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A Comprehensive Review on Applicability and Bioactivity of *Rogan-I-Kunjad* (Sesamum indicum L-Oil): Unani Prospective

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Abstract

Sesame oil has a long history of usage as a food and medicine. It is the most used oil as a medicine or as a base oil for preparations of many compound drugs in the Unani system of medicine. It has a wide range of biological activities as mentioned in Unani classical text; this review highlights its pharmacological activities and their possible mode of action. Searched many Unani classical literature online and offline and simultaneously did parallel search on databases like PubMed, and Science Direct, and extraction of data related to sesame oil, sesame seeds with its pharmacological activities, mode of action, then interpretation and summarization of all related data. Sesame oil possesses many biological activities like anti-inflammatory, antihyperlipidemic, antiatherosclerotic, hepatoprotective, antiasthmatic, analgesic, emollient, antipruritic, and wound healing effects, which were scientifically demonstrated as mentioned in Unani literature. Sesame oil has a hopeful effect on modulating diseases with no significant toxic effect; so, there is a need to identify its safety and efficacy on human subjects to develop a new potential drug.

Keywords

- pharmacology
- *Rogan-I-Kunjad*
- sesame oil
- Sesamum indicum L.
- Unani medicine

Introduction

Rogan-I-Kunjad (sesame oil) is obtained from the seeds of Sesamum indicum L., a herbaceous annual plant in the family of the Pedaliaceae, and is grown for its palatable seeds, oil, and therapeutic uses.[1] Sesame seeds are said to be the earliest oil seeds used by humans. Their farming practices were more than 5000 years old. Sesame comes in various varieties, with the earliest wild species being found in Africa and India. It is widely used as food, medicine, and in ceremonial or spiritual rites. The entire seeds are commonly utilized in cuisines throughout Asia and the Middle East. In North America and Europe, it is used as a flavoring and garnish for a variety of meals, including bread.[2] Sesame oil is regarded as a nutrient-dense food since it is stable, rich in medicinal benefits, and has a high nutritional quality, giving it an edge over other vegetable oils.[3] Nowadays with increasing awareness of the importance and long-term benefits of traditional or herbal medicines, the global traditional medicine industry has started growing rapidly, resulting in a rise in demand and use of herbal products. In 2020, the global market for herbal medicine was estimated to be around US$ 185 billion. The industry is estimated to expand at a Compound Annual Growth Rate (CAGR) of around 11% to around US$ 430 billion by 2028.[4] This has contributed to an increased demand

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for sesame seeds, as they are considered nutritious and have various health benefits. However, it is important to note that the status of sesame seeds in the Indian market is that India is one of the largest producers of sesame seeds in the world and will produce 0.75 million metric tons of sesame seeds in FY (Financial Year) 2023.[5] India exported 328.46 tons of sesame seeds worth 3012.31 in 2015 to 2016.[6] Sesame oil exports from India totaled 798.58 thousand tons in April 2023 FY, down from 880.51 thousand tons in the corresponding period of April 2022 FY.[7]

Sesame oil, seeds, roots, and leaves are utilized for a variety of therapeutic purposes. African and Asian nations use sesame more frequently as a traditional medicine.[8] Sesame seeds and their oil have great importance in the Indian System of Medicine, especially in the Unani system of medicine dealing with a wide range of illnesses like inflammation, asthma, dry cough, constipation, dysuria, wounds, neurodegenerative disorders, musculoskeletal pain and stiffness, alopecia, and thrombosis.[9,10]

The purpose of the current review is to summarize and keep updated the information that is currently accessible on the morphology, ethnobotanical, phytochemical, and most significantly, pharmacological utilization of *Rogan-I-Kunjad* (*Sesamum indicum* L. oil), along with their possible mode of action based on scientific parameters. The main goal of this review is to perform a critical analysis of the Unani medical literature about the nutritional and therapeutic benefits of *Rogan-I-Kunjad*, which will be supported by data from reliable sources. Such observations may help researchers to gain insight to develop novel therapeutic approaches for a wide array of diseases with minimal toxicity, promoting the overall health and well-being of society.

Methodology

In this review article, the data are generated by COPE guidelines. The source of data is Avicenna's "Al-Qanun-fil-Tibb" (The Canon of Medicine), Al-Jami-li-Mufradat-Al-Adwiya-wal-Aghziya, Muhit-I Azam, Khazain-ul-Advia, etc. The source was searched for equivalent (Urdu, Persian, Arabic) words for "*Rogan-I-Kunjad*," "*Tukham-I-Kunjad*," "simsim," and "Til. A parallel search in databases like PubMed and Science Direct was done by using keywords with appropriate Boolean operators ("AND" and "OR") "Sesamum indicum," "Sesame oil," "phytochemical," "pharmacology" "Medicinal use," "preclinical study," and other relevant terms. Title, abstract, and keywords were used for screening the literature for potential articles. The full text of these articles was then used to extract relevant data. The duration of data was from the past 10 years (►**Table 1**). The author visited the library of the National Research Institute of Unani Medicine for skin disorders to find out relevant data from unpublished Unani literature and the botanical name of the plant was confirmed from an authentic website namely The plant list.

Observation

Taxonomy of *Kunjad* (*Sesamum indicum* L.)

Kingdom: Plantae—(plant), Subkingdom: *Tracheobionta*(vascular plants), Superdivision: *Spermatophyta*—(seed plants), division: *Magnoliophyta*—(flowering plants), Class: *Magnoliopsida*—(dicotyledons), Subclass: *Asteridae,* Order: *Scrophulariales,* Family: *Pedaliaceae*—Sesame, Genus: *Sesamum*, Species: *S. indicum* L.[11]

Habitat and Distribution of *Kunjad* (*Sesamum indicum* L.)

The suitable soil for satisfactory production of sesame is on soils with a pH range of 5.4 to 6.7 but is seriously affected by pH below this critical range. These, however, do not tolerate heavily salted or waterlogged soils well. Ninety to one-hundred twenty frost-free days are required for commercial sesame production. Over 23°C (73°F) warm conditions are favorable for growth and yields.[12] It is cultivated all over the world and in India it is widely distributed in Assam, Bihar, Gujarat, Uttar Pradesh, Madhya Pradesh, and Rajasthan.[13]

Morphology of Seeds of *Sesamum indicum* L

Macroscopic

The seeds are compressed, ovoid, pear-shaped pointed at one end and broader at the other end, approximately 3 to 4 mm long, 2 mm broad, 1 mm thick; an indistinct longitudinal ridge running through the center of one of the sides represents the position of the raphe, other ridges run around each side near the edge, and the hilum lies at the pointed end.[14] The color of sesame seeds is of three types that are white, black, and Red/brownish red.[9,15] It has an oily taste without any characteristics or smell.

Microscopic

The diagrammatic transverse section (TS) of the seed is oval with four elevated arrow ridges, one at each end of its long axis, and a faint raphe ridge on one side. Underneath the testa, a narrow, hefty cotyledon is positioned in the center, taking up most of the section. The longitudinal section of the seed has an oval shape that narrows at one end, and the embryo is straight, with two big piano-convex cotyledons and a little cylindrical radicle pointing in the direction of the hilar end.[14]

A detailed TS of the seed reveals the outer epidermis of the testa, which is made up of compact, palisade-like cells with sinuous walls that are radially extended. At the periphery of each cell is a cluster crystal of calcium oxalate that is encased in a thick spherical membrane. The cell under the ridges is devoid of such crystals; beneath this layer is a short band of tegmen and a collapsed parenchyma. The endosperm is a narrow, parenchymatous layer that varies in thickness, being 2 to 3 in rows at the margins and 4 to 5 on the sides. The embryo's cotyledons exhibit one layer of palisade cells next to the inner epidermis, with isodiametric cells making up the majority of the ground tissue. There were aleurone grains and oil globules inside the embryo and endosperm cells.[14]

Characteristics of *Rogan-I-Kunjad* (*Sesamum indicum* L.)

As per the Unani classical text, sesame oil of the Indian verity is pale yellow to golden in color, while some of its verities are also dark yellowish in color. The oil which is extracted from

Table 1 Keys terms used for search in this review

Database	Keyword used	No of articles
PubMed	Sesamum indicum, sesamum indicum AND phytochemistry, sesame oil, animal study on sesame	471
Science Direct	Sesamum indicum and transcriptional regulation, pharmacological action	608

Gair Mukasher (with covering) and *Gair Birya Kunjad* (without roasted) is known as *Dahn-ul-Heel* and its color is dark brownish, having a different flavor with mucilaginous, more stickiness, and the oil that is derived from *Gair Mukasher* (with covering), and *Birya Kunjad* (roasted seeds) is light pale yellow in color with less stickiness.[9,11] According to a study, the amount of lignans, tocopherols, and sterols in the oil produced from roasted sesame seeds was much lower than that of unroasted sesame seeds. The extracted oil's oxidative stability, and antiradical activity were all increased by roasting. In seeds oil roasted at high temperatures, a modest increase in Trans Fatty Acids (TFA) as well as triglyceride and diglyceride dimers was noted. For obtaining highly nutritious grade oil within the allowable range, 210 °C was the ideal roasting temperature. Utilizing this roasted sesame oil seed in plant-based diets may result in improved nutritional quality due to the great antioxidative efficacy of roasted seed oil.[8,16] Refined sesame seeds have more oxidative stability than unrefined sesame seeds oil due to more saturated fatty acids (SFA) and less Mono Unsaturated Fatty Acid (MUFA) and Polyunsaturate Fatty Acid (PUFA). The TOTOX value of refined sesame oil was significantly lower than unrefined. Oil processing was introduced as an effective way to reduce heavy metals.[17] The temperament of the sesame oil is *Har* (hot) and *Ratab* (moist) in second grade and it is liquid at standard room temperature (37 °C).[9,10,18,19] As mentioned in classical Unani literature called "Muhit-i-Azam" by renowned Unani philosopher "Hakim Azam Khan," the oil derived from the white luster surface seeds of sesame is the best form of sesame oil.[9] Much research has been done on its genotypic verities with their significant characteristics. The comparison of the quality of oil derived from different types of sesame seeds showed that oil from white-colored seeds is the best in terms of its oil-yielding capacity, protein, PUFA, and MUFA. This indicates that light-colored seeds that are white and tan are superior in their quality and good for consumption as well as medicinal use[20] (►**Table 2**). Its life span is 2 years for its best use because it is prone to decomposing its phytochemicals and starting to oxidize and become rancid.[21]

Composition of *Rogan-I-Kunjad* (*Sesamum indicum* L.)

Sesame seed oil is rich in polyunsaturated fatty acids that is (43.62%) monounsaturated fatty acids (41.53%) and only (14.85%) SFA natural antioxidants, lignans like sesamin 0.07–0.61%, sesamolin 0.2–0.48%, sesaminol, sesamol, pinoresinol), (►**Fig. 1**) sterols (16–40%), and tocopherol derivatives (10–12%) claiming that oils with this quality are suitable for consumption.[1,8,22] The sesame oil extracted from white and black varieties of seeds contains 14.90% and 14.70% carbohydrates, respectively.[23] Its seeds are a rich source of protein (18–40%).[24] These bioactive components improve sesame oil's stability and shelf life, as well as provide several health advantages (►**Table 3**).

