenerative disorders (NDDs), which include Alzheimer's, Parkinson's, Huntington's, and amyotrophic lateral sclerosis (ALS), are brought on by a progressive loss of neuronal degeneration, or the loss of structure or function of neurons, which ultimately culminates in the death of nerve cells in the human brain. Most of these NDDs cause behavioural problems, including depression, problems with mental functioning, or ataxias, or trouble moving [1]. These non-communicable diseases are growing increasingly widespread and a concern to global health due to a lack of effective treatments and changes in the population. While the precise etiology of neurodegeneration remains unclear, current studies indicate that a variety of established factors, such as elevated intracellular calcium (Ca2+) levels, oxidative stress, chronic inflammatory conditions, and damage to neuronal mitochondria, are responsible for neurotoxicity [2]. In the end, these elements cause a loss of motor, sensory, and cognitive abilities (such as memory impairment). Furthermore, because nutraceuticals can act simultaneously on many pathways linked to neuronal cell death, there is rising interest in employing them to reduce the clinical symptoms associated with non-degenerative diseases (NDDs) [3].

Novel nutraceuticals such as curcumin, α-lipoic acid, astaxanthin, coenzyme Q10 (ubiquinone), L-sulforaphane (isothiocyanate compound), tert-butyl hydroquinone, blueberries, resveratrol, carnosic acid, eugenol, emodin (3-methyl-1,6,8-trihydroxyanthraquinone), rosmarinic acid, aged garlic extract, anthocyanins, epigallocatechin-3-gallate (green tea flavonoid), mustard oil glycoside, retinoic acid, vitamin D, vitamin E, omega-3 polyunsaturated fatty acid, apigenin, soy isoflavones, and isoflavones have been shown to positively impact NDDs by altering one or more of the previously mentioned mechanisms of neurodegeneration. [2].Nutritional supplements like turmerin, astaxanthin, curcumin, β-carotene, α-lipoic acid, quercetin, lutein, lycopene, coenzyme Q10 (ubiquinone), resveratrol, and β-carotene contain antioxidants that have been shown to protect against oxidative stress-mediated diseases, aging, and neurodegenerative diseases[4].Worldwide, there are about 50 million cases of dementia, with 60–70% of cases being diagnosed as AD [5]. 5.8 million Americans over 65 suffer from AD; this number is expected to rise by 6.7% between 2020 and 2025 [6].Parkinson's disease (PD) is the second most common neurological disease that affects mobility, after Alzheimer's disease (AD). About 2% of adults over 65 have Parkinson's disease (PD); 6.1 million people globally had the illness in 2016 [7].Due to dietary choices and sedentary lifestyles, non-communicable diseases (NDDs) are becoming far more common in western countries as compared to Asian countries. Most non-disruptive disorders (NDDs) share a common pathophysiology that includes autophagy, increased stress on the endoplasmic reticulum (ER), altered cellular energetics (mitochondrial dysfunction), neuro-oxidative stress, and apoptosis[8].To treat NDDs and slow the rate of neuronal degeneration, any nutraceuticals with potent anti-inflammatory, anti-apoptotic, anti-protein aggregation, and antioxidant properties that can cross the blood-brain barrier (BBB) and suppress aberrant mitochondrial dynamics (multifaceted and high bioavailability rate) would be beneficial [9,10].

Scientific and technological developments have led to a rebirth of research on human nutrition and health, and they have also stimulated the discovery and use of various phytochemicals with medicinal qualities derived from both plant and non-plant sources. The effective use of food products in medicine and illness treatment has been proved by Indian civilizations for a very long time; Sumerians, Chinese, and Egyptians have been using Ayurveda for 5,000 years [11]. Philosophers had long endorsed nutrition for both personal and societal health before the nutraceutical idea was developed. From 2000 years ago, during the time of Hippocrates (460–377 BC), to the advent of modern medicine, it has been known that dietary practices have an impact on the range of illnesses that people now suffer from [12]. The educational non-profit for Innovation in Medicine in New York first used the term "nutraceuticals" in 1989 in an attempt to hasten scientific research [13]. It is requiring to establish an awareness to increase readers' understanding of the utilization of readily available substances with demonstrated neuroprotective benefits to treat neurodegenerative and psychotic illnesses using nutraceuticals.

Nutraceuticals Classification

On the basis of food source Nutraceuticals are classified into seven different types [1].

1. Dietary fibres-. Fruits, barley, oats, lignin, cellulose, and pectin are a few examples of fibres.
2. Probiotics- live microbial feed supplements are probiotics. When given in sufficient amounts, aid in restoring the host's gut microbial equilibrium. Examples include Sacromycescervicea, Bifidobacilli, and Lactobacilli.

3. Prebiotics-These are the nutritional components,Examples include beans, tomatoes, bananas, and chicory roots.
4. Polyunsaturated fatty acid- Fatty Acids Omega 3 Examples include docosahexaenoic acid, α-linolenic acid, and eicosapentaenoic acid.Fatty acid omega-
5. Antioxidant- Carotenoids, vitamin C, and vitamin E are a few of these.
   variety of fruits and vegetables are source of Antioxidant. They have the ability to inhibit lipid peroxidation and quench singlet oxygen.
6. Polyphenols- Plants produce these phytochemicals to guard against reactive oxygen species and photosynthetic stress. Anthocyanins, flavonoids, and phenolic acids are a few examples [14].

Mechanisms of Nutraceuticals in Neurodegenerative disease

Nutraceuticals work in a number of ways those are: (1) antioxidants and ROS/free radical scavenging; (2) antioxidants that target mitochondria or preserve mitochondrial homoeostasis; (3) anti-inflammatory; (4) anti-excitotoxic; (5) anti-apoptotic and caspase inhibitor; (6) alteration of cell signaling pathways; and (7) metal chelation.
Coenzyme Q10, resveratrol, astaxanthin, α-lipoic acid, curcumin, and isothiocyanate, have shown therapeutic benefit against numerous NDs. [15,16,17,18,19].
Numerous molecular signaling pathways that are known to be involved in cell survival and stress response are under the molecular control of nutraceuticals. Mitogen-activated protein kinase (MAPK) [22], protein kinase C (PKC) [23], Janus kinase -Signal Transducer and Activator of Transcription (JAK-STAT) [24,25], MEK/ERK/CREB, PI3K/AKT [26,27], and Nrf2/ARE are a few of these signaling pathways that incorporate insulin [20,21].

Nutraceuticals Targeting Oxidative stress

Many nutraceuticals scavenge reactive oxygen species (ROS) and, in a nuclear factor erythroid 2 -dependent manner (Nrf2-dependent approach), induce the production of cytoprotective proteins, which has both direct and indirect antioxidant benefits [28,29,30]. For the treatment of neurodegenerative disorders, the Nrf2-antioxidant response element (ARE) signaling pathway is a viable target [31]. In mesencephalic dopaminergic neurons and cell lines, pre-treatment with isothiocyanate compounds, such as L-sulforaphane, appears to prevent the neuronal death triggered by dopamine quinone. This is accomplished by decreasing membrane damage, stopping DNA fragmentation, and lowering the buildup of reactive oxygen species (ROS). Additionally, in a neuron-astrocyte culture, tert-butylhydroquinone and L-sulforaphane protected astrocytes and neurons against hydrogen peroxide-induced OS by activating the Nrf2-ARE transcriptional pathway [32,33].Blueberries are well-known for their ability to modulate ROS signaling through the MAP-kinase and CREB signaling pathways, hence providing neuroprotection. [34,35].Resveratrol's antioxidant qualities, capacity to alter Aβ processing, and capacity to upregulate the longevity-associated gene sirtuin1 all contribute to its neuroprotective effects. [36]. Because of their ability to scavenge ROS, rosmarinic acid and carnosic acid evoked a neuroprotective response both in vitro and in vivo [37]. It is commonly known that aged garlic extract prevents Aβ peptide-induced apoptosis in PC12 cells by reducing DNA fragmentation, caspase-3 activation, and reactive oxygen species (ROS) production [38]. The nutraceutical eugenol produced from cloves stopped the 6-OHDA-induced reduction in dopamine in the rat striatum by lowering lipid peroxidation [39]. Consuming foods high in antioxidants, such vitamin

C and E, has been linked to a decreased risk of non-communicable diseases (NDs), such as Alzheimer's and Parkinson's [40].

Nutraceuticals Targeting Inflammation

Anthocyanins are known to function by inhibiting phospholipase A2, which plays a negative part in a complex signaling network that connects pro-inflammatory cytokines and pro-oxidants to the production of eicosanoid and the release of arachidonic acid [41, 42]. Blueberries showed neuroprotection by altering the expression of genes linked to inflammation [43].Curcumin inhibited Aβ-induced cell death and nuclear factor kappa light chain enhancer of activated B cells (NF-κB) activation in the human neuroblastoma cell line, indicating a possible role for the substance in Alzheimer's disease treatment [44]. The flavonoids epigallocatechin3-gallate and curcumin, which are found in mustard oil, are well known for their ability to block pro-inflammatory molecular signaling through NF-κB or toll-like receptors, thereby maintaining the blood-brain barrier in patients with multiple sclerosis [45]. Extract of Korean red ginseng also enhanced the activation of p38, NF-κb, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinases (ERK) mitogen-activated protein kinase (MAPK) signaling pathway in the striatum of a model of Huntington's disease-like mice produced by 3-nitropropionic acid and inhibited the activation of microglial cells and proinflammatory mediators [46].

Nutraceuticals Targeting Mitochondrial Dysfunction

Curcumin, a substance derived from turmeric, lessened the neurotoxicity caused by 6-OHDA in MES23.5 cells by increasing the activity of Cu-Zn SOD, reducing elevated intracellular ROS and NF-κB translocation, and partially restoring the potential of the mitochondrial membrane [47]. α-lipoic acid, a potent mitochondrial stabilizer, shielded neurons against hypoxia, chemotherapy, Aβ, and other neurotoxins resulting from toxicants in both vivo and in vitro settings by maintaining mitochondrial activity [48]. Without producing more reactive oxygen species, astaxanthin enhanced energy production and shielded the mitochondria of the cultured nerve cells [49, 50]. Another strong antioxidant, coenzyme Q10, shields neurons in striatal excitotoxic injuries caused by the mitochondrial toxin and other NDs by maintaining mitochondrial activity [51, 52]. According to this research, nutraceuticals can stop NDs from developing and starting since they mainly maintain mitochondrial homeostasis.

Nutraceuticals Aimed toward Excitotoxicity and Calcium Overload

Extract of Blueberry has been shown to offer neuroprotection against Aβ by increasing intracellular Ca2+ and reducing ATP leakage brought on by aggregated Aβ [53]. Despite being a successful treatment approach, the potential of nutraceuticals to prevent and reverse intracellular Ca2+ excess has not been thoroughly studied [54].The extract of Uncariarhynchophylla induces the action of free radical scavenging and decreases lipid peroxidation in a rat model of excitotoxicity caused by kainic acid [55].From the ethyl acetate portion of the methanol extract of Eiseniabicyclis (brown algae), phloro-fucofuroeckol A, phloro-tannins eckol, and 7-phloroeckol were extracted. These compounds show their neuroprotective effect by reducing the amount of intracellular ROS production and intracellular Ca^{2+} in PC12 cells that are cytotoxicated by Aβ.

Furthermore, it has been discovered that diphloretho-hydroxy-carmalol inhibits intracellular Ca2+ levels and lowers the generation of ROS [56].