Uses of *Rogan-I-Kunjad* as per Unani System of Medicine

According to the Unani system of medicine, this oil has a hot and dry temperament, which is very much favorable for the person of *Sawdāwī-al-Mizāj* (Malencolic temperament—A type of temperament caused by the predominance of Sawda' (black bile) in the body, which is cold and dry; individuals with this type of temperament have thin built with prominent veins, blackish skin—Standard Unani Medical Terminology [SUMT]-UMI-0161, WHO International Standard Terminology on Unani Medicine [IUMT]-3.1.176).[9] This oil is widely used as a medicine in the Unani system of medicine for the management of many diseases like *Iltihāb* (inflammation—(SUMT)-UMI-2358), *Surfa Yabsiyya* (dry cough—[SUMT]-UMA-0311, National Unani Morbidity Code [NUMC]-D-7.6, International Classification of Diseases [ICD-10]-R.05), *Dīq-un-nafas* (asthma[SUMT]-UMA-0247, [NUMC]-D-4, [ICD-10]-J-45), *Sudda* (obstruction—[SUMT]-UNI-0484), *Waja' al-Mafāṣil* (arthritis [NUMC]-L-4), *Ḥudār* [rheumatism [NUMC]-L-3), *Dā' al-Tha'laboratory* (alopecia areata [NUMC]-J-16), *Ṣudā'* (cephalgia/headache [NUMC]-A-36), *Shūṣa* (pleurodynia [NUMC]-D-11), *Ramad Sawdāwī* (melancholic conjunctivitis [NUMC]-B-1.4), *Qīḥ al-Ṣadr* (Empyema thoracis [NUMC]-D-26), *Qishr al-Ra's* (seborrheic dermatitis [NUMC]-J-15) *Saraṭan* (carcinoma [NUMC]-M-17] as well as all types of *Qurūh* (ulcer—[SUMT]-UMA-

Table 2 Characteristics features of *Rogan-I-Kunjad* from different types of sesame seeds

Sr. no	Coat color of seeds	Oil yielding capacity	Protein%	MUFA	PUFA	SFA	Ref
1	White	51.80%	20.00%	37.61%	46.03%	5.48%	20
2	Black	34.33%	14.87%	37.91%	44.34%	4.48%	
3	Red/ brownish red	42.48%	18.02%	37.41%	46.66%	4.53%	

Abbreviations: MUFA, Mono Unsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids; SFA, saturated fatty acids.

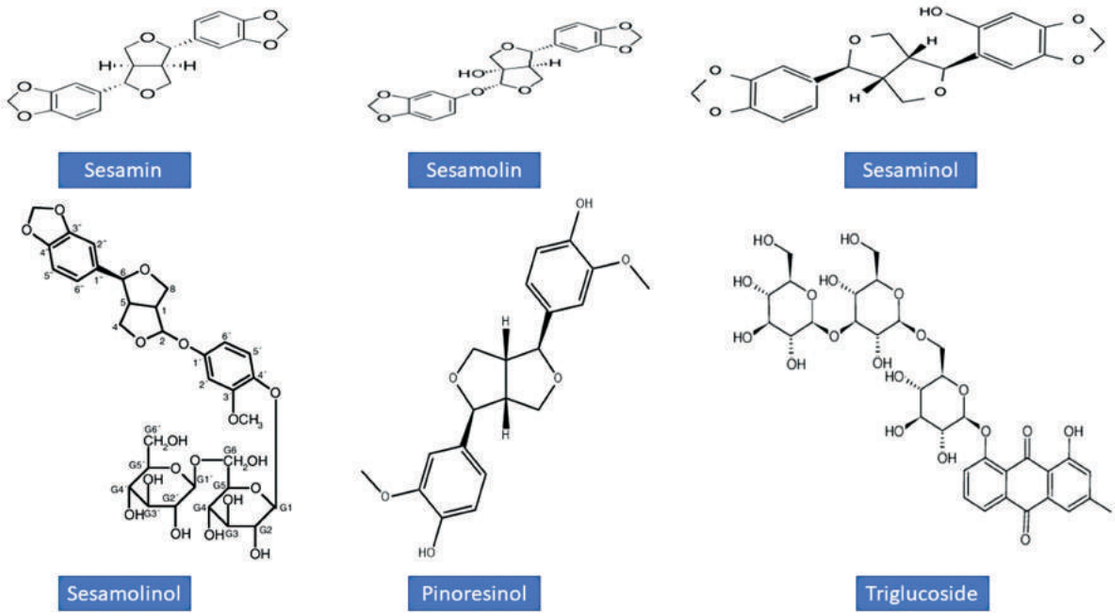

Fig. 1 Lignans of *Rogan-I-Kunjad* (sesame oil).

0200) as its properties are described in Unani medicine as a *Muliyyan* (laxative), *Muḥallil* (resolvent—[SUMT]-UMI-1910), *Mufattiḥ* (obstruent—[SUMT]-UMI-1942), *Mufattiḥ-i-urūq* (vasodilator—[SUMT]-UMI-0753), *Muḥallil-i-waram* (anti-inflammatory), *Mundamil-i-qurūh* (cicatrizing), *Mualid-e-mani* (spermatogenic[SUMT]-UMI-1937),[24–26] *Hikka* (pruritis—[SUMT]-UMI-1162, [NUMC]-J-57, [ICD-10]-L29-L29.9).[6,7,16,19,25–29]

Rogan-I-Kunjad compound compositions have long been employed in Unani medicine. Here, some of the compound compositions that are most frequently utilized were discussed. In case of alopecia areata, seborrheic dermatitis, or dandruff, a mixture of *Rogan-I-Kunjad* and *Barg-I Murad* (leaf of Myrtus communis L.) is applied over the scalp. *Rogan-I-Kunjad* with *Aspaghol* (*Plantago ovata*) is used as a liniment in xerosis, burn, and ulcer. *Rogan-I-Kunjad* in the form of *Qairooti* (Cerate or salve) is used for cosmetic purposes. Eye ointment made up of *Rogan-I-Kunjad* with egg white is intended to treat conjunctivitis and other inflammatory condition of the eye. Preparation in the form of ear drop made up of *Rogan-I-Kunjad* with *Filfil safaid* (*Piper nigrum* L.) and *Mastagi* (*Pistacia lentiscus* L.) is used for otalgia and ear obstruction. *Rogan-I-Kunjad* with Munaqqa (*Vitis vinifera* L.) is used in *Ḥikka* (pruritus) and a solution of *Rogan-I-Kunjad* with *Rogan-I gul* (rose oil) in *Ṣudā'* (headache) is used as an oral administration.[9,18] Some of the marketed compound formulations of *Rogan-I-Kunjad* are mentioned in ►**Table 4**.

Phytochemicals in *Rogan-I-Kunjad* (*Sesamum indicum* L.)

There have been various types of phytochemicals isolated from seeds, seed oil, and other parts of the sesame plant, including polyphenols (whole plant), Phytate 5.18%, oxalic acid 2.2%, tannins 5.26mg/100 g, sterols (seeds), phenols (seeds), naphthoquinones (roots), anthraquinones (roots), triterpenes (seeds), cerebroside (flower), fatty acids (seeds), vitamins (seeds), essential amino acids (seeds, leaf, stems, flower), carbohydrates (seeds), and other organic compounds.[2] (►**Table 3**) However, higher amounts of sesamin (2.45 mg/g seed) and sesamolin (1.10 mg/g seed) were observed for Indian sesame species when compared with 65 sesame seeds harvested in Texas, United States (1.63 mg/g seed for sesamin and 1.01 mg/g seed for sesamolin).[30]

Pharmacological Activities of *Rogan-I-Kunjad* (*Sesamum indicum* L.)

Antinociceptive Effect

In the Unani system, sesame oil has *Muskin-I Alam* (analgesic) and *Muhalil-I warm* (anti-inflammatory) properties and is used for the treatment of pain.[9,10] In an animal model of pain induced by acetic acid and formalin-induced writhing response, tail immersion, and hot plate latency test. It was shown that sesame oil caused significant reduction in abdominal writhes and sesamin inhibited biphasic paw licking response as compared with morphine. In the hot plate, latency time was increased after 60-minute administration of the drug[31] (►**Fig. 2**).

Inflammation and Sesame Oil

Sesame oil showed a potential anti-inflammatory effect on carrageenan-induced inflammatory animal models. Sesame oil reduced paw edema after 3 hours of drug administration. In model of pleurisy induced using intrapleural carrageenan, sesame oil inhibits accumulation of exudate as compared

Table 3 Constituents of *Rogan-I-Kunjad*

Sr.no	Bioactive compounds	Name of components	Organ studied	Quantity	References
1	Fatty acids	SFA Palmitic acid (16:0) Margaric acid (17:0) Stearic acid (18:0) Arachidic acid (20:0) Behenic acid (22:0) Lignoceric acid (24:0)	Seeds Seeds Seeds Seeds Seeds Seeds	14.20 g/100 g 8.9 g/100 g 4.8 g/100 g	63
		MUFA Palmitoleic acid (16:1) Oleic acid (18:1) Gadoleic acid (20:1)	Seeds Seeds Seeds	39.70 g/100 g 0.2 g/100 g 39.3 g/100 g 0.2 g/100 g	63,64
		PUFA Linoleic acid (18:2) Linolenic acid (18:3)	Seeds Seeds	41.7 g/100 g 41.3 g/100 g 0.3 g/100 g	63,64
2	Lignans	Sesamin Sesamolin Sesaminol Sesamolinol Triglucoside Pinoresinol	Aerial part, seeds Seeds Seeds Seeds Seeds	6.20 mg g^{-1} 2.45 mg g^{-1} 0.01 mg g^{-1} 58 mg/100 g	65,66
3	Tocopherol derivatives	Y Tocopherol	Seeds	0.68 mg g^{-1}	63,67
4	Phytosterols	B Sitosterol Campesterol stigmasterol 5 avenasterol Sitostanol Campestanol	Aerial part, Flower Flower Areal part Seeds Seeds Seeds	2.63 mg g^{-1} 1.35 mg g^{-1} 0.47 mg g^{-1} 0.82 mg g^{-1} 0.04 mg g^{-1} 0.02 mg g^{-1}	68,69
5	Carbohydrates	D-glucose D-galactose D-fructose Sucrose Raffinose Stachyose Planteose Sesamose Pentasaccharides Hexasaccharides	Seeds Seeds Seeds Seeds Seeds Seeds Seeds Seeds Seeds Seeds	3.24% 0.06% 2.63% 0.17% 0.24% 0.23% 0.59% 0.38% 0.16% 0.08%	70
6	Protein	Albumin Globulin Prolamin Glutelin	Seeds Seeds Seeds Seeds	8.6% 67.3% 1.4% 6.9%	71
7	Phenolic acid	Ferulic, Vanillic Cinnamic p-coumaric 4-hydroxybenzoic Protocatechuic acid Gallic acid			72,73

with indomethacin.[31] Another study on ($LDLR^{-/-}$) female mice showed a significant reduction in proinflammatory cytokines like monocytes chemoattractant protein-1, normal T-cells expressed and secreted, interleukin-1α (IL-1α), IL-6, and chemokines ligand 16 and upregulation of anti-inflammatory gene IL-13, IL-1β, and macrophage inflammatory protein-3β and 3α.[32] An in-vitro study was conducted on RAW 264.7 cells, which showed an inhibitory effect on tumor necrosis factor-α (TNF-α), TNF-1, TNF-2, IL-6, monocyte chemoattractant protein-1, vascular cell adhesion protein-1, and granulocytes-macrophage colony-stimulating factor by application of aqueous extract of sesame oil.[33] In an animal model wherein acute inflammation was induced by monosodium urate monohydrate crystals, the study showed sesame oil significantly reduced inflammation after 6 hours of administration, and significantly decreased total cell count, TNF-α, IL-1β, IL-6, and mast cells in tissue pouch and skin tissue[34] (►**Fig. 2**).

Table 4 Compound formulation of *Rogan-I-Kunjad*

Sl. no	Drug name	Action	Therapeutic use	Therapeutic doses/ route of drug administration	References
1	Raughan-E-Gul	Anti-inflammatory, refrigerant, laxative/ softener	Acute arthritis Headache Constipation	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
2	Raughan-E-Kaddu Shireen	Hypnotic agent	Insomnia Meningitis Melancholia	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
3	Raughan-E-Kahu	Hypnotic agent Sedative	Insomnia Epilepsy Melancholia	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
4	Raughan-E-Luboob-E-Saba	Hypnotic agent	Insomnia	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
5	Raughan-E-Luboob-E-Saba Barid	Hypnotic agent Sedative	Insomnia, headache	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
6	Raughan-E-Turb	Analgesic	Otalgia	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
7	Raughan-E-Babuna Sada	Anti-inflammatory, Analgesic	Arthritis, pneumonia	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
8	Raughan-E-Banafsha	Sedative Hypnotic agent	Headache Insomnia Ankylosing arthritis	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
9	Raughan-E-Amla	Muqawwi-e-Shar (drug which enhance hair growth) Musawwid-e-Shar (drug which enhance hair color)	Alopecia Gray hair	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
10	Raughan-E-Amla Sada	Muqawwi-e-Shar, Musawwid-e-Shar	Alopecia Gray hair	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
11	Raughan-E-Chahar Barg	Anti-inflammatory, Analgesic	Arthralgia Arthritis	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
12	Raughan-E-Haft Barg	Nervine tonic Analgesic	Paralysis Facial palsy Arthritis Flaccid palsy	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
13	Raughan-E-Qust	Nervine tonic	Paralysis	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
14	Raughan-E-Surkh	Anti-inflammatory	Arthritis Gout	Quantum Satis (Q.S)/ Local Application (L.A)	[74]
15	Raughan-E-Hina	Anti-inflammatory, analgesic	Arthralgia ulcers	Quantum Satis (Q.S)/ Local Application (L.A)	[75]
16	Raughan-E-Bars	Blood purifier rubefacient, sedative	Vitiligo, pityriasis alba	Quantum Satis (Q.S)/ Local Application (L.A)	[75]
17	Raughan-E-Dhatura	Nerve stimulant, Nervine tonic	Paralysis Facial palsy Arthritis Flaccid palsy	Quantum Satis (Q.S)/ Local Application (L.A)	[75]
18	Raughan Beer Bahooti	Aphrodisiac	Erectile dysfunction	Quantum Satis (Q.S)/ Local Application (L.A)	[76]
19	Raughan-E-Benazeer	Brain tonic	Cerebrasthenia baldness Asthenopia/Amblyopia	Quantum Satis (Q.S)/ Local Application (L.A)	[77]
20	Raughan-E-Muqawwi-E-Asab	Nerve stimulant, nervine tonic Analgesic anti-inflammatory	Neuralgia Paralysis Facial paralysis Polyarthritis Myalgia	Quantum Satis (Q.S)/ Local Application (L.A)	[77]
21	Marham-E-Dakhliyun	Anti-inflammatory	Uterine ulcer Vaginitis	Quantum Satis (Q.S)/ Local Application (L.A)	[76]

Table 4 (*Continued*)

Sl. no	Drug name	Action	Therapeutic use	Therapeutic doses/ route of drug administration	References
22	Marham-E-Kafoor	Antiseptic	Ulcer	Quantum Satis (Q.S)/ Local Application (L.A)	76
23	Marham-E-Saeeda Chob Neemwala	Anti-inflammatory	Piles	Quantum Satis (Q.S)/ Local Application (L.A)	76
24	Tila Aroosak	Nerve stimulant, Nervine tonic	Erectile dysfunction	Quantum Satis (Q.S)/ Local Application (L.A)	76
25	Tila Benazeer	Aphrodisiac	Erectile dysfunction	Quantum Satis (Q.S)/ Local Application (L.A)	77
26	Zimad Muqawwi	Aphrodisiac	Erectile dysfunction	Quantum Satis (Q.S)/ Local Application (L.A)	77

Pulmonary Diseases and Sesame Oil

In a study on an animal model of allergic asthma, ovalbumin (10 mg intraperitoneal) induced pulmonary edema and inflammation in female BALB/c mice, sesame oil showed a significant reduction in lung weight (wet-to-dry) as a result of improvement in pulmonary edema and significant decreases in proinflammatory cytokines (IL)-1β and IL-6, as well as total cell count and neutrophils; biomarkers like nitric oxide, inducible nitric oxide synthase, and immunoglobulin E (IgE) were also downregulated. This showed that sesame oil has the potential in treating allergic asthma.[35] Another preclinical study on an allergic asthma model treated with lignan of sesame oil called sesamin showed a significant decrease in cytokines of T-helper cell-2 (IL-4, IL-5, IL-13) and increase in interferon-gamma, and inhibited the production of IgE as well as decreased NF-κB P65 level by blocking the phosphorylation of IκB-α and P38 MAPK pathways[36] (►**Fig. 2**).

Cardiovascular Diseases and Sesame Oil

Sesame oil (5%) significantly reduced total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apo A, serum glutamate pyruvate transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT) in comparison to the hypercholesteremic diet (1% cholesterol) in the rat model.[37]

A randomized, triple-blind, crossover clinical study was conducted to demonstrate the effect of sesame oil on the APOA-1 gene in adults with and without type 2 diabetes mellitus. The result showed no significant differences in TC, triglycerides, LDL-C, HDL-C, apo A, apo B, or lipoprotein (a); however, significant differences were observed in LDL:

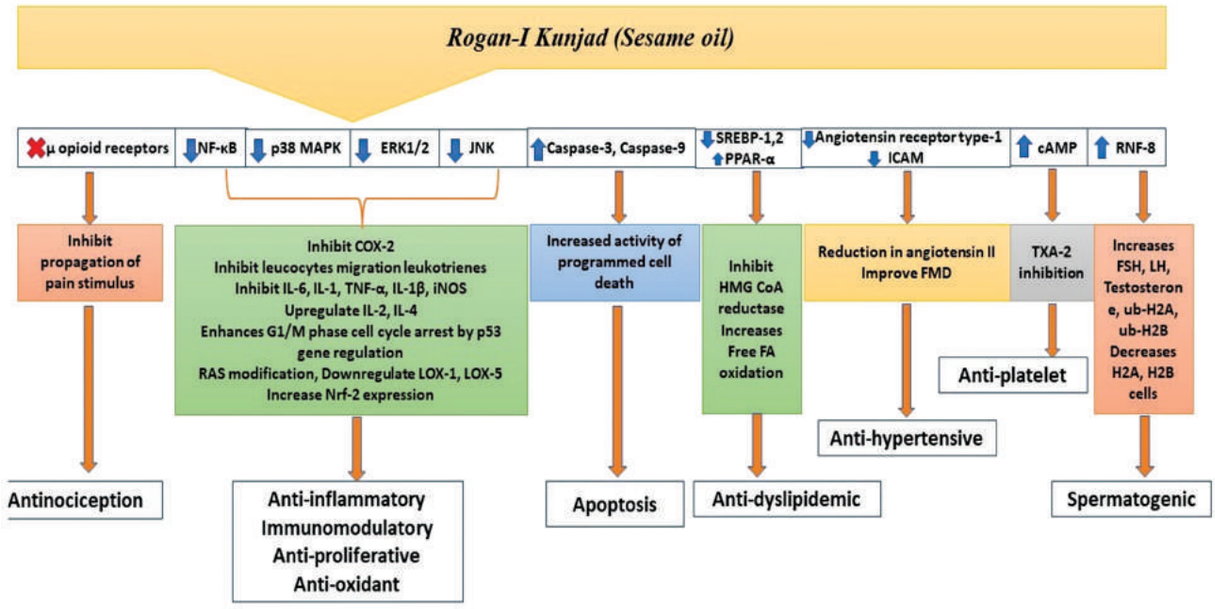

Fig. 2 Pharmacological target for *Rogan-I-Kunjad* (sesame oil).

HDL and TC:HDL ratios, blood pressure, as well as cardiovascular risk scores.[38] A study was conducted on a hypertensive animal model induced with cardiac hypertrophy using an injection of deoxycorticosterone and 1% sodium chloride. Sesame oil showed significant reduction in the size of cardiomyocytes, cardiac renin, angiotensin-converting enzyme, and angiotensin- II, and downregulated the expression of angiotensin receptor type-1, p38 MAPK, and JNK.[39] In a two-phase clinical trial, the first phase (acute phase) involved 30 hypertensive participants who consumed 35 g of sesame oil and were given corn or olive oil as a comparison. After 15 minutes, the objective parameters were observed, and the second phase (chronic phase) involved 60 days of treatment before the participants were evaluated. The study showed significant improvement in flow-mediated dilatation, and a significant decrease in intracellular adhesion molecule was observed after 60 days of treatment.[40] An antithrombotic activity was examined in animals by inducing thrombus using He–Ne laser; the sesame seeds significantly inhibited thrombosis in mice[41,42] (►**Fig. 2**).

Hepatic Diseases and Sesame Oil

In a study on C57BL/6J mice, a steatohepatitis animal model, sesame oil showed a significant reduction in hepatic fatty acid and cholesterol synthesis by downregulating both sterol element binding proteins (SREBPs)—SREBP-1 responsible for the synthesis of FAS enzyme and SREBP-2 responsible for synthesis of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase)—and increased fatty acid oxidation by upregulating Peroxisome Proliferator Activated Receptor Alpha (PPAR-α) expression responsible for synthesis of CTP-1. Endoplasmic reticulum (ER), stress and apoptosis maskers like glucose-regulated protein-78, protein kinase like endoplasmic reticulum kinase, phosphorylated eukaryotic initiation factor-2α, and X-box-binding protein-1 were significantly reduced; these markers showed there was significant improvement in ER stress, and apoptosis biomarkers like BAX, caspase-3, caspase-9, and C/EBP homologous proteins were also reduced.[43] On an animal model another study on nutritional fibrosing steatohepatitis was conducted, and it was found that there was a significant decrease in the degree of steatosis, activity of matrix metalloproteinase-2, 9 (MMP-2, 9), α-SMA expression, fibrotic collagen, SGPT, SGOT, and upregulated PPAR-ɣ expression.[44,45] (►**Fig. 2**).

Carcinoma and Sesame Oil

A preclinical study was conducted on C57BL/J6 mice, in which the mice survived that received whole-body ɣ-radiation with sesame oil compared with the control group due to the ability of sesame oil to diminish MN frequency in nucleated bone marrow cells and eliminate apoptotic cells. This study also demonstrated significant improvement in B-cell (CD 19) and T-cell (CD 4 and CD 8), decrease in TBARS, and significant enhancement in splenocytes proliferation in pretreated sesame oil mice.[46] An in-vitro study was conducted on human head and neck squamous cell carcinoma, that is, HSC-3 (tongue cell line), FaDu (pharynx cell line), and Ca9–22 (Gingiva cell line) to understand the anticancer and anti-metastatic effect of sesamin. This study showed there was a marked reduction in cell mortality, invasion, and migration in all cell lines at 40 μM of sesamin. There was downregulation of MMP-2 expression in HSC-3 and FaDu by 23 and 19%, respectively, and also downregulation of P-p38 and pJNK1/2 expressions leading to inhibition of MAPK pathway. It indicates that sesamin inhibits the synchronized movement of the tumor cell population.[47] In an in vitro study on colon cell lines, Human Colorectal Adenocarcinoma Cell Line (HT-29) and Human Colon Cancer Cell Line (HCT-111) demonstrated that ethanolic extract of *Sesamum indicum* L (ESL) significantly enhanced the radical scavenging activity and decreased cell growth in HT-29 and HCT-116 cells, respectively. The same study revealed a 3.2-fold increased population in sub-G1, G1/M by 2.3–6.6 population and reduction in G0/G1 and S phase of cell cycle as compared with control, which indicates that ESL significantly arrests cell cycle at G2/M.[48] An in vitro study was conducted on a lung adenoma cancer cell line to demonstrate the anticancer effect of sesame oil which showed that there was marked decrease in cell viability and enhancement in apoptotic activity by upregulation of Bax, PARP, TRAIL-R1, and TRAIL-R2 and downregulation of Bcl-2 expression.[49] An in vivo study was conducted to demonstrate the anticarcinogenic effect of sesame oil on serous papillary ovarian carcinoma. The result of this study indicated that there was a significant reduction in the size of the tumor, as well as downregulation of many metabolic enzymes responsible for cellular metabolism and growth and singling pathways like hypoxia inducible factor-1, ER pathways, and proteoglycans.[50] Another study on adult UChB, an experimental model of ovarian cancer, showed the anticancer effect of melatonin, while the result showed there were significant increases in plasma melatonin and downregulation of Her-2, p38 MARK, and p-AKT expression[51] (►**Fig. 2**).