Apoptosis-targeting nutraceuticals

Product of beehives that is honey, uses its antioxidant and anti-apoptotic qualities to counteract the harmful effects of kainic acid, thereby protecting the brain from neuronal death and dementia. Ishigefoliacea is the source of diphlor-etho-hydroxy-carmalol, which has anti-apoptotic properties by increasing cell survival and inhibiting caspase-9 and caspase-3 [57]. The carotenoid compound called crocin is derived from Crocus sativus. It has been shown to have neuroprotective effects in vivo and in vitro in various CNS disorders. Additionally, it has been shown to decrease the expressions of CHOP and binding-immunoglobulin protein (BiP), which inhibits the pro-apoptotic factor caspase-12 activation in PC12 cells [58,59]. By inducing AMP activated protein kinase of the mammalian silent information regulator 1 signalling pathway, the naturally occurring phytoestrogen resveratrol enhances the autophagic breakdown of α-Syn in PC12 cells [60].

Conclusion

Owing to the constantly evolving human lifestyle, oxidative stress is frequently caused by an overloading of the antioxidant defence systems. Additionally, as people age, their levels of antioxidant defence mechanism decline noticeably. Numerous diseases could arise as a result of them. As a result, research during the previous few decades has mostly concentrated on various nutraceuticals. Products containing antioxidants can either naturally scavenge free radicals (such vitamins and polyunsaturated fats) or they can specifically boost the body's defences.

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The Hidden Side: A Study on The Lived Experience of Being A "Separated Child"

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Abstract

The present study explores the "lived experience" of 3 individuals whose parents are separated in Kolkata. In-depth interview was conducted with these participants. Interpretative Phenomenological Analysis was used as a method of analysis and interpretation. Four superordinate themes (Usage of defense as a part of dealing, Coping as a part of dealing, Resources as a mediating factor, Affective emptiness) and nine subordinate themes emerged from the analysis (Abreaction as a defense, Rationalization as a defense, Identification as a defense, Resource and resilience in the form of family, Resource and resilience in the form of mother, Resource and resilience in the form of friends, Emotional suffering and a sense of vacuum, Deficiency of nurturance need and Deficiency of need for affection). The findings have been discussed in terms of attitude expressed, coping, affective regulation, and various defense mechanisms used by participants to deal with the situation.

Keywords: Parental separation, childhood experience, defense mechanisms, coping mechanisms.

Introduction

In India, the law validates and guards the rights of both parties in a marital union during their judicial separation and divorce. In judicial separation, the partners continue to be legally married but do not cohabit. Whereas in divorce, the marriage is dissolved, and the partners are legally allowed to remarry [1]. In attachment theory, people should have a reliable emotional support system, or "primary attachment figure," to lean on no matter how challenging life may be [2]. There are four types of attachment: secure attachment, avoidant attachment, preoccupied attachment and disorganized attachment.

Attitude refers to people's lasting evaluations towards any object, person or situation [3]. These involve various kinds of behaviours, which develop due to positive and negative feelings resulting in inclining towards or avoiding the particular object, person or situation [4]. Allport [5]said that an attitude is a mental or neural state of readiness developed through experience, exerting a direct influence upon the individual's response to different objects. Attitude has three main components [6]:

Affective - refers to the emotions associated with the said stimulus, which might be pleasant or unpleasant

- Behavioural - referring to the way of expression or behaviour that results from the attitude.

- Cognitive - which includes the thoughts and beliefs that lead to the development of attitude towards an object, person or situation.

Parenting refers to the aspects of raising a toddler and there are several styles often employed by parents to raise their children [7]. Behaviours such as antisocial externalizing, temperament, parent and child negative affectivity, can be diminished from children through parenting [8]. Good nurturing helps a child to shape the future's social, cognitive and emotional development. During infancy, strong attachment is required between a child and a parent, for good bonding, better mental health for the child in the future [9].

Current research about the psychological impact of parental separation seems to focus on the affective dimension of the parent-child relationship [10]. The psychological well-being of the child is associated with how emotionally close they were with their parents prior to the separation [11]. Research has also indicated that children who come from families with separated parents, tend to psychosocially struggle to keep up with their peers from intact family units [12].

Method

- The present study aimed to explore the subjective experience of 'living' as a "separated child" and their "attitude towards their parents". The present study adopted a qualitative approach to data collection and analysis as these experiences and attitudes (attitude of young adults towards divorced or separated parents) can be thought to be relative to each individual and specific to the social context under study.

- **Understanding interpretative phenomenological analysis (IPA)**

IPA is an approach to qualitative research that is concerned with exploring and understanding the lived experience of a specified phenomenon. IPA involves the detailed examination of participants'

- Life-worlds
- Their experiences of a particular phenomenon
- How they have made sense of these experiences
- The meanings they attach to them

The other distinctive feature of IPA is the concept of 'double hermeneutic'. Smith and Osborn used the term 'double hermeneutic' to emphasize that two layers of interpretation:

1. The first is the participant's meaning-making
2. The second is the researcher's sense-making
3. Thus, there is an inevitable circularity in the process involving questioning, uncovering meaning, and further questioning; this circular process of understanding a phenomenon is called the "hermeneutic circle"

- **Operational definition of key constructs**

Parenting: defined as all actions related to the raising of offspring.

Separated Children: Separated children are those separated from either one of the parents or both parents, or from their previous legal or customary primary caregiver, but not necessarily from other relatives [13].

Parenting styles:

Researchers have described different human parenting styles—ways in which parents interact with their children [14]:

- Authoritarian parenting
- Authoritative parenting,
- Permissive parenting
- Rejecting–neglecting parenting

Attachment:

It is defined as the emotional bond between an infant and its parent figure or caregiver [15].

Divorce or Separation

The legal dissolution of marriage, leaving the partners free to remarry [16]. When the couple has children, the term **parental separation** is often used [17].

Attitude

An attitude is a mental and neural state of readiness, organized through experience, exerting a directive or dynamic influence upon the individual's response to all objects and situations with which it is related [5].

Table 1. Selection criteria for participants

Inclusion criteria	Exclusion criteria
• Age- 18-25 years • Sex- can be of either sec • Educational level- class 8 or above • Having divorced or separated parents.	• Presence of significant psychiatric illness • Presence of significant chronic medical illness

Sampling:

Purposive sampling was used for data collection which is a technique used in qualitative research for the identification and selection of information-rich cases for the most effective use of limited

resources. This involves identifying and selecting individuals or groups who are especially knowledgeable about or experienced with a phenomenon of interest.

The final sample consisted of 3 participants-

1. S.K.K. (23 Years)
2. S.M (18 Years)
3. D.K (22 Years)

Tools Used:

Information Schedule: A semi-structured interview was designed to elicit information pertaining to the name, age, SEX, educational level of the participants.

In-Depth Interview:

An in-depth interview was conducted to enable the participants to tell their daily lifestyle, life stories and to explore their experiences of being 'separated child' [18]. The interviews for each participant were conducted till "saturation" was achieved. Typically, each interview lasted for about 30 minutes to 45 minutes. The interview was conducted keeping in mind the following:

- The overall experience of living as a separated child.
- The kind of attitude they retain towards both parents.
- The kind of avowal towards parenting and attachment with parents.

Questions Of Interview: An in depth semi structured interview was conducted. The index questions are for reference. Leading questions and probe questions were asked during the interview.

Procedure:

The IPA approach was used to assess participant's perspectives of their experience and interpret their views. Participants were selected through 'purposive sampling' considering the inclusion and exclusion criteria. Data was collected through semi-structured interviews of each participant after building a proper rapport. Then, data was transcribed and coded (open code, focus code, and axial code). Then the coded data were analyzed and interpreted.

***Triangulation and validation*:** The data was transcribed by the researcher and simultaneously coded and interpreted by three researchers (the researcher, her supervisor and another researcher). Only those codes were sustained which were corroborated by at least two researchers.

***Ethical issues*:** The data was kept strictly confidential, and the identity of the participants was not disclosed. Since the participants would be discussing life experiences which are extremelypersonal and might evoke unpleasant emotions, participants were told that they can opt out of theresearch at any point even if they had given prior consent. This was done to ensure that the well- being of the participants is not affected.

Data Analysis

This section summarizes data that emerged from three in-depth individual interviews.

The data transcript was coded at all the levels of analysis and categorized into exploring themes emerging themes and superordinate themes by authors of the present study. The super-ordinate themes and emerging themes obtained from the interpretative phenomenological analysis are described as following:

Usage of defense as part of dealing a part of dealing

***Abreaction as defense*: -** participant has relived her past where she longed for her childhood:

"choto belar kotha boli…ghum theke otha, school e jawa, tarpore bikel bela khelte jawa theke khub bhalo bhabe hoto…baba amaye agge jamon baccha belaye agle rakto..."

***Rationalization as defense*: -** A sense of justification was reflected about their lives and the reason behind their parental separation:

"surroundings e dekhcho tomar friend circle o ache tader baba-maa dujon kei dekhcho..tokhon bujhte parcho tomar ekta life e keu ekjon nei but…tumi ekjoner karor story sune you can't judge..."

***Identification as defense*: -** Participant had shown an unconscious identification with a parent's views:

"baba actually prochondo…very kind hearted person khub e bhalo….kintu aage prochondo short tempered chile..mane aita chere diye as a person prochondo bhalo amni hisebe…"

Coping as a part of dealing

Most participants use coping strategies to deal with their situations in their lives. They have oscillated between various adaptive and maladaptive ones:

"akta social media addiction hoye gechilo, totally prochur addiction hoye gechilo…ami just ekhan theke beronor joono, eta theke berobo ki kore etai bhabchilam"

Resource as mediating factor

***Resources and resilience in the form of family*: -** Oneparticipant conveyed that she had received support from her family which helped her to deal with the situation:

"*Otyadhik close chilo amar sathe dadu. Dadu-I actually mul father figure bolle chole…Ma ektu fulfil korechilo but purotai dadu fulfil korto"*

***Resource and resistance in the form of mother*: -** Two participants said their mothers played a role in fostering a sense of growth and resilience within them**:**

"Maa j amay duto space deten dubhabe, okhanei ekta maa er figure ekta babar figure jeta ami pai ni seta actually fulfilled by one person…maa…Duto position e thik ache!"

***Resource and resilience in the form of friends*: -** A participants expressed how his friends have supported him in times of distress:

"*Amar friends circle besi boro na chotoi cho saat jon ache most of them are school friends….amra j kono jinis discuss korte chaile korte pari."*

Affective emptiness

***Emotional suffering and a sense of vacuum*: -** One of the participants expressed how distressing emotions impacted her life:

"Ar jakhon baba-maa totally emotionally unavailable hoye jaye…maa ke oo kichu bolte pari na tara emotionally kano ato unavailable thake ami sotti ii jani na"

***Deficiency of Nurturance needs*: -** One participant expressed a lack of nurturance in his life. He wished to have both the parents together as one family:

"*tahole erakomi dekhte hobe, je khane baba bujhbe, maa bujhbe…chele meye der just etai."*

***Deficiency of need for affection*: -** A participant conveyed that she felt a lack of affection among her family members:

"*…family te jhogra o hoi bhalobasha theke jekhane Jai chole asuk they willbe inseparable….. but at the end of the day oita mane joto khon tomara Amar kache toh oitai in the sense family."*

Discussion

This research study presents an overview of the attitudes of children towards their divorced or separated parents. The separation of their parents have led each individual to experience changes within themselves and in their dynamic relationship to their external environments. The presence of similarities and differences among the lived experiences of the participants has been noted. There were 4 superordinate themes and 10 sub-ordinate themes that were identified from the interview transcripts.

Usage of defense as part of dealing

The interview narratives of all the participants indicate the presence of defense mechanisms as a way of dealing with the separation of their parents. Children of divorce have tended to possess a more negative perspective of their external environment and often compare their situation to other children who belong to families that are intact [19].