Male Sexual Health and Sesame Oil

An animal study was conducted on streptozotocin-induced diabetic rats to demonstrate the effect of sesame on testicular structure and male reproductive parameters. Sesame oil showed a significant improvement in histopathological changes and prevented testicular atrophy as compared with diabetic control; also, there were significant increases in male reproductive parameters, i.e., follicular stimulating hormone, testosterone, and luteinizing hormone, and decreases in serum glucose level in sesame-treated group as compared with control group.[52] Male Sprague Dawley rats were used in an animal study to show the impact of melatonin on epididymis and sperm characteristics after exposure to fructose and bisphenol A. This study showed marked improvement in epididymis morphology, enhancement of sperm motility, and reduction in apoptotic sperm cells in the 20 mg/kg melatonin-treated group as compared with the fructose and bisphenol A group.[53] Another animal study was conducted on cyclophosphamide-induced spermatogenic dysfunction in which sesamin showed significant increases in the weight of testis, sperm concentration and motility,

Johnsen's score, RNF8 expression, ub-H2A, and ub-H2B, and reverse morphological damage of sperm as well as testicular tissue derangement, significantly lowering the H2A and H2B[54] (►**Fig. 2**).

Arthritis and Sesame Oil

In an animal model of osteoarthritis, sesame oil significantly reduced muscular oxidative stress by decreasing LPO and ROS, and increased GSH level, GPx, and Nrf-2 expression after 7 days of administration.[55] A randomized, double-blind, clinical trial on knee arthritis was conducted to find out the effect of topical application of sesame oil, in which the result showed significant improvement in participants' walking speed, knee flexion angle, VAS, and WOMAC, after 4 weeks of intervention; this result suggested that sesame oil has a significant effect on OA.[56] The effect of sesame seed extracts on Freund's complete adjuvant-induced rheumatoid arthritis model showed that sesame oil markedly reduced paw volume, body temperature, IL-6, TNF-α, and ESR, and significantly upsurges spontaneous activity, body weight, hemoglobin, and RBC[57] (►**Fig. 2**).

Discussion

Rogan-I-Kunjad (sesame oil) is derived from seeds of *kunjad* (*Sesamum indicum* L.) widely used as edible oil as well as a medicine in many traditional systems of medicine; for example, in the Unani system of medicine *Rogan-I-Kunjad* is a well-known drug for the treatment of various *Sawdāwī-Amrāz* (melancholic diseases) diseases like asthma, arthritis, rheumatic arthritis, xerosis, pruritus, ulcer, obstructive pathology, headache, and carcinoma.[58] Additionally, *Rogan-I-Kunjad* has hot and moist temperament, owing to anti-inflammatory, analgesic, laxative, emollient, obstruent, resolvent, diuretic, and vasodilator properties of this drug. According to Unani philosophy of temperament, it has been well documented that the drugs having hot and moist temperament are used in the management of *Sawdāwī-Amrāz* (melancholic diseases).[59,60] Sesame oil is a versatile oil, so it is important to look at the current pharmacological research on it to learn about its traditional uses as well as its possible mechanism of action. In this review, we discovered that the various ethnomedical uses of sesame oil, including their anti-inflammatory, anticholesterol, antifungal, antibacterial, antidiabetic, antiulcer, wound healing, and anti-infertility properties, have been confirmed by contemporary pharmacological investigations using a variety of in-vitro and in-vivo techniques.

The findings reported in all the studies suggest a promising effect of *Rogan-I-Kunjad* (sesame oil) targeting antinociceptive and anti-inflammatory effects by inhibiting prostaglandin synthesis, especially PGE_2 and $PGF_{\alpha 2}$ synthesis from arachidonic acid by downregulation of the cyclooxygenase 2 (COX-2) enzyme. It acts as a COX-2 inhibitor, blocking μ-opioid receptors. It also inhibits the migration of inflammatory cells like leucocytes and leukotrienes synthesis.[61] Sesame oil, which contains active compounds such as sesamin, sesamol, melatonin, and pedaliin, acts as an anticancer agent by suppressing the phosphorylated JNK, p38 MAPKs, NF-κB, and ERK1/2 signaling pathways, thereby reducing abnormal cell proliferation and proinflammatory cytokines such as IL-6, IL-1, and TNF-α, and increasing the activity of the immune cell cytokine IL-2.[33,62] Pedaliin also arrested the cell cycle at G2/M phase and induced apoptosis by regulating caspase-3 and caspase-9.[48] Sesamin and episesamin have potent anti-dyslipidemic effects by blocking SREBP-1, SREBP-2, and HMG-CoA reductase and activating PPAR-α.[38] Sesame oil reduces oxidative damage and inhibits MAPK activation to modify cardiac RAS to alleviate LVH.[39] Through a rise in cyclic nucleotides (i.e., cAMP) and inhibition of MAPK phosphorylation, sesamol has significant antiplatelet action.[42] The limitation of this review article is that the studies included in this manuscript are of particular time duration and set field. As far as strength is concerned the manuscript included some of the clinical studies, preclinical studies, toxicological studies, in vivo, in vitro, in silico studies, and phytoconstituents. The main goal of this review article is to perform a critical analysis of the Unani medical literature about the nutritional and therapeutic benefits and their mechanism of action of chemical constituents found in *Rogan-I-Kunjad*.

Conclusion

Rogan-I-Kunjad is a medication that has been used since antiquity to cure and prevent a variety of ailments. However, it has been shown that the mechanism of action of *Rogan-I-Kunjad* showed its phytopharmacology. The in vitro, in vivo, and in silico models have sufficiently validated its phytopharmacology. The traditional literature showed the significance of *Rogan-I-Kunjad*. But there are still certain gaps that need to be filled. To begin with, *Rogan-I-Kunjad's* traditional pharmacological actions demand additional new pharmacological interpretations to clarify its fundamental mechanism; however, just a few recent researches have been conducted so far. Second, it has frequently been mixed with other Unani medicine, so drug interactions should be studied more thoroughly by traditional therapy. However, some of the clinical studies were conducted on cardiometabolic marker modulation, diabetes mellitus, hypertension, etc., but there is a need to conduct more clinical as well as toxicological studies to generalize stronger evidence. It is important to document the complete pharmacological profiling of *Rogan-I-Kunjad* for its future prospective.

Conflict of Interest

None.

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Current Perspective on Vitamins and SARS-CoV-2 Disease (COVID-19)

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a highly pathogenic and transmissible coronavirus, has resulted in a pandemic named coronavirus disease 2019 (COVID-19). It has taken over the world in no time causing nearly 5 million deaths and almost 500 million people being affected as of June 2022 causing an extensive burden on healthcare facilities globally. Though the disease onset is via respiratory tract, but it affects almost all organs of the body and due to induction of mutations in the virus, combating with the disease is extremely difficult. The major damage associated with disease is driven through inflammatory pathways in tissues with accompanying cytokine storm mediated mainly by macrophages. Building a strong immune system requires maintenance of a healthy diet along with keeping vitamin and coenzyme deficiencies away. The review focuses on the importance of the vitamins for maintaining a good immune system to reduce the susceptibility to SARS-CoV-2 infection, to fight the infection efficiently, and to reduce the impact of the disease. Vitamins play an essential role in modulating the immune responses to infection via altering the signaling pathways, which can act as potential weapons against the disease. Various water- and fat-soluble vitamins like vitamin B, C, D, and E have crucial roles in mediating primary interferon response, improving innate as well as adaptive functions of immunity and antioxidant properties. The current understanding about the supplementation of various vitamins as an adjunct therapeutic strategy to fight COVID-19 disease has also been discussed.

Keywords
- COVID-19
- vitamins
- vitamin D
- micronutrients
- inflammation
- cytokine storm

Introduction

Coronavirus disease or COVID-19 is an extremely widespread infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus, member of the *Coronaviridae* family with positive-sense single-stranded RNA genome. Previously, human coronaviruses were known to be linked to mild respiratory tract problems, causing 15 to 25% of all "common colds," until the outbreak of SARS in 2002 and Middle East respiratory syndrome (MERS) in 2012.[1] The first case of novel coronavirus was reported in December, 2019 in Wuhan, China, which has now rapidly spread to other countries across the world due to its high mutation rate and rapid evolution, thus developing a severe pandemic. Despite tremendous efforts and vaccination drive as of April 2021, more than 149 million

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cases of COVID-19 have been recorded worldwide along with 3 million deaths as reported to the World Health Organization (WHO).[2]

COVID-19 is predominantly acquired orally with a wide range of clinical manifestations from asymptomatic forms to mild symptoms like dry cough, fever, fatigue, and severe cases including acute respiratory distress syndrome (ARDS) and multiorgan dysfunction.[3] Although the exact pathophysiology of SARS-CoV-2 still needs to be explored, it is already known that the virus uses angiotensin-converting enzyme 2 (ACE-2) as receptor via the spike protein for access to the host cells in the respiratory tract and ultimately lead to ARDS and pulmonary edema.[4] Patients infected with SARS-CoV-2 also show increased circulating levels of proinflammatory cytokines, the so-called "cytokine storm," leading to a hyperinflammatory state.[5]

Till date, some vaccines and treatments have been approved for the disease but none of the above are entirely effective against it, making it necessary to maintain optimum levels of personal hygiene and good immunity for its prevention. One of the major preventive factors is maintaining a diet rich in essential proteins, vitamins, and minerals. Healthy diet is the best way to strengthen the immune system and to reduce the susceptibility to viral infections including COVID-19.[6] With existing knowledge regarding the vitamins playing pivotal roles in supporting both innate and acquired immune responses, the vitamins supplementations have gained much attention during the current pandemic. Also, anti-inflammatory properties of several vitamins are well elucidated.[7] Notably, uncontrolled inflammation is a key component in COVID-19 pathogenesis, and in the absence of any specific treatment strategy, vitamin supplementation can act as an important weapon against COVID-19. This review aims to investigate the current findings on identifying the potential value of vitamin supplementation in the fight against COVID-19.

Potential Role of Vitamins Supplementation against COVID-19

The contribution of vitamin supplementation in enhancing the immunity and exhibiting their anti-inflammatory role has long been known. Vitamin A and vitamin B complex are already known for regulating the production of proinflammatory cytokines. Not only this, vitamin D also promotes the synthesis of anti-inflammatory cytokines and antimicrobial peptides that help in inhibiting cytokine storm.

Evidences suggest that different vitamins like vitamin C, vitamin K, and vitamin D and members of vitamin B mediate anti-inflammatory actions by inhibiting NF-κB, an important member of inflammatory cascade.[8,9] Moreover, vitamin C is gaining attention of many researchers due to the fact that antioxidant properties of vitamin C can be used against cytokine storm-mediated oxidative stress in ARDS.[10]

Notably, vitamin deficiencies can lead to weakened immune system and can decrease resistance to infections. Epidemiological studies have confirmed the link between vitamin D deficiency and increased susceptibility to respiratory viral infections.[11] Thus, exploring the role of each vitamin in context to COVID-19 will be of great use.

Vitamin A

Vitamin A, a fat-soluble vitamin, enhances the immune related functions and plays a regulatory part in cellular and humoral immunity. Retinoids stimulate natural killer (NK) cells, dendritic and innate lymphoid cells.[12] It plays a crucial role in maintaining morphology and functional maturation of the epithelium thus enhancing antiviral properties. Thus, reduced vitamin A status has been correlated with improper functioning of neutrophils, macrophages, T-and B-cells and an increased risk of acquiring viral infections. Also, the subjects with deficit vitamin A levels showed morphological changes in pulmonary epithelial and parenchymal lining, causing respiratory dysfunction, as reviewed by Iddir et al.[13] This information is relevant while considering the COVID-19 effects on lung function. Yuan et al depicted that an agonist for retinoic acid (RA) receptor α (Am580) serves as an effective inhibitor of SARS-CoV by disrupting sterol regulatory element binding protein-mediated lipogenic pathways.[12] Another study suggested vitamin A as a treatment of coronavirus infection.[14] A scientific report proved that dietary supplementation with vitamin A increased antibody generation in response to the vaccine in calves. Moreover, lower vitamin A with viral infestations led to an increased damage to epithelium in chickens. These observations also correlate with clinical reports, which stated an increased susceptibility to SARS-CoV with reduced vitamin A amounts in various diseased models. Vitamin A oral supplementation is currently being tested as COVID-19 therapeutics.[7] Vitamin A supplementation to deficient subgroups reduces infection with *Mycoplasma pneumoniae*, a secondary infection seen associated with COVID-19.[15] With bioinformatics-based tools, several potential targets of vitamin A against COVID-19 like "*MAPK1, IL10, EGFR, ICAM1, MAPK14, CAT* and *PRKCB*" have been identified.[16] Also a literature report suggested a possibility of vitamin A usage for rectifying olfactory dysfunction in COVID-19 infection that might help in regenerating olfactory neurons.[17]

However, some other observations from pooled analysis suggested no effect on symptoms or incidences of lower acute respiratory tract infection (RTI) after vitamin A administration. Also, in old age individuals, poor association was found between circulatory carotene and retinal levels with immune response to vaccine, signifying that the immune function was not appreciably influenced upon varying these dietary micronutrients in elderly population. Even in children, vitamin A administration had no influence upon risk assessment of the lower respiratory diseases. Similar results were revealed by a meta-analysis study, where acute RTI incidence did not corelate with the supplemented subset in developing nations as reviewed by Iddir et al.[13] Taking above observations into consideration the role of vitamin A is protective against respiratory tract infections and hence must be considered for its therapeutic value against COVID-19.

Vitamin B Complex

SARS-CoV-2 infection results in increased proinflammatory cytokines mediating its effect on both innate and adaptive immune response. Vitamin B is important for the activation of both innate and adaptive immune responses and it also assists in maintaining endothelial integrity, reducing proinflammatory cytokine levels, preventing hypercoagulability; therefore, it is imperative to unveil the effects of vitamin B complex in reference to inflammatory pathways and COVID-19.[18]

Vitamin B1

Thiamine or thiamine pyrophosphate is an essential water-soluble vitamin crucial for regulating cellular metabolism. Vitamin B1 exhibits an anti-inflammatory role by influencing proapoptotic proteins, cytochrome C release, and MAPK activity.[9] It has also been reported that vitamin B1 deficiency induces cytokine storm by producing high amounts of proinflammatory cytokines like IL-1, tumor necrosis factor-alpha (TNF-α), and IL-6 indicating that adequate amount of thiamine is essential during COVID-19 infection.[19]

Vitamin B2

Vitamin B2 or riboflavin is an important precursor of coenzymes needed for flavoprotein enzymatic reactions. According to Lindsay et al, pathogen reduction with riboflavin-ultraviolet (UV) decreases the infectivity of CoV-2 in whole blood and plasma, thus suggesting that vitamin B2 infusion can be helpful against COVID-19. Not only this, riboflavin insufficiency can induce the intensification of proinflammatory activity of adipocytes that leads the way to chronic inflammation.[20]

Vitamin B3

The immense demand for vitamin B3 or niacin is due to its pivotal role in oxidation–reduction reactions. One of the clinical studies suggests that niacin is a strong agent to decrease proinflammatory cytokines interleukin-1 (IL-1), IL-6, and TNF-α.[21] Besides this, niacin also inhibits the enzymatic activity of M^{pro} protease by binding to its catalytic pocket.[22] On molecular levels as a part of the innate response, PARPs (poly ADP ribose polymerase) get activated due to DNA damage and are required for the inhibition of viral replication.[23] Continued activation of PARP will lead to their depletion at one time. Previous data indicated that nicotinamide adenine dinucleotide (NAD) infusion may restore PARPs' function for inhibition of viral replication and thus support in innate response towards COVID-19.[24] Furthermore, studies indicated that supplementation of NAD precursors like nicotinamide ribose helps combat COVID-19 infection by increasing activity of Sirtuins, reducing oxidative stress and are therefore considered as potential therapeutics for reducing hyperinflammation.[25]

Vitamin B5

Vitamin B5 usually known as pantothenic acid is used for the synthesis of acetyl CoA required in the metabolic pathway of proteins, carbohydrates, and fats. Apart from this, it also plays an important role in decreasing inflammation, improves mental health, and is an important regulator of innate and adaptive response. However, vitamin B5 or the mechanisms of its action in context to COVID-19 has not been studied yet.

Vitamin B6

Pyridoxine is a water-soluble vitamin; its active form pyridoxal phosphate (PLP) has a supporting role in inflammation, metabolism, and immunomodulation. In this context, PLP supplementation has been proposed to decrease proinflammatory cytokines, prevent hypercoagulability, and support endothelial integrity which tends to diminish COVID-19 symptoms.[26] Insufficient levels of PLP have also been observed in type-2 diabetes patients, persons with long suffering cardiovascular disease, and in the elderly, the groups who are at an increased risk of poor COVID-19 outcomes.[27] According to Qian et al, pyridoxine deficiency decreased the lymphocyte number and interleukins production that are an important part of adaptive response during COVID-19 infection.

Vitamin B9

Vitamin B9/ folate is a water-soluble vitamin involved in one carbon metabolism and is essential for DNA and protein synthesis. It has been studied that folic acid inhibits furin protease and $3Cl^{pro}$ (coronavirus main proteinases).[28] Hence, folic acid decreases the rate of replication in these two ways. Folic acid supplementation reverses the uncoupling of epithelial nitric oxide synthase (eNOS) restoring nitric oxide production along with an affect on endothelial function therefore, showing an anti-inflammatory and antioxidant activity.[29] Moreover, the expression of nuclear factor kappa B (NF-κB; proinflammatory molecules) and cyclooxygenase-2 is also under the control of eNOS. As NF-κB is a regulatory factor for proinflammatory cytokines, folic acid is known to reduce homocysteine induced NF-κB.[30]

Vitamin B12

Vitamin B12 is necessary for the synthesis and division of red blood cells and white blood cells. Vitamin B12 deficiency gives rise to hyperhomocysteinemia further leading to increased inflammation, reactive oxygen species (ROS), and endothelial dysfunction. Patients with COVID-19 show the symptoms like elevated homocysteine, hypercoagulopathy, and increased oxidative stress that are parallel to that of vitamin B12 deficiency.[31] Romain et al showed that vitamin B12 also exhibits anti-inflammatory effects similar to folate by regulating NF-κB. Interestingly, a recent study manifested that methylcobalamin supplementations tends to decrease COVID-19-related symptoms and organ damage.[32] Various roles of vitamin B have been compiled in ►**Table 1**.

Vitamin C

Vitamin C is a water-soluble vitamin known for its antioxidant role. COVID-19 patients show increased number of neutrophils but are lymphopenic. Researchers also reported that vitamin C suppresses antiviral T cells through

Table 1 Role of different vitamins of B complex

Vitamin	Physiological role	Role in COVID-19	Recommended (per day)
B1 (Thiamine)	• Important cofactor for enzymes involved in carbohydrate and amino acid metabolism[68] • Antioxidant role	• Anti-inflammatory (inhibits NF-κB pathway)[9] • Antioxidant role[19]	• 1.1 mg
B2 (Riboflavin)	• Precursor of enzymes needed for reactions of flavoproteins[69]	• Riboflavin-UV in combination reduces the infectivity of CoV-2 in whole blood and plasma[70]	1.1mg
B3 (Niacin)	• Cofactor in oxidation reduction reactions[71] • Reduction in pro-inflammatory cytokines[21]	• Potent inhibitor of M^{pro} protease (needed for viral replication)[2263,64] • Increase sirtuins activity[72] • Relieves oxidative stress	14 mg
B5 (Pantothenic acid)	• Synthesis of coenzyme A (essential for carbohydrate, fatty acid, proteins metabolism)[73] • Wound healing[74]	• Not studied yet	5 mg
B6 (Pyridoxine)	• Red blood cell synthesis[75] • Vital for metabolic pathway of fats, carbohydrates and proteins	• Prevents hypercoagulability • Inhibits release of proinflammatory cytokines.[26]	1.3 mg
B9 (Folate)	• DNA and protein synthesis • Transfers one carbon units in metabolic pathways (one carbon metabolism)[76]	• Inhibits furin protease (viral entry)[28] • Inactivates protease $3Cl^{pro}$ (virus replication)[77] • Anti-inflammatory (regulate NF-κB) and antioxidant activity[78]	400 µg
B12 (Methylcobalamin)	• Important for synthesis of DNA • Red blood cells' and white blood cells' division	• Exhibits anti-inflammatory effects by regulating NF-κB[79] • Methylcobalamin supplements tends to decrease COVID-19 related symptoms[32]	2.4 µg

Abbreviations: COVID-19, coronavirus disease 2019; NF-κB, nuclear factor-kappa beta; UV, ultraviolet.

augmentation of interferon (IFN) production and thus it is helpful in maintaining normal neutrophils to lymphocytes ratio.[33]

Neutrophils bind to platelets that induce them to form neutrophil extracellular traps (NETs).[34] NETs play both protective and detrimental roles during viral infections. Small pathogen like viruses gets entrapped inside NETs as found during influenza and syncytial respiratory virus infection.[35] On the other hand, NET-associated antimicrobial factors may have toxic effects for the host and their increased production may cause tissue damage.[36] Additionally, vitamin C has a prime role in clearance of dead neutrophils from the site of infection; vitamin C also weakens NET formation in healthy people.[37] Furthermore, it counteracts oxidative stress, thus preventing NF-κB-driven cytokine storm too. However, vitamin C treatment must be started before the requirement of intensive care unit to maintain normal neutrophil to lymphocytes ratio that may prevent SARS-CoV-2 patients to worsen toward ARDS.[38] Vitamin C may also help in decreased alveolar epithelial damage by preventing excess activation and aggregation of neutrophils.[7] In addition, SARS-CoV downregulates ACE-2 receptor via its spike protein that contributes to multiple organ injury in COVID-19. Moreover, SIRT-1 is expressed next to the promoter region of ACE-2 gene; hence, ACE-2 transcript is under the epigenetic control of SIRT-1. Vitamin C is known to upregulate SIRT-1 consequently leading to increased expression of ACE-2.[39] Therefore, vitamin C is a potential rescue therapy for COVID-19.

Vitamin D

In context to COVID-19 pandemic, vitamin D is one of the most extensively studied vitamins due to its crucial role in immune response against viral diseases. The potential roles of vitamin D with respect to its structure and other cellular and immune related functions has been summarized in the ►**Table 2**. Scientific literature has discovered that 91% of indoor workers possessed suboptimal vitamin D status.[40] Its role as immune modulator in viral acute RTIs came from a study involving patients possessing mutated vitamin D receptors.[15] Jain et al illustrated that administration of vitamin D with L-cysteine decreased oxidative stress in vitamin D deficient mouse model.[41] In a rat model of ARDS, calcitriol upregulated ACE-2 and downregulated renin and angiotensin-II indicating vitamin D hinders the advancement of infection induced ARDS. Furthermore, a meta-analysis demonstrated a 12% shielding effect of vitamin D supplementation and risk of acute RTIs that raised up to 19% either through a daily or weekly treatment relative to a monthly bolus. Although, 70% of the protective effect was observed when the deficiency was corrected.[7] A current analysis including 11,000 subjects depicted a protective role of vitamin D administration in acute RTIs in 25 random control

Table 2 Potential roles of vitamin D

	Roles	References
Structural-related functions	-Maintenance of lung structure, size, volume, and functions -Upregulation of ACE-1, ACE-2	7,40
Cellular functions	-Induction of autophagy -Stimulation of apoptosis	41,47
Immune-related functions	Differentiation and killing potential of monocytes	14
	Supports antigen presentation	14
	Augmentation of natural protective barriers	7
	Downregulates NF-κB that led to increased anti-inflammatory, reduced proinflammatory factors (IL-6, IL-8, IL-12, COX-2, GM-CSF, IL-4, IL-5, VCAM-1, ICAM-1, E-selectin)	80
	Affects the production of enzymes, i.e., iNOS, PLA2	81
	Generation of antimicrobial peptides like cathelicidins (LL-37), defensins	45,81
	Immunosuppression by activating T-regulatory cells	45
	Antioxidant functions	41

Abbreviations: ACE-1, angiotensin-converting enzyme 1; Cox2, cyclooxygenase-2; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM-1, Intercellular adhesion molecule-1; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-kappa beta; PLA2, Phospholipase A2; VCAM-1, Vascular cell adhesion molecule-1.

trials.[42] Similarly in one more clinical trial, a 100,000IU intake of vitamin D once in a month declined acute RTIs in older patients, with respect to a standard dose of 12,000IU/month.[43]