Defense mechanisms are mostly emotion-focused coping strategies because they usually consist of attempts to deny, avoid or cognitively rearrange the building blocks of a triggering situation, in order to deal with the negative emotions that come along with it [20].

There is a relationship between the usage of defense mechanisms and coping behaviours among adolescents of divorced parents [21]. Some demonstrated "other-oriented" externalizing defenses like turning against others, and others have "self-oriented" internalizing defenses like turning against your own self [22]. One of the participants described how he was different from his peers,

as a child of separated parents. As he proceeded to sympathize with and justify his parents neglecting him and their lack of presence in his life, it indicated a denial of the neglect that the participant was subject to, and the usage of a "self-oriented" defense [20].

Coping as a part of dealing

It is evident that all of the participants took their parental separation as a stressful situation [23]. They experienced "negative affectivity" which represents a negative mood marked by anxiety, depression, and hostility [24]. She stopped talking to others and the whole day used her smartphone. Insecure coping practices, poses a risk to smartphone addiction [25]. Negative life events arouse negative emotions that leads individuals to interrupt ongoing goal pursuit to analyse the situation which affects their mental well-being [26]. On the other hand, negative emotion also evokes efforts to cope with the situation by moderating its effects on daily life [27].

Studies state that frequency of participation in leisure activities influence *immediate* adaptive outcomes, such as coping effectiveness and stress reduction, and *distal* adaptive outcomes, that is mental and physical health [28].

The participants also described the presence of "emptiness" at the initial stages of the stressful situation, which is quite normal as the first reaction to the stressful stimuli [29].

After using maladaptive coping, a gradual self-realization of doing inappropriate practices was developed in participants. One participant stopped "scrolling" her smartphone and questioned herself how to quit that situation positively [30]. These stressful events may not only result in negative outcomes but may also lead to some positive changes in people's lives known as "stress-related growth" [31].

Participants started to practice adaptive coping mechanisms. One participant started reading books instead of using the smartphone, which clearly means that she became task-focused and action-oriented [32].

One of the participants took "self-care" as an adaptive coping mechanism. According to"Self-care theory" by Orem [33], self-care is the production of actions directed to the self or the environment to regulate one's functioning in the interests of life, integrated functioning, and well-being. These behaviours help to make a sense of self-esteem and better self-image of the individual [34]

Resources as mediating factor

- Resource and resilience in the form of family:

One of the participants derives a great source of strength from the parent that fulfils the emotional role. It has been discovered that vital relationships with their immediate community or kin help children of divorced or separated parents to thrive emotionally [35].

- Resource and resilience in the form of mother:

All participants have evolved gradually from a negative to positive mental state. A sense of "self-realization" gave birth to a strong resilience in these participants.

One participant realized the struggles, responsibilities, and love of her mother. She made a strong bond with her and replaced all emptiness with that loving bond. Thus, she started to walk in the path of self-realization [36].

This pathway of self-realization means overcoming boundaries through personal involvement in actions that reflect moral and humanistic values[37]

The participant took her mother as an inspiration to her life. Inspiration is evoked by a person or object in the external environment [38]. The individual is moved by what is good or beautiful about that person or object [39].

The participant also started to make a close relationship with her mother and their relationship had evolved from a mother-daughter relationship to friendship. Friendships often involve cooperative interactions that are separated in time [40]

- Resource and resilience in the form of friends:

The participants usually form a small group of non-judgmental friends to avoid criticism and gossip about their personal life [41]. Parental divorce has an effect on the perception of the friendship and a somewhat greater effect on the experience of it[42]

Affective Emptiness

Children of divorced parents show lower self-esteem, relationship difficulties, insecure attachment, feelings of loss, abandonment and guilt [43]. The participants reported that feelings of numbness heightened when their peers talked about their own parental support and affection.

- Emotional suffering and a sense of vacuum:

Participants revealed that they were subject to bullying at a tender age, when their peers learned that they did not have both parents in the family [42]. They disclosed that bullying often made them feel less and they often put their luck or fate to question.

Due to lack of emotional support, they reported refraining themselves from socializing from the fear of judgement being passed about their family [44]. There are effects of divorce upon child behaviour in significant areas such as - peer relations, stress, aggression, work effectiveness at school [45].

They reported that their parents were dealing with their own issues and were unable to extend emotional support towards them [46].

- Deficiency of nurturance need:

The participants have had a suppressed need for nurturance. Children who experience parental divorce, exhibit more signs of psychological maladjustments, poor social coping and poor self-concept.

- Deficiency in need for affection:

Some of their constant need for support, love and nurturance, led them to identify with fictional characters (from books, television programs etc. Shortly after the separation children need more attention, care and affection from the immediate caregiving parent, as they identify the two parents in one parent

Conclusion

This study contributes to our understanding of the lived experiences and attitudes of individuals whose parents have separated, specifically highlighting the importance of emotional regulation, coping mechanisms, attachments and resilience. Despite experiencing significant adversities, participants have expressed a sense of hope, optimism, emotional maturity and presence of external as well as internal resources to deal with the situations. To build inner strength, utilize coping mechanisms, be intrinsically and extrinsically motivated and find meaning in their life experiences was evident among the participants. Their hope and resilience emerged as psychological protective factors that helped them to navigate and deal with the hardships they have encountered.

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APPENDIX 1: INFORMATION SCHEDULE

INFORMATION SCHEDULE:

NAME :

AGE :

GENDER :

EDUCATIONAL QUALIFICATION :

AGE AT WHICH GET TO KNOW ABOUT PARENTAL SEPARATION

DETAILS OF FAMILY MEMBERS :

NUMBER OF FAMILY MEMBERS :

RELATIONSHIP WITH THE FAMILY MEMBERS :

HABITAT :

APPENDIX 2: INTERVIEW QUESTION

Domains	**Questions**
Psycho-social Aspect of the individuals' life	Can you please tell me about your day-to-day life experiences before and after the separation of your parents.
Emotional Aspect of the individual	When you first heard the news of separation, what kind of feelings did you experience? If you can disclose it to us, it will be really helpful.
Attitude of the individual	How do you perceive an ideal family picture?
Positivity in the life of the individual	What factors have helped you to deal with the situation?

Nanotherapeutics: a non-invasive approach to bypassing the blood-brain barrier via nasal drug delivery

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Abstract

Nanoparticle-based systems have shown remarkable potential in overcoming the challenges of delivering drugs to the brain, particularly through the intranasal route. The blood-brain barrier (BBB) presents a significant obstacle to the systemic administration of therapeutics, limiting drug efficacy in treating neurological diseases such as Alzheimer's and Parkinson's disease. Intranasal delivery bypasses the BBB, utilizing the olfactory and trigeminal pathways for direct brain targeting, reducing systemic side effects. Nanoparticles, including polymers, lipids, and drug nanocrystals enhance drug bioavailability and absorption in the brain. These carriers are designed to navigate physiological barriers, improve drug stability, and offer controlled release. Surface modifications by various ligands, and permeation enhancers further optimize brain targeting, as demonstrated in various preclinical models. Despite promising preclinical results, clinical translation remains limited, especially for drugs against neurodegenerative diseases. Continued innovation in nanotechnology holds potential for increasing therapeutic efficacy, improving patient outcomes, and facilitating regulatory approval for nasal drug delivery to the brain.

Keywords: Nanotherapeutics, non-invasive approach, blood-brain barrier, nasal drug delivery, controlled release

Introduction

Nanoparticle-based drug delivery systems have gained attention for treating central nervous system (CNS) disorders by bypassing the blood-brain barrier (BBB), a major obstacle to drug delivery. The BBB limits the entry of therapeutic agents, making it challenging to treat conditions like Alzheimer's disease, Parkinson's disease, and brain tumors. Traditional methods, such as oral or intravenous delivery, often fail due to low bioavailability caused by the restrictive BBB [1, 2].

Nose-to-brain (NtB) drug delivery offers a non-invasive alternative, using the direct connection of nasal cavity to the brain through the olfactory and trigeminal nerves. It bypasses the BBB, enhancing therapeutic outcomes while reducing systemic side effects [3]. Nanoparticles (NPs) are critical to this approach, encapsulating drugs and protecting them from degradation. Biopolymer-based NPs, known for their biocompatibility and ability to release drugs in a controlled manner, are particularly effective in reaching the brain [4-6]. Studies show NP-based NtB systems improve

bioavailability, therapeutic efficacy, and reduce side effects. As research advances, more sophisticated NP designs could transform the treatment of neurological diseases, offering new hope for patients [7].

Challenges and Barriers for Nose-to-Brain Drug Delivery and Its Solutions

Clearance of the Mucociliary Tract

This natural defensive system decreases the amount of time that drugs remain on the nasal mucosa, while also assisting in the removal of toxins and foreign objects from the cavity inside the nose. Consequently, drugs are frequently rapidly excreted from the body before they have a chance to fully enter the brain. This fast clearance particularly affects large particles and formulations that aren't meant to stick to the mucosal surface, which reduces their efficacy. The bioavailability of drugs in the brain is further complicated by the various environmental influences, and diseased condition. Consequently, techniques like mucoadhesive polymers and mucopenetrating carriers are being investigated to prolong the drug retention in the nasal cavity [8-10].

Drug Attributes

Large molecular size of the drug limits the efficiency of nose-to-brain delivery by impeding absorption. A drug's ability to pass through the nasal epithelium is influenced by its water-lipid affinity, while nasal enzymes and pH can compromise its chemical stability. Metabolism and efflux transporters also reduce active drug bioavailability [8].

Nasal Architecture

The nasal cavity's complex structure, including mucosal thickness, surface area, and airflow patterns, impacts drug absorption and brain delivery. Variations in these features can lead to inconsistent drug distribution. Structural issues like septum deviations or polyps can further hinder drug delivery. Adaptable delivery systems are needed to address these anatomical differences for effective brain targeting [8].

Formulation Stability

The nanoformulations face challenges such as pH fluctuations, enzymatic activity, and rapid mucosal turnover, affecting drug stability and absorption in nose-to-brain delivery. Nasal enzymes degrade drugs, while pH variations destabilize sensitive formulations. High mucosal turnover limits adhesion, reducing absorption time. Drug transport to the brain may be enhanced, retention can be prolonged, and stability can be improved by using strategies such as particle encapsulation, mucoadhesive polymers, enzyme inhibitors, and penetration enhancers [8].

Limited Dose Size

A notable constraint of nasal drug administration is the maximum dosage size that may be given. One nostril can only receive 50–250 μL of drug at a time. This constraint poses a challenge for drugs that require high doses to achieve therapeutic effects, as such drugs cannot be effectively delivered via the nasal route. Consequently, the effectiveness of treatments needing larger doses is compromised, limiting the applicability of nasal drug delivery for certain therapeutics [9].

Pathways for Nose-To-Brain Delivery

Olfactory nerve pathway

The olfactory mucosa allows the drugs to enter the nose and pass through the BBB. Neurons called olfactory receptors, which are in charge of transduction, comprise the olfactory mucosa. Within the cilia of the olfactory receptor is where transduction takes place. The compounds are transported via paracellular and transcellular pathways to the olfactory receptor neuron. It enters the brain through the cribriform layer and is carried via nerve bundle axon to the surface-level olfactory bulb. The intestinal fluid that is present in the brain helps the drug to be transported from the cerebrospinal fluid to the brain [9, 11].