The cocooning or indoor confinement might be the main cause of vitamin D deficiency that was aggravated during COVID-19 lockdown. The prevalent vitamin D deficiency in Northern Hemisphere has been linked to inflammation suppressive role of vitamin D as evident in severe COVID-19 subjects.[40] The correction of vitamin D deficient levels in COVID-19 individuals represses an adhesion-related molecule (CD26/DPP4) via which the COVID-MERS viruses are thought to access a host cell. The interaction of spike protein with ACE-2 translocates the virus inside the host cell, thereby diminishing ACE-2 and probably improving the pathogenesis of respiratory infections. Also, a negative association was revealed between vitamin D levels and COVID-19 mortality in many European countries as reviewed in ref.[7] Similarly, SARS-CoV-2 patients in Switzerland had significantly lower vitamin D status (11.1 ng/mL) relative to the negative cases (24.6 ng/mL).[42] Overall, the usage of multivitamins including vitamin D might decrease inflammation in SARS-CoV-2 infection and can serve as an adjunct in COVID-19 therapeutics. Vitamin D is known to affect the risks, severity, morbidity, and mortality of numerous pulmonary diseases and may also have similar effects in case of COVID-19. However, Rhodes et al suggested that a few nations have low mortality, which signifies the role of vitamin D in determining outcomes from COVID-19.[44] Also, the circulatory 25(OH)D declines with age, which parallels the mechanism of increased COVID-19 fatality with increasing age.[45] In relation to COVID-19, khoramipour considered that 1000 to 2000 IU/d of vitamin D was sufficient to enhance respiratory immunity.[46] Moozhipurath et al depicted an inverse connection of UV-B exposure, which is related to vitamin D production thus showing inverse correlation of vitamin D production with COVID-19 mortality. There is 17.3% risk of COVID-19 among individuals with severe vitamin-D deficiency; however, it was 14.6% in individuals having vitamin D within normal limits. Vitamin D diminishes proinflammatory cytokines that are involved in COVID-19 manifestation, shifts cell population from Th1 to Th2 type, thus preventing cytokine storm. The data from studies worldwide correlates severe 25(OH)D deficiency with COVID-19 that caused coagulopathies, disrupted immune activity, thrombocytopenia, and raised prothrombin time, suggesting vitamin D supplementation as a potential therapeutic regime as reviewed by Vyas et al.[41]

Adding to the advantages of vitamin D, recent findings showed raised number of white blood cells after vitamin D ingestion in COVID-19 cases.[47] Further as treatment to COVID-19, the combination of vitamin D with melatonin and a triple therapy comprising of quercetin, calcitriol and estradiol is advised.[48] A trial conducted by University of Granada involving 200 subjects proposed a dosage of 25,000 IU of calcitriol in preventing and curing COVID-19.[49]

Surprisingly, contrasting evidences to the above-mentioned information do exist, as investigations of UK biobank and The Royal Society revealed no clue to conclude vitamin D as a solution in COVID-19 infections. However, they discerned that COVID-19 disproportionately affects Black and minority ethnic subsets irrespective of vitamin D levels.[42] Studies on RTIs detected nonsignificant differences between the high-dose (2000IU/d) versus standard-dose (400IU/d) except in the severe deficient cases.[43] "Scientific Advisory Committee on Nutrition" verified that the present evidences do not support calcitriol administration to avert acute RTIs in the individuals.[40] Moreover, calcitriol deficiency has also been linked with numerous disorders such as diabetes, hypertension, cognitive dysfunctions, and cardiac illness, making one more prone to COVID-19. The reason for

complicated role of vitamin D might be due to its different doses, course of therapy, study settings, study subjects and interval, definitions, and verification of the outcomes.[40]

Thus, the existing studies suggest a preventive role of vitamin D supplementation against COVID-19; however, exceeding supplementation causing hypervitaminosis must also be taken into consideration as a consequence.

Vitamin E

Vitamin E is a fat-soluble vitamin with an antioxidant activity along with important immunomodulatory effects. Excessive ROS production and lipid peroxidation have been observed in ARDS as well as in vitamin E deficiency.[50] Vitamin E supplementation improves the immune function by T cell activation, enhancing NK cell activity by modulating NO levels, increasing macrophage phagocytic activity along with the decreased IL-12 production and regulating function of dendritic cells.[51] In analysis of male Finnish smokers, vitamin E infusion was found to reduce the risk of pneumonia in older subjects (>60 years) but no significant effect was found on respiratory infection in another study of Dutch older adults.[52] A study in year 2020 showed the mitigating effect on cardiac injuries in COVID-19 patients with combined supplementation of vitamin C and E. However, another group of researchers found that risk of pneumonia was increased in 50 to 69 years old smokers due to vitamin E administration.[52]

A randomized double-blind trial reported that vitamin E supplementation (200IU/d) for 1 year decreased the risk of upper respiratory tract infections.[53] Therefore, it can be speculated that vitamin E might be helpful in prevention of COVID-19 infection. Not only this, one study had already indicated that vitamin E administration is more helpful in reducing elevated oxidative stress than vitamin C in influenza patients, but both vitamin C and E in combination even reduce lipid peroxidation.[54] Study by Dewald in 2021 indicated the greater number of COVID-19 patients (36%) were linked with high dose of vitamin E (>15.5mg/mL) that is attributed to vitamin E supplementation. Patients with intracranial hemorrhages also have the vitamin E levels ranged between 23 and 46.7 mg/mL and the deceased patients show even higher levels of vitamin E (111.7mg/mL). Altogether, this suggests that although vitamin E has protective effects in context to COVID-19, it is necessary to evaluate the necessary supplement intake and patients' needs in order to avoid overdosing and thus toxicity.

Vitamin K

Vitamin K is another fat-soluble vitamin recognized for its crucial role in coagulation via prothrombin (clotting factor II). Vitamin K also activates anticoagulant protein S, required for thrombosis prevention. These pleiotropic roles of vitamin K in blood clotting could be very important in the context of severe COVID-19 where coagulopathy has been well known. The key mediators behind this hypercoagulable state are the increased levels of proinflammatory cytokines, the cytokine storm observed during COVID-19 infection. Therefore, special attention should be paid to vitamin K levels in COVID-19 patients with heart-related comorbidities especially those taking vitamin K antagonists.[55]

Apart from this, vitamin K is also essential for carboxylation and activation of many proteins including osteocalcin in bone and matrix Gla protein (MGP) that protect against vascular calcification.[56] Increased circulating levels of inactive MGP (desphospho-uncarboxylated MGP) in COVID-19 patients exhibiting elastic fiber pathologies indicate the link between vitamin K deficiency and COVID-19.[57]

Other than its role as cofactor for different enzymes, vitamin K also shows significant anti-inflammatory effects. One mechanism for such effects is the inhibition of IL-6, a major cytokine in NF-κB signaling. Taking into consideration the anti-inflammatory actions of vitamin K, another study suggested that vitamin K deficiency can cause activation of the Th2 storm with increased production of IL-6 and thus suggesting its link with cytokine storm in COVID-19 patients.[58] However, further investigations are required to understand the interrelationship between vitamin K and COVID-19.

Discussion

COVID-19 had caused drastic health crisis worldwide. Although a few vaccines and treatments have been approved for COVID-19, due to the lot of people being infected in the COVID-19 scenario, clinical preventive measures are required to reduce the catastrophic effects of COVID-19. One such approach is maintaining optimum vitamin levels and a balanced diet to strengthen the immune system. In this review, we have highlighted the potential role of vitamin supplementation in the fight against COVID-19 taking into consideration the pleiotropic roles played by vitamins that are required for a healthy immune system (summarized in ►**Fig. 1**). Retinoids (vitamin A) perform various immune functions by regulating multiple processes like actions of IFN-I, phagocytic and oxidative activity of macrophages, function of immune cells, production of IL-2, TNF-α, and differentiation of Th1 and Th2 cells.[12,59] In relation to coronavirus it has been known that retinoid signaling inhibits coronavirus and RA receptor α (Am580) serves as a potential SARS-CoV inhibitor.[60] Also, vitamin A ingestion via diet raised the antibody production in response to inactivated bovine coronaviruses.[61] Apart from this, there are existing reports that suggested either negative or no associations of vitamin A supplementation with risk of reducing COVID-19 infections. However, as contradictory studies exist in the present literature, it is difficult to say whether vitamin A intake is fully advantageous against COVID-19.

Evidences from literature also demonstrated anti-inflammatory, antioxidative, as well as anticoagulopathic function of vitamins that have been proven to be beneficial in fighting disease. Vitamin C, D, K, and B have a peculiar regulatory function in mediating the innate immunity via regulation of pro- and anti-inflammatory pathways. These vitamins either inhibit the production of proinflammatory cytokines like IL-1, IL-6, and TNF-α or promote their anti-inflammatory

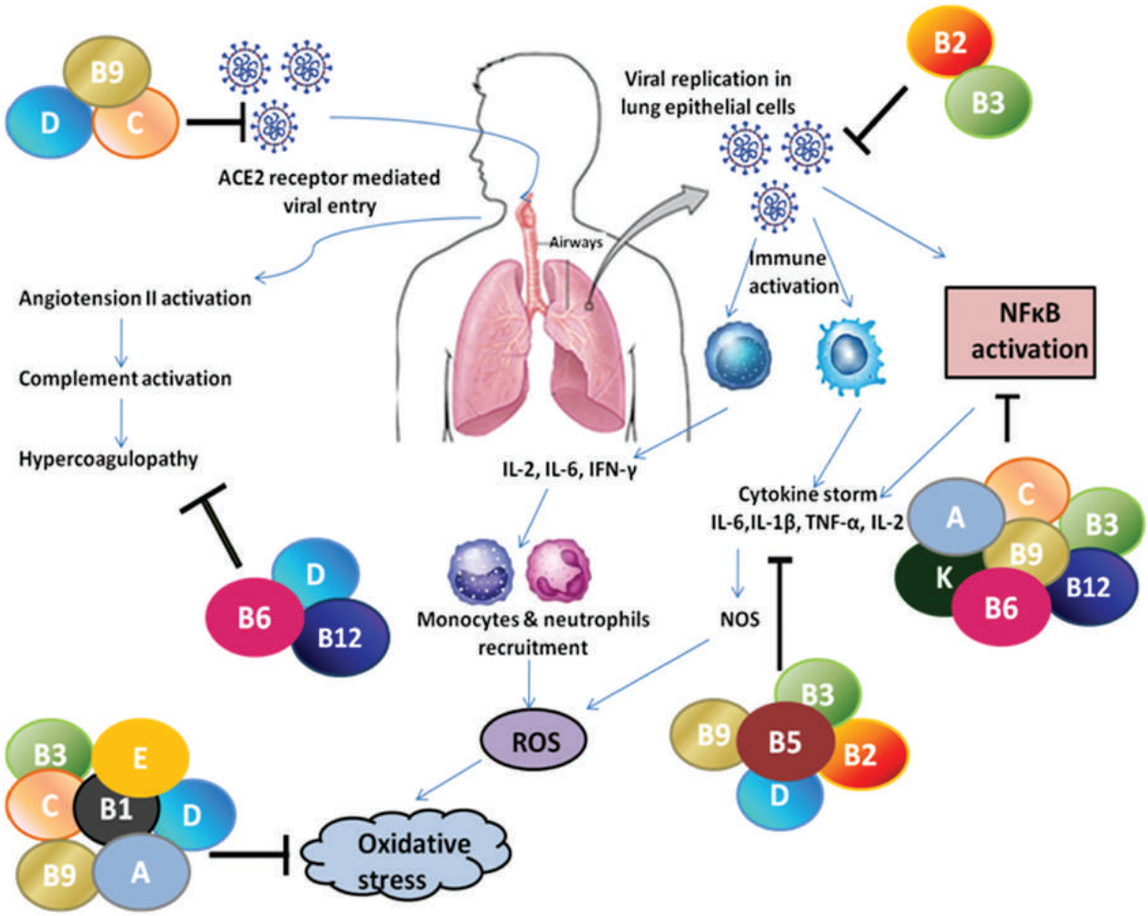

Fig. 1 Potential role of various vitamins against the pathogenesis of coronavirus disease 2019 (COVID-19). ACE-2, angiotensin-converting enzyme 2; IFN-γ, interferon γ; IL-2, interleukin-2; NOS, nitric oxide synthase; NF-κB, nuclear factor-kappa beta; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α. Vitamins are depicted by the alphabets and numbers.

effects by inhibiting NF-Kb.[9,26] Moreover, both vitamin E and B9 modulate NO production that add to their anti-inflammatory activities.[29,62] In addition to their anti-inflammatory role, vitamin B family is found to be beneficial in respiratory tract infections like COVID-19 because of their role as antioxidants. As inflammation is also accompanied by increased ROS production and lipid peroxidation in COVID 19, vitamin E, C, B1, B9, and B12 mediate their vital functions by acting as strong antioxidant agents.

Some of the vitamin B complex are also known to inhibit the activity of the enzymes essential for SARS-CoV-2, like folate inhibits the furin protease and niacin blocks M^{pro} protease.[63,64] Moreover, vitamin C is a potential rescue therapy for COVID-19 because its supplementation opposes oxidative stress-induced cytokine storm by preventing NF-κB activation and neutrophil sequestration.[10] Furthermore, combined treatment of vitamin C and E reduces oxidative stress and lipid peroxidation.[54] Vitamin D has a versatile impact in combating lung diseases by functions such as monocyte differentiation to macrophages, inflammatory cytokine production, antigen presentation, maintenance of cell-to-cell junctions, and production of antimicrobial peptides like cathelicidins and defensins. Moreover, the sufficient consumption of vitamin D might help to reduce the prevalence, symptom severity, death, and recovery rate of COVID-19 patients.[15] Further as therapeutics or prevention strategies to COVID-19, a few combination therapies have been suggested including quercetin or estradiol.[65]

Besides this, it has been noted that severe 25(OH)D deficient environment with coronavirus infection can cause disruption in coagulation profile namely reduced platelet count and raised prothrombin time that might be rectified after 25(OH)D supplementation.[41] Vitamin B6 and B12 are also known to prevent hypercoagulopathic state and thus sum up to be beneficial in COVID-19 infection. Apart from this, vitamin K also has a pleiotropic role in blood clotting.

Till scientific evidence come forward, excessive doses of vitamins should be avoided. Excessive vitamin D intake could result in hypercalcemia and renal stones.[66] Also, vitamins can act as epigenetic modulators and can alter transcriptional and translational switch of diverse inflammation related markers.[67] All these evidences suggest that adequate vitamin consumption is essential for precise body functioning in the COVID-19 scenario.

Data Availability Statement

As this is a review article, thus the data added in has been taken from the available literature.

Authors' Contributions

J.K. supported the conceptualization and the objective of the article. D.R., H.G., R.S. carried out the writing of the article. D.L. contributed by editing the article so as to make it a crisp read. J.K. also contributed by conceptually revising the article on a critical level.

Conflicts of Interest

None declared.

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Measurement of Intact Serum Parathormone and Corrected Serum Calcium after Thyroid Surgery

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Abstract

Postoperative hypocalcemia is an important complication of thyroid surgery. The present study was undertaken to measure the serum levels of intact parathormone (iPTH) and corrected serum calcium (CSC) in the immediate postoperative period and then sequentially till 1 month after thyroid surgery. A total of 36 patients undergoing total thyroidectomy and 44 undergoing hemithyroidectomy had measurement of the level of iPTH and CSC at 1 hour, 1 day, 1 week, and 1 month after surgery. A mean drop of 9.3% in CSC, 40% in iPTH, and 10% in ionic calcium levels was noted 1 hour after total thyroidectomy. All the patients recovered to near preoperative levels at the 1-month follow-up. Among hemithyroidectomy patients, significant postsurgery drop in levels was not observed. The importance of the study is early recognition of a hypoparathyroid state at 1 hour after surgery and institution of calcium replacement, thereby sparing the patient from unpleasant symptoms of hypocalcemia. Furthermore, patients with a drop in the iPTH levels below the defined hypoparathyroidism levels should have careful evaluation of their thyroidectomy specimen for identification and possible autotransplantation of the parathyroid gland intraoperatively or in the immediate postoperative time frame.

Keywords
- corrected serum calcium
- serum parathormone
- thyroid surgery

Neurological injuries and hypocalcemia are the most common complications that can occur after thyroid surgery. Although the incidence of these complications has been steadily declining over the years, when they occur they can lead to serious morbidity and occasional mortality as well.

Postoperative hypocalcemia is secondary to inadvertent removal of the parathyroid glands or their devascularization during dissection. The incidence of hypoparathyroidism after thyroid surgery has been reported to vary from 14 to 60% for temporary hypoparathyroidism and 1.5 to 11% for permanent hypoparathyroidism.[1] Clinical manifestations of hypocalcemia may vary from perioral, hand, and foot numbness to muscle spasm, depression, hallucination, weak and brittle nails and easily fracturing bones. Furthermore, there are several

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confounding factors such as age, gender, vitamin D status, pH of blood, thyroid function status, and pathology (benign vs. malignant), which can affect postoperative calcium status besides the inadvertent damage to the parathyroids.

The present study was undertaken to evaluate the serum levels of intact parathormone (iPTH) and corrected serum calcium (CSC) in the immediate postsurgery period and in the follow-up period up to 1 month after thyroid surgery.

Patients and Methods

This prospective observational study was conducted over a 3-year period from April 2017 to March 2020, during which 80 patients requiring thyroid surgery were recruited. Out of these, 44 patients underwent hemithyroidectomy and 36 were subjected to total thyroidectomy. The exclusion criteria for patient data accrual for this study were the following:

- Preoperative abnormal serum iPTH (<15 or >51 pg/mL).
- Preoperative abnormal CSC level (<8.5 or >10.5 mg/dL).
- Patient on calcium supplement.
- Patient with abnormal thyroid function, renal function, and hypoalbuminemia.

The intent of including patients undergoing hemithyroidectomy was to demonstrate that there were no extraneous factors, such as anesthetic agents or perioperative medication, that could have influenced the iPTH and CSC levels.

The diagnosis of goiter was confirmed on the basis of clinical evaluation, thyroid ultrasound, thyroid function test, and fine-needle aspiration cytology (FNAC). All the patients had a preoperative measurement of the serum level of intact parathormone (iPTH), CSC, and ionized serum calcium. Repeat evaluation of these parameters was done 1 hour, 1 day, 1 week, and 1 month after surgery.

For iPTH measurement, 2 mL of blood was collected in ice-cooled ethylenediaminetetraacetic acid (EDTA) vial using an overnight ice-cooled syringe. The sample was immediately centrifuged in a refrigerated centrifuge at 4°C and the lab estimation by chemiluminescence method was done as quickly as possible. For ionic calcium, 1 mL of arterial blood was collected in a heparinized syringe free of air. The ionic calcium estimation was done by the ion-selective electrode (ISE) method used for arterial blood gas estimations. CSC was estimated by the following formula: CSC = patient serum calcium (mg/dL) +0.8 (4-patient serum albumin in g/dL).

The normal lab reference range of the three parameters were the following:

- CSC: 8.5 to 10.5 mg/dL.
- iPTH: 15 to 51 pg/mL.
- Ionic calcium: 1.0 to 1.30 mmol/L.

Evaluation for clinical features of hypocalcemia was done by (1) asking patients about the perioral and acral paresthesia, tingling, or numbness, (2) Trousseau's sign of latent tetany, (3) Chvostek's sign, and (4) evidence of spontaneous carpopedal spasm or generalized tetany. Trousseau's sign was elicited by inflating the blood pressure cuff to pressure greater than systolic blood pressure and holding it for 3 minutes. Spasm of muscles of the hand and forearm indicated a positive sign. Chvostek's sign was elicited by tapping in front of the ear. Ipsilateral facial muscle spasm indicated a positive sign.

The end point of the study was 1 month after the thyroid surgery at which time CSC, iPTH, and ionic calcium levels were measured again. Values less than the lower limit of reference range were considered hypocalcemic (<8.5 mg/dL of CSC or <1.0 mmol/L of ionic calcium) or hypoparathyroid (<15 pg/mL of iPTH).

Observations

A total of 80 patients who underwent hemithyroidectomy (44) or total thyroidectomy (36) over a 3-year period were included in the study. The mean age of the patients was 43 years (range: 22–68 years) and the gender distribution was 19 males to 61 females (M:F::1:3.2). All patients had a goiter and the preoperative diagnosis were the following: (1) solitary thyroid nodule ($n = 44$); (2) multinodular goiter ($n = 27$); (3) well-differentiated thyroid cancer ($n = 5$), and (4) chronic lymphocytic thyroiditis ($n = 4$).

All patients underwent surgery under general anesthesia with muscle relaxation. The salient features of the operative procedure were the following:

- Capsular ligation of the branches of the inferior thyroid artery.
- Routine attempt at identification of at least one or two parathyroid glands prior to vessel ligation.

Among the 36 patients undergoing total thyroidectomy, we could reliably identify 1 or 2 parathyroid glands in 28 patients. On examination of the resected total thyroidectomy specimen, we could identify one or two parathyroid glands in three specimens. However, autotransplantation was not done in any of the patients because intraoperative preservation of at least one parathyroid gland was demonstrated in the thyroid bed.

Among the 44 patients who underwent hemithyroidectomy, the mean baseline (preoperative) iPTH levels were 35.6 ± 11.6 pg/mL. One hour after hemithyroidectomy, the mean iPTH was 33.4 ± 10.2 pg/mL, which was a 6% drop over the baseline value. At 1 day after surgery, the iPTH rebounded to 43.8 ± 9.8 pg/mL, it was 41.5 ± 10.4 pg/mL at 1 week, and stabilized at 38.4 ± 9.1 pg/mL at 1 month after hemithyroidectomy (►**Table 1**).

Among the 36 patients who underwent total thyroidectomy, the mean baseline (preoperative) iPTH was 39.4 ± 11.9 pg/mL, which dropped by 40% to 23.4 ± 17.5 pg/mL 1 hour after surgery and gradually recovered to 28.6 ± 17.8 pg/mL at 1 day, 30.2 ± 16.2 pg/mL at 1 week, and stabilized at 36.1 ± 13.0 pg/mL 1 month after total thyroidectomy (►**Table 1**, ►**Fig. 1**).

The mean value of CSC in the two groups followed a similar pattern. In the hemithyroidectomy patients, the mean baseline CSC was 9.55 ± 0.77 mg/dL, which dropped by 2.8% 1 hour after surgery and recovered to normal levels at 1 day postoperatively. Among total thyroidectomy patients, the mean baseline CSC was 9.5 ± 0.68 pg/dL, which dropped

Table 1 Serum iPTH levels at baseline and at 1 hour, 1 day, 1 week, and 1 month after surgery

	Hemithyroidectomy ($n = 44$)	Total thyroidectomy ($n = 36$)
iPTH baseline (pg/mL)	35.598 ± 11.6320	39.348 ± 11.9700
iPTH 1 h after surgery (pg/mL)	33.427 ± 10.2710 (–6%)	23.416 ± 17.5015 (–40%)
iPTH after 1 d (pg/mL)	43.883 ± 9.8528 (+23%)	28.615 ± 17.8183 (–27%)
iPTH after 1 wk (pg/mL)	41.598 ± 10.4965 (+17%)	30.275 ± 16.2366 (–23%)
iPTH after 1 mo (pg/mL)	38.445 ± 9.1708 (+8%)	36.133 ± 13.0558 (–8%)

Abbreviation: iPTH, intact parathormone.
Note: Values in bracket represent percentage change over base line values.