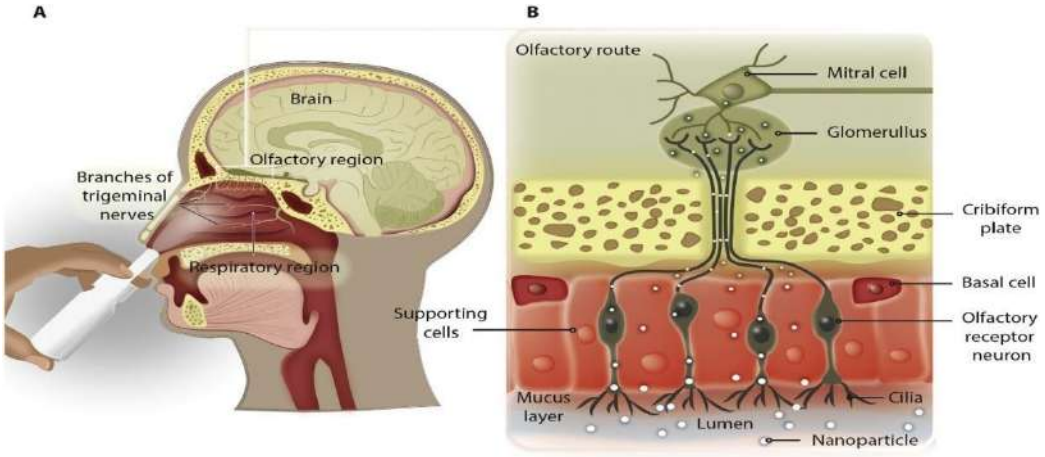

Fig.1. Anatomy of Olfactory nerve pathway and Trigeminal nerve pathway [12]

Trigeminal nerve pathway

The spine, pons, and other parts of the brain's posterior region are connected by the trigeminal pathway. Axonal transport, also known as endocytosis or intracellular transport, is the method to distribute the drug through the trigeminal nerve route or nose. This largest of the five cranial nerves is the trigeminal nerve, which has three branches: the ophthalmic, mandibular, and maxillary. Since, the drug enters the nasal mucosa through neurons and ends in part in the olfactory bulbs, the ophthalmic and maxillary branches of those three branches are the most important for the nasal- to-brain drug delivery pathway[1, 7].

The dorsal region of the anterior nose and the nasal mucosa are innervated by nerves in the ophthalmic portion of the trigeminal pathway. Drug enters the nasal cavity through the mucosa and proceeds to the trigeminal nerve located in the olfactory respiratory region via the brain stem transporter or the axonal route. The region of trigeminal nerve which passes through cribriform region may get involved in delivering the therapeutic moiety from nasal cavity to forebrain [11-14].

The potential use of nanotechnology in nose-to-brain delivery

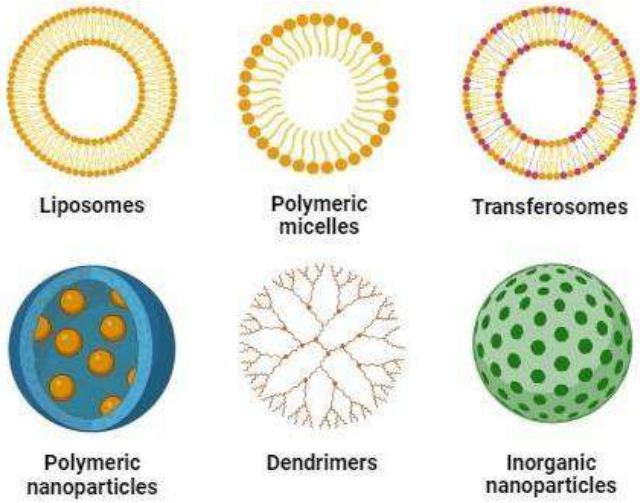

Figure 2: Various nanocarriers implemented for nose-to-brain drug delivery

Lipid-based nanoparticles

For nose-to-brain delivery of drugs, lipid-based nanoparticles such nanostructured lipid carriers (NLCs) and solid lipid nanoparticles (SLNs) show great promise. These lipids, which might be synthetic or natural, provide improved bioavailability, biocompatibility, and biodegradability. SLNs have a solid lipid matrix, ideal for lipophilic drugs, while NLCs blend solid lipids and oils for versatile drug delivery. Their small size and lipophilic nature enable efficient drug transport across the nasal epithelium with controlled and prolonged drug release [15, 16].

Nanostructured lipid carriers (NLC)

Advanced lipid nanoparticles known as nanostructured lipid carriers (NLCs) were developed to get over the drawbacks of solid lipid nanoparticles. They are made up of liquid and solid lipids, which increase the solubility of hydrophobic compounds and raise the effectiveness of drugs encapsulation. This makes them particularly suitable for nose-to-brain drug delivery. NLCs use biocompatible lipids such as triglycerides and fatty acids, ensuring lower toxicity and better drug protection. Their structure increases drug-loading capacity, minimizes leakage, and offers controlled release. While NLCs excel at encapsulating hydrophobic drugs, their capacity for hydrophilic drugs is limited [15, 17].

Noorulla et al., prepared and modified Chitosan based NLCs to transport buspirone to the brain through the nasal drug delivery. They utilized solvent diffusion evaporation technique to make these nanocarriers CH-coated. Oleic acid and glycerol monostearate was chosen as the lipid mixture, while tween 80 acted as the surfactant. This formulation showed an impressive 3.06-fold increase in AUC value as compared to intravenous dosing in nasal delivery [18].

Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are colloidal particles intended for transporting drugs from the nose to the brain. SLNs are between 100 and 400 nm in size and are made of a biodegradable lipid

substrate that remains solid at body temperature. Their hydrophobic core, supported by an outer layer of surfactant, may encapsulate hydrophilic and lipophilic drugs. Since this structure promotes drug absorption through nasal mucous membranes and facilitates drug dispersion, SLNs are perfect for nose-to-brain applications [8, 15, 17].

Yasir et al., developed solvent-free SLNs that contain buspirone (BUS) for nose to brain administration. The method was optimized using a three-factor, three-level Box-Behnken design, with particular attention paid to the drug-to-lipid ratio (A), surfactant concentration (B), and sonication time (C). Compared to BUS-Sol, BUS-SLNs exhibited superior ex-vivo permeation. The drug's confinement within the lipid core was verified by differential scanning calorimetry and X-ray diffraction. When delivered intranasally, BUS-SLNs demonstrated 2.18 times more brain bioavailability than BUS-Sol. [19] As a possible substitute for polymeric nanoparticles, SLNs have advantages including longer nasal cavity retention duration, regulated drug release, and biocompatibility. However, challenges like non-uniform particle formation and limited shape options can affect drug release consistency. Despite these drawbacks, SLNs have a high degree of stability and minimal toxicity, which supports their potential for nose to brain drug transport [8, 15, 17].

Liposomes

Liposomes are phospholipid bilayer membranes that are enclosed and have hydrophilic cores. Lipid-soluble substances can be imbedded in phospholipid bilayer membranes, while water-soluble substances can be contained in hydrophilic regions. Liposomes' surface charge increases the duration of contact between the drug and the mucus membrane, increasing the bioavailability of the drug while also shielding the encapsulated biomolecules from the enzymes in the nasal mucosa. Liposomes are safe for long-term usage and do not harm the nasal mucosa. They also don't irritate the cilia or induce toxicity [16-17, 20].

Saka et al., formulated imatinib mesylate liposomes that allows for prolonged drug release for up to 96 h for Alzheimer's disease, with a particle size of less than 150 nm. Tests for cytotoxicity on N2a cells revealed no harm at doses up to 25 μg/ml. When delivered intranasally, the liposomes considerably boosted brain deposition compared to normal drug solutions, with greater drug penetration and longer brain retention [21].

Nanoemulsions

Drugs may be efficiently delivered from the nose to the brain with the help of nanoemulsions (NEs). They are composed of two immiscible liquids, usually water and oil, that are stabilized by surfactants to produce emulsions that are either water-in-oil or oil-in-water, with droplet sizes ranging from 20 to 200 nm. The small size of these droplets enhances stability, preventing issues like sedimentation or coalescence, while giving the emulsions a transparent or milky appearance. [15, 17, 22].

Bayanati et al., prepared an in-situ gel nanoemulsion containing temozolomide. The mucoadhesion was higher than the control. In addition, in situ and traditional nanoemulsions showed improvements in nasal penetration. Moreover, brain accumulation of the nanoemulsion was verified by gamma scintigraphy investigations [23].

These nanoemulsions improve drug solubility, protect against environmental factors like pH and oxidation, and facilitate drug absorption. Mucoadhesive polymers such as chitosan can be used to

increase nasal residence time, enhancing drug delivery efficiency. While nanoemulsions can be unstable over time, their ability to improve drug absorption and deliver therapies directly to the brain makes them a valuable tool for treating neurological conditions [15, 17].

Polymeric nanoparticles

Therapeutic substances can be more efficiently transported from the nose to the brain with the help of polymeric nanoparticles. These nanoparticles, which are typically 100–200 nm in size, are composed of either synthetic or natural polymers. While synthetic polymers such as polycaprolactone offer flexibility in drug loading and stability, natural polymers like chitosan, albumin, and gelatin offer biocompatibility. Drugs can be entrapped or chemically conjugated within or on these nanoparticles, enabling controlled and sustained release [8, 15-16].

Ahmad et al., synthesized chitosan nanoparticles by ionic gelation with sodium TPP serving as the cross-linking agent, and PLGA nanoparticles by solvent evaporation techniques. The chitosan nanoparticles exhibited the best encapsulation effectiveness for L-Dopa, and zeta potential of 46.2
± 2.3. FTIR showed no chemical changes upon encapsulation, whereas XRD verified L-Dopa's amorphous form. C2 showed a nearly two-fold increase in AUC compared to L-Dopa solution, despite a 90-mindelayed T_{max}, and demonstrated improved drug release due to chitosan's mucoadhesive properties [24].

Surface modifications, such as using chitosan for mucoadhesion or thermosensitive poly lactic acid for targeting brain tumors, enhance their effectiveness. By protecting drugs from degradation and ensuring a steady release, polymeric nanoparticles minimize dosing frequency and reduce potential toxicity. Their capacity to enhance the solubility and stability of drugs, together with targeted delivery makes them potential carriers for drug delivery to specific organs and tissues [15, 16].

Polymeric micelles

Nanostructures known as polymeric micelles play a pivotal role in the efficient transfer of drugs from the nose to the brain. Amphiphilic block copolymers with hydrophilic and hydrophobic chains are used to manufacture these micelles. These copolymers self-assemble into spherical micelles when dissolved in water above a critical micelle concentration (CMC). The micelles consist of a core-shell structure, whereby the hydrophilic shell solubilizes hydrophilic drugs and stabilizes the micelles in water, while the hydrophobic core encapsulates drugs that are hydrophobic. This arrangement makes it possible to transport various drugs directly to the brain. Polymeric micelles can bypass the P-glycoprotein efflux system through receptor-mediated endocytosis, enhancing drug permeability and retention at the nasal mucosa, leading to improved therapeutic outcomes [8, 15].

Sipos et al., examined the permeability and dissolution tests of polymeric micelles conducted *in vitro* to fulfill the requirements for intranasal administration. Compared to the pure drug, polymeric micelles had five times more nasal permeability and almost twenty times quicker drug dissolution [25].

Transferosomes

Transferosomes represent a novel class of vesicular drug delivery vehicles intended for precise, regulated nose-to-brain administration. They consist of an edge activator, generally a single-chain

surfactant, coupled with a lipid bilayer, mostly composed of phosphatidylcholine. By destabilizing the lipid bilayer and increasing its flexibility and deformability, this surfactant enables transferosomes to pass through narrow pores in biological membranes [15, 26].

Because of its deformability, transferosomes can effectively deliver the drug to the brain through the nasal mucosa, avoiding the blood-brain barrier. Designed for controlled release, transferosomes ensure steady drug levels, improving treatment efficacy for neurological disorders while minimizing off-target effects. Their stability and biocompatibility make them suitable for a range of drugs [27, 28].

Mirza etal., manufactured transferosomes loaded with cefepime using the Box-Behnken design optimization process and thin film hydration technique. It exhibited higher deformability index and sustained drug release. Ex vivo studies showed a 34-fold higher permeation than conventional gel, while in vivo studies confirmed improved brain targeting through intranasal delivery [29].

Dendrimers

Dendrimers are highly branched, nanoscale polymeric structures suitable for delivering drugs from the nose to the brain. They have a central core from which many symmetrical branches extend outward, giving them a remarkable three-dimensional shape in aquatic environment. The core, with at least two identical chemical functionalities, initiates the growth of the dendrimer. Repeated branching forms concentric layers or ‘generations’, becoming denser at the periphery and more open at the core [30].

This structure allows dendrimers to encapsulate substantial amounts of therapeutic agents. Their surface can be chemically modified to enhance interaction with specific cells, improving targeted delivery and reducing off-target effects. PAMAM is one example of a dendrimer that may enter brain cells and pass across BBB, offering potential for treating neurological diseases with reduced toxicity [16, 20].

Singh et al., compared to the control donepezil (DZ) solution, the DZ (ester)-polyamidoamine (PAMAM) (PDZ) formulation dramatically enhanced DZ bioavailability in Sprague-Dawley rats. The bioavailability of PDZ was almost four times more than that of DZ alone. Additionally, the PDZ formulation improved DZ brain penetration, which might be probably owing to PAMAM conjugation [31].

Inorganic nanoparticles

Inorganic nanoparticles composed of metals, such as silver and gold, or metal oxides, including silica and iron oxide. Due to their unique characteristics, they show promise for nose to brain drug delivery. They enhance imaging and therapy through high surface areas, tunable pore sizes, and ease of functionalization. Their capabilities include controlled drug release and targeting specific brain regions. For instance, mesoporous silica nanoparticles (MSNs) offer extended drug release, while magnetic nanoparticles can be guided by external fields for precise delivery. Quantum dots and gold nanoparticles enable real-time tracking through fluorescence. However, potential nasal mucosa irritation and toxicity require careful assessment to ensure patient safety and effective delivery [16].

Marketed drug delivery system for the nose to brain

Table 1. Marketed drug delivery system for the nose to brain

Device Name	Company Name	Formulation	Active ingredients	Indication	References
TOSYMRA™	Upsher-Smith Laboratories	nasal spray	sumatriptan	migraine	[32-35]
ONZETRA® Xsail®	Currax Pharmaceuticals LLC	nasal powder	sumatriptan	migraine	[32,33]
ZOMIG®	Amneal Pharmaceuticals	nasal spray	zolmitriptan	migraine	[32,33,36,37]
MIGRANAL®	Bausch Health Companies Inc.	nasal spray	dihydroergotamine (DHE) mesylate	migraine	[32,33,36]
IMITREX®	GlaxoSmithKline	nasal spray	sumatriptan	migraine	[32,33,36,37]
Goprelto	Genus Lifesciences Inc.	Nasal solution	Cocaine hydrochloride	Local anesthetic	[33,34,38]
SPRAVATO®	Janssen Pharms	nasal spray	Esketamine Hydrochloride	Treatment resistant depression (TRD) in adults	[33,35]

Conclusion and Future Outlook

Bypassing the blood-brain barrier, nose-to-brain delivery of drugs using nanoparticles provides a viable treatment option for diseases and disorders of the central nervous system (CNS). This non-invasive strategy reduces systemic adverse effects while improving drug targeting. While advances have been made in the field of nanotechnology, challenges such as low bioavailability and potential brain toxicity persist. Future developments in nanotechnology, precision medicine, and intranasal gene therapies could transform this field. However, further research is required to optimize nose- to-brain drug delivery systems, reduce toxicity, and develop delivery devices for selective drug targeting. As research continues, nose-to-brain delivery of drugs has the potential to revolutionize the management of CNS disorders.

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Parenting Style and Family Type: Connecting Them to Subjective Wellbeing and Self Concept

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Abstract

The study aimed to explore the association between parenting style (authoritative parenting, authoritarian parenting and permissive parenting) and family type (joint family and nuclear family) and to explore the association between the family types (joint family and nuclear family) in terms of subjective well-being and self-concept among emerging adults (18-29 years). The research design was quantitative. The study involves 80 participants from nuclear and joint families. The tools used in this study were Perceived Parenting Style Scale, Robson Self-Concept Questionnaire and Subjective Well-Being Inventory. Data was analyzed using Chi-Square Test of Association and Student's t-test in SPSS 20. The study investigated the nature of parenting style, subjective well-being, self-concept in the context of family types (nuclear family and joint family). The findings suggest no significant association between parenting style and family type and; no significance mean difference between the types of familyconsists of nuclear and joint family in terms of subjective wellbeing and self-concept among emerging adults.

Keywords: Family types, parenting styles, self-concept, subjective wellbeing, emerging adult, quantitative study, association

Introduction

Parenting styles significantly influence a child's development, with styles ranging from authoritative to permissive. Early researchers developed various styles, including ambivalent parenting, submissive and dominant parenting, emotionally overinvolved and uninvolved, over controlling-neglectful, inhibiting-permissive. Baumrind's theory on parenting style in the 1960s and 1970s identified three designs: authoritarianism, authoritativeness, and permissiveness. These styles have different outcomes on children's social competence and cognitive development.

Baumrind's parenting style, characterized by parental demandingness and responsiveness, was updated by Maccoby & team who classified permissive parenting into permissive-indulgent and permissive-neglecting styles, with Baumrind's focus on permissive parenting. (1)

Types of parenting styles are described below:

Authoritarian parenting involves strict rules and one-way communication, with children expected to obey without questioning or negotiation. This style is rigid and expects no errors. Children with authoritarian parents often behave well at home to avoid punishment, but may struggle with

emotional maintenance, self-esteem, and decision-making. Drawbacks include difficulty maintaining emotions and making decisions independently.

Authoritative parenting is a nurturing approach where parents establish clear boundaries and maintain open communication with their child. This style encourages independence and encourages open communication. Discipline is not used as punishment but as a support mechanism, fostering good communication between parents and children. This approach yields the healthiest outcomes, requiring patience and effort from both parents.Authoritative parenting styles foster responsibility, confidence, self-regulation, and emotional resilience in children. These styles encourage independence, self-esteem, and achievement, leading to higher academic achievements and better school performance.

Permissive-indulgent parenting involves those parents who are emotionally sensitive, nurture their child's emotional and physical needs, provide adequate attention and care. Here parents prioritize their child's happiness over rules, setting few rules. This style often lacks self-discipline and self-control, but can lead to high self-esteem in children raised by permissive-indulgent parents.

The term **"family type"** refers to the different structures and compositions of families based on various factors such as marriage, residence, ancestry, and size. These types of families are essential in understanding the social dynamics and roles within a family.

A **nuclear family** consists of two parents and their children, known for their close relationship and defined roles. It is seen as a common and modern family form, offering emotional support, financial stability, and a sense of belonging to its members.

A **joint family**, also known as an extended family, consists of several generations and relatives staying together in a shared household. It emphasizes unity, with grandparents, aunts, uncles, cousins, and extended family members included. This structure is common in traditional societies, providing security, support, and continuity.

Subjective well-being (SWB) is individuals' self-assessment of their lives, consisting of cognitive judgments and emotional reactions. Coined by Diener SWB includes life satisfaction, positive affect, and negative affect. (2) Life satisfaction reflects overall quality of life, while positive affect concerns positive emotions like love and joy, and negative affect relates to negative emotions such as anger and stress. Higher SWB is associated with life satisfaction and frequent positive emotions, while lower SWB is linked to dissatisfaction and frequent negative emotions.

Self-concept is how one views themselves, including attitudes, feelings, and beliefs about their abilities and roles. Coined by Carl Rogers, it consists of the ideal self, real self, and self-image, influencing behavior and development.

The spheres of self-concept include:

The **ideal self** is the person one aspires to be, shaped by personal desires and societal influences. It motivates individuals to attain desired qualities and guide them towards self-improvement.

The **real self**, or actual self, is an individual's true nature including characteristics, inclinations, and feelings shaped by experiences.

Self-image is how one sees oneself, including physical appearance, personality, and values. It is crucial for well-being, with positive self-image as seeing oneself as smart, attractive, and happy.

Self-esteem is the level of self-confidence and self-satisfaction a person has, including self-respect and self-worth, affecting self-perception and value. It's influenced by genetics, upbringing, experiences, health, and thoughts, changing over time.

Emerging adulthood the term proposed by psychologist Jeffrey Arnettis a distinct developmental period bridging adolescence and young adulthood, typically from ages 18 to 29. It involves exploration, self-focus, and uncertainty, as individuals are no longer fully dependent but not fully adult responsibilities. This phase lays the foundation for adulthood through diverse experiences and challenges in career, relationships, and personal growth.
Thus the aim of the current study is to explore the association between parenting style (authoritative, authoritarian and permissive) and family type (joint family and nuclear family) in terms of subjective well-being and self-concept among emerging adults (18-29 years).

Method

***Objectives*:**

- To explore the association between parenting styles & family type.
- To examine the effect of family type on subjective well-being & self-concept.

***Hypothesis*:**

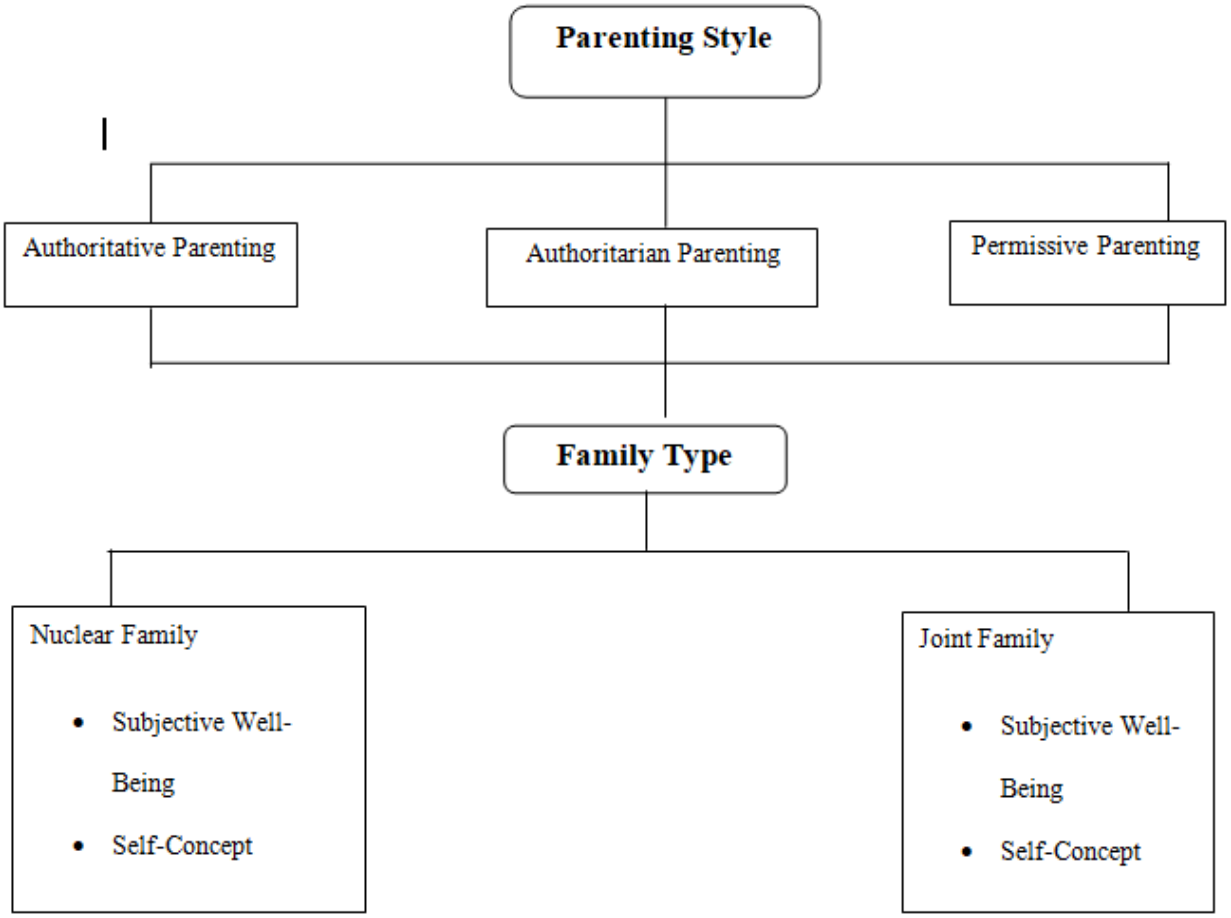
Ho1: There is no significant association between parenting styles (Authoritative, Authoritarian and Permissive) and family type (nuclear and joint).

Ho2: There is no significant mean difference between 2 types of family namelynuclear family and joint family in terms of subjective well-being for emergingadults.

Ho3: There is no significant mean difference between 2 types of family namelynuclear family and joint family in terms of self-concept for emerging adults.

***Research Design*:**

This study is conducted under a quantitative research paradigm.



***Sample*:**

There are 80 emerging adults (N= 80), 39 emerging adults (18 to 29 years) from joint family, and 41 emerging adults (18 to 29 years) from nuclear family. The mean age of emerging adults is 25.05 years and SD = 6.01 years.

Inclusion –

i. All participants were between 18-29 years of age.
ii. All participants are currently living with their families.

Exclusion –

i. All individuals whose parents are divorced or one of them deceased or orphans.

Tools Used

Perceived Parenting Style Scale (PPSS)

The Perceived Parenting Style Scale, was developed to measure children's perception about the behavior of their parents. It measures the perceived parenting style of the subject in

three dimensions such as authoritarian parenting, authoritativeparenting, and permissive parenting. The scale consists of 30 items in whichresponses are elicited on a five-point Likert scale.Instructions are given in clear and simple language in both English and Malayalam language. The responses on the items are elicited interms of 5-point Likert scale such as Strongly Agree (5), Agree (4), Neutral (3), Disagree (2), and Strongly Disagree (1). All the items on thescale are worded positively and scored 5 to 1. All three perceived parentingstyles are scored separately. The items of authoritative parenting are 1, 4, 7, 10, 13, 16, 19, 22, 25, 28; items for authoritarian parenting are 2, 5, 8, 11, 14, 17, 20, 23, 26, 29 and items for permissive parenting are 3, 6, 9, 12, 1, 18, 21, 24, 27, 30.

To determine the scale's reliability, the Cronbach Alpha coefficient wascomputed for each parenting style. It was found that the authoritativestyle has an Alpha coefficient of 0.79, authoritarian 0.81, and permissive 0.86. All the styles of the perceived parenting style scale have an acceptable level of reliability. The authors claim that the scale hasface validity. In the current study the scale is used to measure the parenting style that the participants perceive they received.

The Subjective Well-being Inventory (SUBI)

The Subjective Well-being Inventory is a questionnaire that deals with howindividuals feel about some aspects of their lives. It consists of 40 items intotal with 19 positive items and 21 negative items.The Subjective Well-being Inventory can be scored by attributing the values 3, 2, and 1 to the response categories of the positive items, and 1, 2, and 3 tothe response categories of the negative items. Thus, the minimum andmaximum scores that can be obtained for the items are 40 and 120 respectively. The total score can be interpreted summarily in terms of threebroad score ranges: 40-60, 61-80, and 81-120 to have an overall picture ofthe well-being status. The mean score on normal adult Indian samples is 90.8 with a standard deviation (SD) of 9.2. There is a different way ofinterpreting the scores in terms of sub-scores on the two sets of positive andnegative items. The minimum and maximum scores that can be obtainedfrom the positive items are 19 and 57 respectively. The minimum and maximum scores that can be obtained from the negative items are 21 and 63 respectively. The mean score on normal adult Indian samples on positiveitems is 2.9 with a standard deviation of 4.6. The mean score on normaladult Indian samples on negative items is 47.9 with a standard deviation of 5.1. In the current study this scale has been used to measure the subjective understanding of their own well-being by the participants.

Robson Self Concept Questionnaire (Robson SCQ)

Robson Self Concept Questionnaire measure the belief and attitude of any individuals. There are a total of 30 items in thisquestionnaire and the options against each question are Completely disagree (0 or 1), Disagree (2 or 3), Agree (4 or 5) and completely agree (6 or 7). Some items are scored normally but other questions are reversed. The 14 ‘normal’ items are 1, 2, 3, 6, 9, 10, 12, 15, 16, 18, 24, 26, 29, and 30 whichare having full stop after the question number (e.g. 2.) and there are 16 ‘reversed’ items, these are 4, 5, 7, 8, 11, 13, 14, 17, 19, 20, 21, 22, 23, 25, 27, and 28 which are having colon after the question number (e.g., 4:). Scoring for the ‘normal’ items are straight according to the scale but scoringfor the ‘reversed’ items are reversed (i.e. 0 = 7, 1 = 6, etc.). In this current research, self-concept has been measured through this scale.

Procedure

According to the area of study, scales were chosen at the beginning. Then thequestions were given to the participants aged between 18 to 29 (emerging adults) in the form of Google forms that were filled out online. The Google formcontained socio-demographic data (i.e. email, age, sex, family type, etc.) alongwith 3 types of scales – i) Perceived Parenting Style Scale, ii) The Subjective Well-being Inventory, and iii) Robson Self Concept Questionnaire. The Google formalso contained informed consent along with the other questionnaires and it wasmade to assure that all information provided would remain completely anonymousand they were informed that the data collection was for research purposes only. Following the instructions, participants were instructed to fill up the Google formwith the most appropriate answer of their choice.

Statistical Analysis

Chi square test of association is done to measure the association if any between parenting style & family type.

t-test is done to find out the impact of family type if any on the subjective well-being & self-concept.

All analyses are conducted using SPSS 20.

Results

Table 1. Showing the chi-square value and significant value for parenting style and family type.

Variables	**Family Type**		
Parenting Style	**Nuclear**	**Joint**	
	Chi Square Value		**p-value**
Authoritative Parenting	1.594		>0.05
Authoritarian Parenting			
Permissive Parenting			

The above table shows that there is no significance association between parenting style and family type.

Table 2. Showing the t-value and significant between the 2 family types for subjective wellbeing and self-concept

Dependent Variable	**Independent Variable**	**t- value**	**p-value**
Subjective Wellbeing	Nuclear Family	0.979	>0.05
	Joint Family		
Self-Concept	Nuclear Family	0.372	
	Joint Family		

The above table shows that there is no significance mean difference between the two family types (nuclear family and joint family) in terms of subjective wellbeing and self-concept.

Discussion

The present study was conducted on emerging adults (18-29 years) to find whether there is any association between family type (nuclear family and joint family) and parenting style (Authoritative parenting, Authoritarian parenting and Permissive parenting); and to find whether there is impact of family types (nuclear family and joint family) on two dependent variables-subjective well-being and self-concept. Three quantitative questionnaires (Perceived Parenting Style Scale, Subjective Well-Being Inventory and Robson Self-Concept Questionnaire) were given and chi-square test of association and student's t-test was used for analysis.

Our results indicate no significant association between parenting styles (authoritative, authoritarian, and permissive) and family type (nuclear vs. joint). This finding suggests that family structure, whether nuclear or joint, does not significantly influence the parenting style adopted by caregivers. This finding corroborates with research in KhonKaen Province, findings suggest no significant relationship between family types and all three parenting styles; rather the most practiced parenting style in the study was uninvolved parenting style. (4). This consistency suggests that cultural or socio-economic factors may play a role in neutralizing expected differences in parenting styles across various family structures. Parenting is a very unique thing, and is mainly depended on the core of the caregiver rather than the whole family.

The analysis also revealed no significant mean difference in subjective well-being between individuals from nuclear and joint families. This finding corroborates with certain prior research suggesting that family type alone may not be a determinant of subjective well-being. (5). It challenges the notion that the more cohesive and supportiveenvironment typically associated with joint families necessarily translates into higher subjective well-being. Other factors, such as individual personality traits, external social support systems, and economic stability, may play more critical roles in influencing subjective well-being.

Similarly, there was no significant difference in self-concept between individuals from nuclear and joint families. This finding suggests that family structure does not significantly impact an individual's self-concept during emerging adulthood. Supporting this, research has found that positive self-concept development is more strongly related to authoritative parenting styles rather than the type of family structure. (6). This corroborates our findings that the influence of parenting style on self-concept appears to be independent of whether the family is nuclear or joint.

Conclusion

Thus it can be concluded that –

1) There is no significance association between parenting style (authoritative parenting, authoritarian parenting and permissive parenting) and family type (nuclear family and

joint family)

2) There is no significance mean difference between the two family types (nuclear family and joint family) in terms of subjective well-being.

3) There is no significance mean difference between the two family types (nuclear family and joint family) in terms of self-concept.

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Physical Fitness Index and Dietary Pattern among Young Athletes and Nonathletes Students: A Comparison Study

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Abstract :

Physical fitness—which includes aerobic capacity, muscular strength, flexibility, and body composition—is essential for day-to-day functioning and general health. This study investigates the Physical Fitness Index (PFI) and dietary patterns among young athletes and non-athletes, aiming to compare their physical fitness and nutritional habits and seeks to describes the association between dietary habits and physical fitness in athletes and non- athletes aged 18-25.This cross-sectional study, conducted from March to April 2024, involved 30 healthy athletes and 30 healthy non-athletes aged 18-25. Physical Fitness Index (PFI) and anthropometric measurements (height and weight) were taken. Aerobic fitness was assessed using the Harvard Step Test with a modified step height of 33 cm. Dietary patterns were evaluated using a modified Food Frequency Questionnaire(FFQ). Data were analyzed using SPSS (version 16.0), with means, standard deviations, and percentages calculated for PFI, BMI, height, weight, and age. Correlations between nutritional status, BMI, and PFI were assessed using chi-square tests, and t-tests compared mean PFI and BMI scores, with significance at 95% confidence interval.The mean score of BMI among non-athletes is higher than athletes and it was not statistically significant. The mean score of PFI among athletes is higher than non-athletes. This is due to regular physical exercise and training of athletes. There is significant association between PFI status and physical activity category($P<0.05$). Athletes typically have an excellent PFI status, however this study also reveals that non-athletes have a significantly good PFI status. Despite the assumption that athletes would maintain higher nutritional standards, there is a discrepancy in dietary frequency data between athletes and non-athletes, with the later displaying better dataThe study found that college students lack physical activity, highlighting a significant health issue. College should encourage regular exercise and organize sports events to promote fitness.

Keywords: Diet pattern, Harvard's Step Test, Physical fitness, Student athletes

Introduction

The ability to perform daily work with energy and vigor, without experiencing undue exhaustion, to engage in leisure activities, and to handle unforeseen emergencies is known as physical fitness [1]. Aerobic fitness, or the heart's and lungs' capacity to pump blood to the muscles, muscular strength and endurance, or the capacity to move joints through their natural range of motion, flexibility, and body composition all contribute to an individual's level of overall fitness. A holistic lifestyle encompassing all aspects of fitness is essential for leading a healthy life. Aerobic fitness, or the heart's and lungs' capacity to pump blood to the muscles, muscular strength and endurance, or the capacity to move joints through their natural range of motion, flexibility, and body composition all contribute to an individual's overall level of physical fitness. The Physical Fitness Index is a metric that assesses an individual's physical preparedness for strenuous physical labor as well as their capacity for recuperation. It is calculated by comparing an individual's accomplished strength index with a norm based on their age, weight, and sex. Body Mass Index is universally considered a marker of health. It is a useful tool for assessing nutritional status and physical fitness [2].Research has demonstrated that BMI is a reliable indicator of the physical health for college students. The present study was undertaken to assess Physical Fitness Index (PFI) among athletes and non-athletes within the age group of 18-25yearsusing modified Harvard's Step Test and to see their dietary habits. As a predictor of VO_2max, the Harvard Step Test is used to assess a client's aerobic fitness. It assesses the cardiovascular system as well as the body's overall ability to recover from and handle greater physical exertion. Physical fitness tests can be used to objectively assess a variety of abilities such as upper and lower body strength, aerobic endurance, and motor agility/dynamic balance that support the behaviors required to carry out daily tasks.

Numerous research has investigated the relationship between certain food nutrients—such as protein, dietary fiber, vitamin D, and certain antioxidants—and physical fitness. The secret to ageing well is maintaining a healthy diet and level of physical activity, and they go hand in hand. Studies have suggested that diet is a major factor affecting muscle mass and muscle function. Maintaining optimal function and increasing skeletal muscle mass can be achieved through healthy dietary habits [3].

Nutrition and diet may enhance physical performance in sports. There are well-established guidelines for sports nutrition regarding the quantity and timing of fluids and macro-nutrients to enhance performance and recuperation. Maintaining a balanced diet helps individuals not only meet their physical needs but also maintain their fitness level by ensuring that their food intake is well-balanced in terms of food types, quantities, and eating frequencies. Sportsmen are impacted by nutrition in a variety of ways. It is fundamentally significant for obtaining and preserving health. An athlete can exercise and compete for longer and recover from training sessions if they consume

an optimal diet that minimizes tiredness. Achieving high levels of success in athletics is mostly dependent on diet.

Objectives

- To assess anthropometric measurements of the study sample.
- To assess PFI.
- To know the nutritional status of the individuals
- To compare the PFI and Nutritional status among athletes and non-athletes.
- To find the association between Nutritional status and physical fitness level.
- To assess and compare the nutritional intake between college athletes and non- athletes.
- To analyze the frequency and types of foods consumed by college athletes and non-athletes.
- To compare the overall dietary patterns between college athletes and non-athletes using Food Frequency Questionnaire.
- To analyze the impact of socioeconomic status and lifestyle factors on the dietary patterns of the college athletes and non-athletes.

Methods and Material

***Sampling and study location*:** This cross-sectional study was done for a periodof2 months from March to April 2024. For this study 30 healthy athletes among which 15 athletes were from Southern Sports Association, and 15 were from NSHM Knowledge Campus College and30healthynonathletes from NSHM Knowledge Campus college, Kolkata were selected. Physical fitness index and physical anthropometric parameters such as height, weight of non-athletes were measured at college campus during their leisure period and for athletes it was measured at the club premises during their resting period and in college it was measure during their free time.

Inclusion criteria

- The athletes were of age group 18-25 years, being trained for at least 3 hours, 5 days a week, for a minimum of 6 months.
- Non athlete college students who did not perform regular physical activity in the form of any exercise, and/or their structured physical activity was less than 20 minutes per day were age matched with athletes from college.

Exclusion criteria

The study excluded participants having a history of smoking or tobacco misuse, acute or chronic respiratory illnesses, cardiovascular diseases, or any other medical conditions, as well as those taking medications.

Data Collection

Harvard's Step Test-At first the participants were asked to remove their shoes, watches that they have worn before test procedure. Then the test procedures were explained to them. Bluetooth head phones were provided to them, so that they can follow a beat being played on the metronome, for stepping up and down the tool.

Initially, for five minutes, or until they become exhausted, the participants move up and down on a platform with a step height of 50.8 cm at a pace of 30 steps per minute (once every two seconds).

The inability to continue stepping at a certain pace for 15 seconds was considered exhaustion. For ease of performance in an Indian setting, a modified version of the Harvard step test with a step height of 33 cm was used in this investigation. Students were required to do a modified version of the Harvard Step Test rhythmically for five minutes, or until they were tired.

After the test is over, the patients sit down right away, and the total number of heartbeats is tallied from one to one and a half minutes, two to two and a half minutes, and three to three and a half minutes. Total duration of the exercise was measured as the time in seconds up to which each subject was able to perform the test [4,5].

Physical Fitness Index- PFI was calculated using the formula:

PFI= Duration of Exercise in Seconds / 2 (P1+ P2+P3)

Where P1, P2 and P3 were pulse rates.

Based on the score ,PFI was graded as:[6]

- Excellent(>90)
- Good(80-89)
- Average (65-79)
- Low Average(55-64)
- Poor(<55)

***Food Frequency Questionnaire (FFQ)* :** FFQ evaluate a person's typical intake during a given time period, usually a lengthier reference period, and inquire about the frequency of food items consumed. They commonly combine a variety of foods with comparable nutritional profiles into a single category.

FFQ offer a more cost-effective alternative to the 24HR because the subject usually self-completes the tool and are generally used in research studies with for large sample sizes. FFQ can be quantitative, semi-quantitative, or qualitative. While the frequency of eating is the only factor considered in qualitative food frequency questionnaires, portion sizes that are typically consumed are also assumed, which lowers participant burden while simultaneously lowering the quality of the data [7-13].

A modified food frequency questionnaire was used in this study. The questionnaire consists of 52 food items with response categories include once a day, twice a day, thrice a day, 2-3 days a week, 4-5 days a week, sometimes and never. The study questionnaire was then distributed to the students who volunteered for the study.

***Recording of Physical Anthropometry*:**

Height (in cm): This was measured with the subject in standing position without footwear

To nearest to 0.1cms.

Weight (in kg): The subject was weighed with a standard machine with minimum of

clothing,to nearest to 0.1kgs.

Body Mass Index or BMI (kg/m^2): The BMI was calculated for each of the participants

Using the following formula–

$$BMI = \text{Weight in kg} / \text{Height in } m^2$$

Table 1 : WHO Asian BMI Classification

Nutritional Status	**BMI(kg/m^2)**
Underweight	<18.5
Normal weight	18.5–22.9
Overweight	23 –24.9

Obese Class 1	25 –29.9
Obese Class 2	>30

Source: Girdhar,S., Sharma,S.,Chaudhary,A.,Bansal,P.,&Satija,M.(2016).An Epidemiological study of overweight and obesity among women in an Urban area of North India. Indian Journal of Community Medicine, 41(2), 154-157.[11].

Table 1 states that Nutritional status can be classified based on Body Mass Index (BMI) as follows: Individuals with a BMI of less than 18.5 are considered underweight. A BMI range of 18.5 to 22.9 is classified as normal weight, while those with a BMI between 23 and 24.9 fall into the overweight category. Obesity is further divided into two classes: Obese Class 1 includes individuals with a BMI ranging from 25 to 29.9, and Obese Class 2 applies to those with a BMI greater than 30.

Equipment:

- **Stadiometer:** This was used for measuring height.
- **Digital weighing machine:** It was used for measuring body weight.
- **Stepping tool:** To perform Harvard's Step test.
- **Stop watch:** To record the time takes to complete three circuits.
- **Bluetooth headphones:** To hear the beat played on metronome.

Statistical Analysis

The data was entered in Microsoft Excel and presented in the form of pie chart and bar diagram. Appropriate statistical methods were used for proper analysis of the . The data was distributed using tables. The statistical package for social sciences (version 16.0, SPSS) was used to conduct the analyses. The means, standard deviations, and percentages for PFI, BMI, height, weight, and age were computed. To evaluate the relationship between nutritional status, BMI, and PFI, the chi square correlation test was employed. To compare mean of PFI score and BMI score, independent sample t-test was used. Statistical results were considered to be significant at 95%confidence interval.

Result

The mean age, height, weight, BMI &PFI of the participants inourstudyare21.01(±1.75), 164(±8), 62.28(±1.28), 22.90(±3.89), 76.15(±2.83) respectively. Table 2 displays the anthropometric evaluation data for both athletes and non-athlete's students. The results showed that the selected subjects were within the normal weight category for their height.

Table No. 2:Mean and Standard Deviation of height, weight, BMI & PFI of participants

Characteristics	Athletes(n-30)		Non-athletes(n-30)		P Value
	M	SD	M	SD	
Weight(kg)	60.25	±11.45	64.30	±14.02	0.23
Height(cm)	165	±7.6	165	±8.4	0.48
BMI(kg/m^2)	22.46	±4.11	23.34	±3.67	0.8
PFI	97.80	±16.15	56.20	±20.50	.00

n-Number of participants ,**M-** Mean, **SD**- Standard Deviation

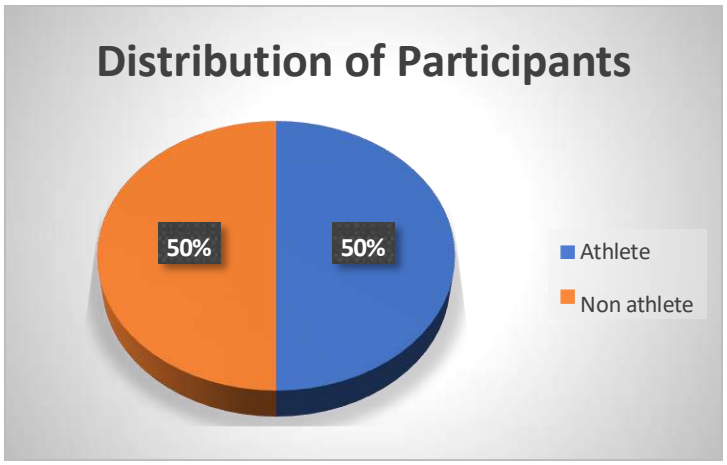

Figure-1: Distribution of participants

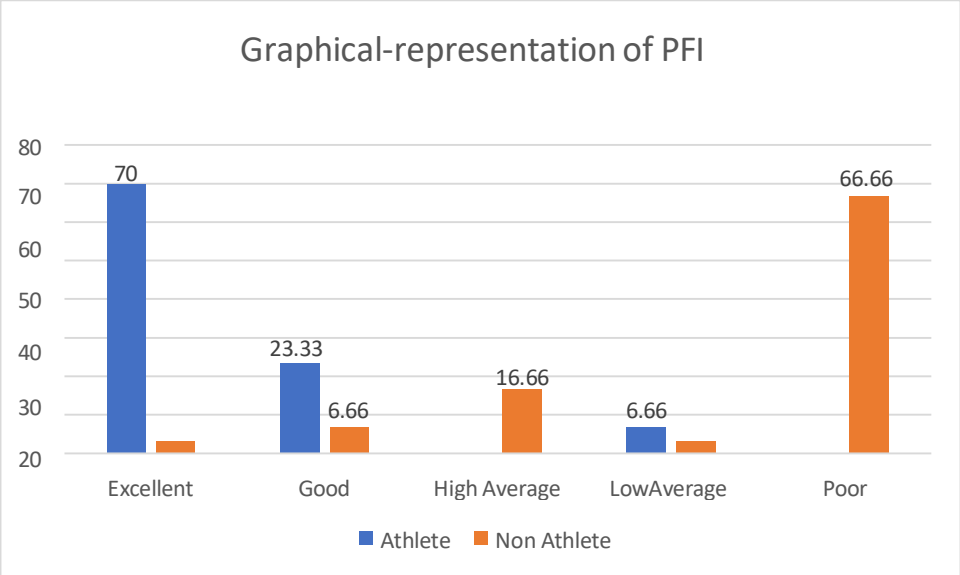

Figure-2: Comparison of PFI category among athletes & athletes

Table No. 3: Association between body mass index and physical fitness index (n=60)

BMI CATEGORY	PFISTATUS					TOTAL	P Value
	Excellent	Good	High Average	Low Average	Poor		
Normal	14	3	2	1	11	31	0.14
Obese1	4	1	1	2	1	9	
Obese2	1	0	0	0	3	4	
Overweight	3	2	2	0	4	11	
Underweight	1	3	0	0	1	5	

In table 3 it is shown that there is no significant association between PFI status and BMI category. Among participants with normal BMI, there was a wide range of PFI scores. Participants in the obese 1 and obese 2 categories tended to have lower PFI score. Overweight participants had PFI scores among poor, good, excellent and high average. Underweight participants had a range of PFI scores.

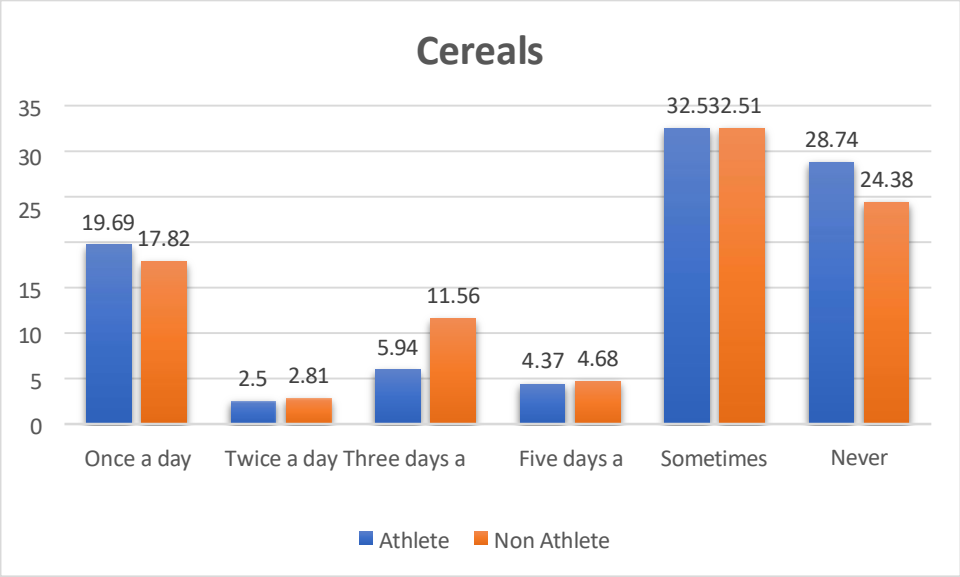

Figure 3: Comparison of Food Frequency Data for cereals Among Athlete and Non Athlete Students

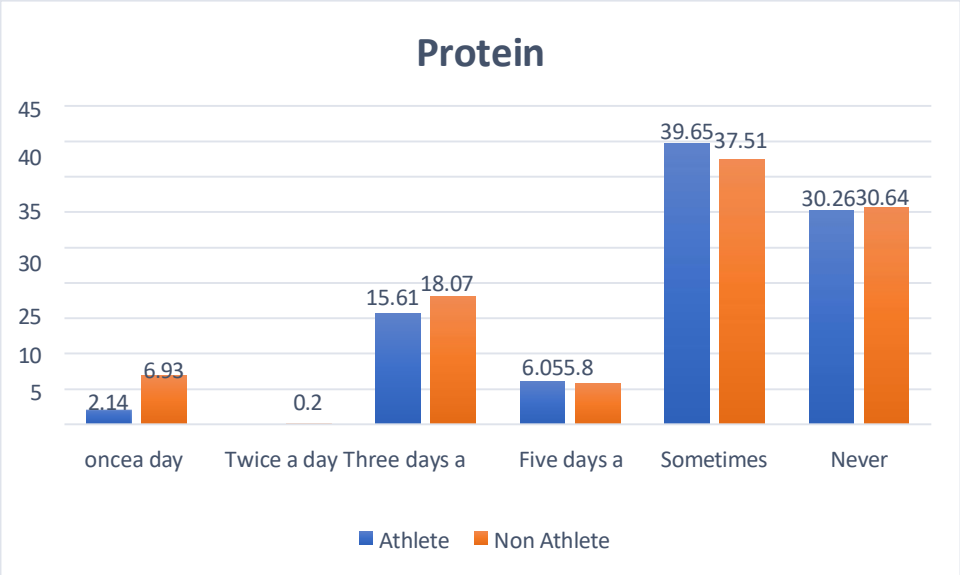

Figure 4: Comparison of Food Frequency for protein Data Among Athlete and Non Athlete Students

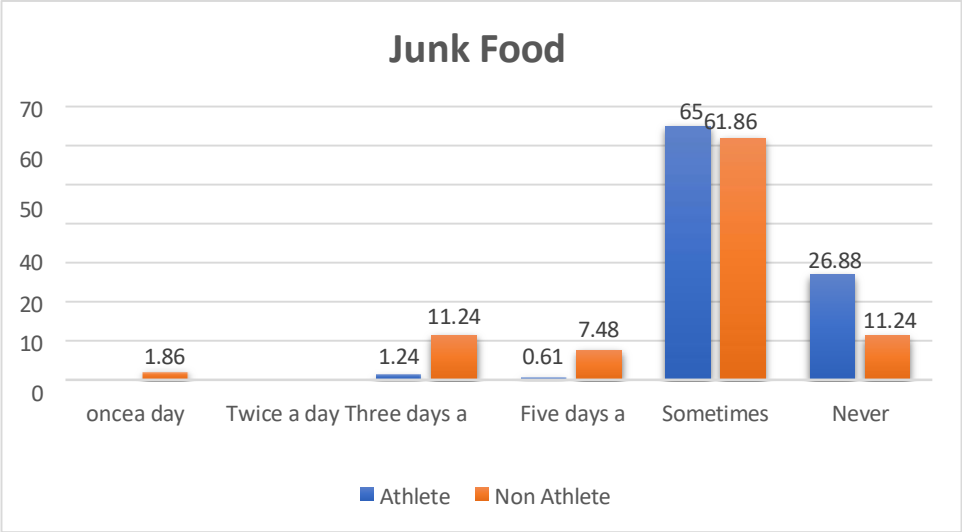

Figure 5: Comparison of Food Frequency Data for junk food Among Athlete and Non Athlete Students

Figure 3,4,5 shows the graphical representation of the comparison of frequency percentages of cereal, protein and junk food consumption between athletes and non-athletes which offer insights into their dietary habits. Notably, the study reveals a higher percentage of protein consumption among non-athlete students compared to their athletic counterparts, contrary to the expected result that athletes would consume more protein to support their physical demands. This finding suggests possible deviations in dietary adherence among athletes or perhaps differences in dietary education or access to protein-rich foods. Similarly, fruit consumption is also higher among non-athletes, which could indicate a lack of emphasis on balanced nutrition within the athlete group. Cereal consumption is nearly identical between the two groups, suggesting that both groups may be following similar patterns for this food category. Finally, the data highlight a in quality in junk food consumption, with non-athletes exhibiting a higher frequency. This finding aligns with expectations, as athletes are often encouraged to avoid high-calorie, low-nutrient foods to optimize performance. These results under line thee need for targeted nutritional education to ensure that athletes meet their specific dietary requirements.

Discussion

Physical fitness has three main aspects. These are static fitness (absence of disease), dynamic fitness (ability to perform strenuous work) and motor skills fitness. Of these three, the dynamic fitness is very important in athletes [6]. There was unquestionably a difference in BMI between players and non-athletes in the current study. BMI was lower in athletes than in non-athletes. Int non-athlete students, there were some overweight even obese students due to lack of physical activity [2]. This may be influenced by an unhealthy diet, lack of physical activity, lifestyle changes, lack of time, tight schedule, age, gender, and health state are some of the variables that influence one's degree of physical activity.[7] As for athletes, those who played football, some of them falls under underweight category. This maybe due to they belong from a low socioeconomic group and can't maintain a proper diet that is required for athlete. Some athletes from weightlifting falls under overweight category, but that is due to increased muscle mass. Athletes had better Physical Fitness Index scores than non-athletes, according to the Harvard's Step Test calculation. In a related study, Arsha Krishnan and Gokul Suresh Revathy discovered

that students who participated in athletics had PFIs that were considerably greater than those of sedentary students.

Total time for doing the steps was also higher in athletes than non-athletes and also the pulse counts between 1 – 1.5 minutes after the test was slightly lower in athletes than non-athletes. These suggests that athletes have faster recovery due to aerobic training. The sympathetic nervous system controls bodily functions during activity; however, following exercise, there is a change in the autonomic balance, and the parasympathetic nervous system puts the body back into a resting condition. The post-exercise heart rate recovery is the outcome of a synchronized process involving sympathetic withdrawal and parasympathetic reactivation. The amount of activity and cardiovascular fitness determine how long the heart rate recovers. Cardiovascular fitness is a measure of how well the heart, lungs, and blood arteries supply oxygen to the working muscles. [7] In this study both male and female athletes exhibited similarly high physical fitness index score. It was observed that poor nutritional status of some athletes did not affect PFI. One major component influencing athletic performance is height. However, some research has indicated that even if a person is not athletic, increasing their height can make the step test relatively simpler to complete. In present study the mean height of athlete and non-athlete were same. In this study, we observed that non-athletes showed better food frequency data compared to athletes, a finding that differs from the anticipated outcome as athletes typically require and, thus, are expected to maintain superior nutritional standards to support their physical activities and optimize performance. Several existing literatures also suggests that athletes often have better dietary habits due tot their focus on performance nutrition. Several potential factors may explain this discrepancy. One possible explanation for this paradoxical outcome is the socioeconomic status of the athletes. There is a strong correlation between socioeconomic status and dietary habits. Most of the athletes from the sports club belongs from lower socioeconomic backgrounds. Although it does not seem to affect their performance, and the Physical Fitness Index Score is better than the non-athletes.

Despite the importance of physical activity, ideal nutrient intake and sound nutritional knowledge have been identified as critical components in enhancing athletes' athletic performance and health status. [9]

A healthy diet is essential to an athlete's optimal physical growth and the achievement of ideal results, so athletes and their training professionals should pay particular attention to nutrition management. An athlete's body can only optimize its adaptability to physical loads and offset increased energy loss and nutritional requirements with sufficient nutrition [11]. Althoughmostofthenon-athleteshavebetterfoodconsumptionfrequency, but some of them consume more junk foods and have poor food intake, which is primarily attributed to living away from home and staying in hostel.

Limitation

With only thirty people in each group, the study's primary sample size restriction was encountered. Finding important relationships in the data was challenging. More accurate results might have been produced if the study had been based on a bigger sample size. The lack of time was also a problem in the study. More subjects would be included in the study if there was more time. Dietary recall

method would have been a better way to estimate dietary pattern as the Food Frequency Questionnaire lack the precision and detail found in the recall method.

Conclusion

This study concluded that the college students lack physical activity. This indicates an important health issue among college students. They must be encouraged to do regular exercise for their physical well-being. And for this college and universities various sport events, encourage students to play in the campus. By focusing on physical fitness, active and habitual participation in exercise can be promoted to students. As for athletes it was observed that some of them falls under underweight category. They need to follow a proper diet suggested by the coach or nutritionist. The nutritional needs of athletes are different from regular person and understanding the demands is of huge value to the coach and sports nutritionist. To address poor food intake among athletes, Institutions or the training specialist can provide access to affordable healthy foods by proper menu planning with the help of registered dietitians.

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