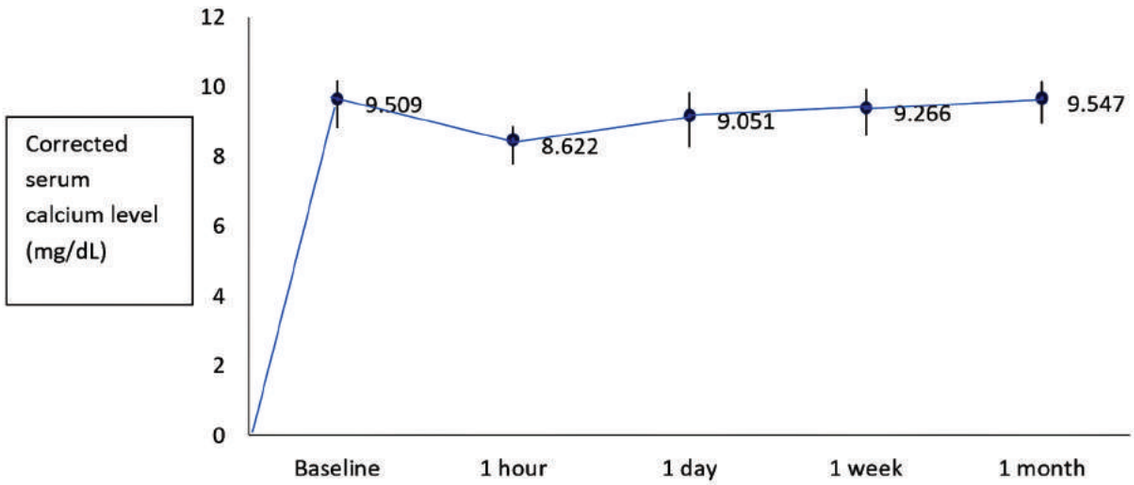

Fig. 1 Sequential values of corrected serum calcium (CSC) after total thyroidectomy ($n = 36$) at baseline, 1 hour, 1 day, 1 week, and 1 month after surgery.

Table 2 Corrected serum (CS) calcium levels at baseline and at 1 hour, 1 day, 1 week, and 1 month after surgery

	Hemithyroidectomy ($n = 44$)	Total thyroidectomy ($n = 36$)
CS calcium baseline (mg/dL)	9.550 ± 0.7793	9.509 ± 0.6827
CS calcium 1 h after surgery (mg/dL)	9.283 ± 0.8031 (–2.8%)	8.622 ± 0.5533 (–9.3%)
CS calcium after 1 d	9.694 ± 0.5536 (+1.5%)	9.051 ± 0.7858 (–4.8%)
CS calcium after 1 wk (mg/dL)	9.886 ± 0.4084 (+3.5%)	9.266 ± 0.6676 (–2.6%)
CS calcium after 1 mo (mg/dL)	9.939 ± 0.3711 (+4.1%)	9.547 ± 0.6025 (+0.4%)

Note: Values in bracket are percentage change over the baseline values.

by 9.3% 1 hour after surgery and remained below the baseline value by 2.6% at 1 week after surgery. Stabilization of the CSC level was seen at 1 month after total thyroidectomy compared to 1 day after hemithyroidectomy (►**Table 2**, ►**Fig. 2**).

At 1 hour post total thyroidectomy, the CSC level was found to be less than 8.5 mg/dL in 12 patients (33%), which is our defined lower limit for normal CSC range. At 1 month of follow-up, none of the patients had CSC value less than the normal range (►**Table 3**).

On clinical evaluation on the evening of surgery, of the 36 patients who underwent total thyroidectomy, occult clinical evidence of hypocalcemia such as perioral or acral tingling or numbness, Trousseau's sign and Chvostek's sign could be evoked by subtle questions or sign elicitation technique in 6 patients. Among these three patients had CSC <8.5 mg/dL and three had CSC >8.5 mg/dL. None of the patients developed spontaneous carpopedal spasm or generalized tetany. All patients had recovery of their clinical signs within 2 to 4 days of surgery. Only 1 of 44 hemithyroidectomy patients complained of perioral paresthesia on enquiry.

In none of the hemithyroidectomy patients did the iPTH level fall to <15 pg/mL, which is the usually accepted definition for hypoparathyroidism. Among the 36 total thyroidectomy patients, the iPTH level fell to <15 pg/mL in 5

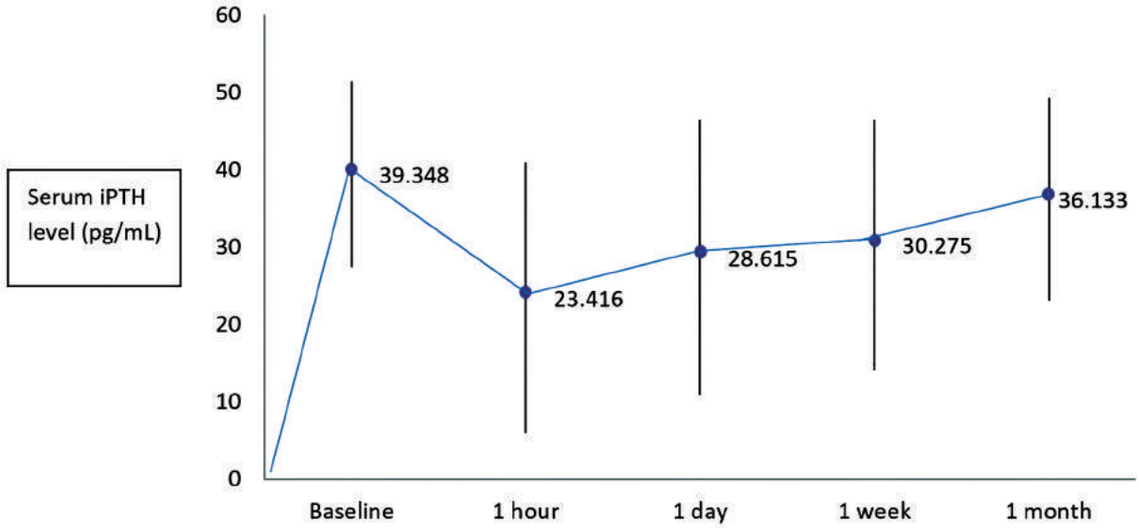

Fig. 2 Sequential values of iPTH after total thyroidectomy ($n = 36$) at baseline, 1hour, 1 day, 1 week and 1 month after surgery.

Table 3 Follow-up of total thyroidectomy patients ($n = 36$) whose immediate postoperative iPTH level fell to less than 15 pg/mL and CSC level below 8.5 mg/dL

	At 1 h postoperative	At 1 mo postoperative
iPTH <15 pg/mL + CSC <8.5 mg/dL at 1 h postoperatively (both less than normal range values)	4 (11%)	Nil
Only CSC <8.5 mg/dL (normal iPTH)	8 (22%)	Nil
Only iPTH <15 mg/dL (normal CSC)	1 (3%)	Nil

Abbreviation: CSC, corrected serum calcium, iPTH, intact parathormone.

patients (14%) immediately after surgery although it showed a gradual recovery to near baseline levels in 3 patients. In the remaining two patients, it was 22 and 25 pg/mL at 1 month postoperatively (►**Table 3**).

Discussion

Hypocalcemia is an important potential complication after thyroid surgery. It is almost exclusively seen after total thyroidectomy specially when central neck node dissection (level VI cervical lymph nodes) is combined with thyroid surgery.[2] The reported incidence of post thyroidectomy hypocalcemia varies widely from 1 to 50%.[3] In the present study, none of the patients who underwent hemithyroidectomy ($n = 44$) experienced significant immediate postoperative hypocalcemia, while there was an average drop of 9.3% in CSC and 40% drop in the iPTH level at 1 hour after total thyroidectomy ($n = 36$). This is only to be expected because during hemithyroidectomy there can be possible damage to a maximum of two parathyroid glands, while during total thyroidectomy, all four parathyroid glands are at risk of unintentional removal, devascularization, or surgical stunning due to manipulation.

An important observation in our study was the finding that biochemical hypocalcemia did not demonstrate a linear association with symptoms of hypocalcemia or their severity. None of our patients exhibited obvious clinical signs of hypocalcemia such as spontaneous carpopedal spasm or generalized tetany. However, occult symptoms or signs were evident on day 1 postoperatively in 6 (of 36) patients who underwent total thyroidectomy and 1 (of 44) patient who underwent hemithyroidectomy. These occult manifestations were perioral and acral tingling or numbness, Trousseau's sign, or Chvostek's sign, all of which needed to be elicited by subtle questions or drawn out by the appropriate sign elicitation technique (►**Table 4**).

Out of 12 patients of total thyroidectomy who had CSC <8.5 mg/dL at 1 hour postoperatively, 3 (25%) exhibited occult clinical features of hypocalcemia. Among the 24 patients who had CSC >8.5 mg/dL, 3 (12.5%) also exhibited occult clinical features of hypocalcemia. In none of these six patients, the signs of occult hypocalcemia could be

Table 4 Clinical features suggestive of hypocalcemia after thyroid surgery

	Hemithyroidectomy ($n = 44$)	Total thyroidectomy ($n = 36$)	
		With CSC <8.5 mg/dL ($n = 12$) at 1 h post-op	With CSC >8.5 mg/dL ($n = 24$) at 1 h post-op
Occult symptoms and signs of hypocalcemia (perioral and acral paresthesia, tingling or numbness, Trousseau's or Chvostek's sign)	1 (2.2%)	3 (25%)	3 (12.5%)
Obvious symptoms or signs of hypocalcemia (carpopedal spasm, generalized tetany)	Nil	Nil	Nil

Abbreviation: CSC, corrected serum calcium.

demonstrated beyond 4 days. It is well known that symptoms of hypocalcemia depend on several other factors besides the absolute serum calcium values. These factors are (1) fraction of ionic serum calcium, (2) pH of blood, (3) patient receiving calcium supplementation in diet or medication, (4) hydration status, and (5) serum magnesium levels. An additional scenario could be patients who have been chronically calcium deficient and have therefore become acclimatized to low serum calcium levels.[4]

Postsurgical hypoparathyroidism is defined as reduction of the serum iPTH level below 15 pg/mL or decline of CSC <8.5 mg/dL or ionized calcium below 4.4 mg/dL or 1.05 mmol/L with or without symptoms of hypocalcemia.[5] Transient hypoparathyroidism is defined as resolution of hypocalcemia without treatment within 6 to 12 months. Permanent hypocalcemia has been reported to occur between 0 and 43%. This large variation is due to heterogeneity among reports as to the definition and duration of hypocalcemia.[6]

The half-life of parathyroid hormone in circulation is about 5 minutes or less.[7] Therefore, intraoperative or very early postoperative measurement of serum iPTH can be a reliable indicator of the status of parathyroid during thyroid surgery. This property of iPTH is also utilized by surgeons for intraoperative assessment of adequacy of parathyroid tumor removal during surgery for hyperparathyroidism. For the purpose of this study, we utilized this attribute of iPTH for parathyroid status assessment even 1 hour after surgery.

The present study has demonstrated that none of our post thyroidectomy patients including the 36 who underwent total thyroidectomy had persistent hypocalcemia at 1 month after surgery. However, there was a drop of 9.3% in the CSC level, 40% in the iPTH level, and 10% in the iCa level at 1 hour after total thyroidectomy. This was followed by a very rapid recovery and the CSC levels improved to –4.8% below the lower baseline at 1 day, –2.6% at 1 week, and +0.4% at 1 month after total thyroidectomy. Similarly the corresponding values for iPTH at 1 day, 1 week, and 1 month were –27%, –23%, and –8% (►**Figs. 1**, **2** and **3**). A total of 12 patients attained the defined level of hypocalcemia at 1 hour after surgery. Importantly, only occult clinical features could be elicited in only three of them as well as three who had a CSC level of >8.5 mg/dL. The immediate postoperative drop in the iPTH and CSC levels could be attributed to one of the following three hypotheses:

- Inadvertent removal of two or three of the parathyroid glands.
- Devascularization of the parathyroids during thyroid lobe mobilization.
- Stunning effect of dissection around the parathyroid glands.

The parathyroid glands are regarded as the "calcium thermostat" of the body. It is pertinent to note that calcitonin-producing cells in the thyroid are removed completely during total thyroidectomy. The normal homeostasis of serum calcium levels is maintained by a fine interplay between iPTH and calcitonin. How this balance of hormones with antagonistic functions is affected after total thyroidectomy has not been satisfactorily explained.

The present study has demonstrated that immediate post-thyroidectomy measurement of iPTH can be a reliable method to assess the possible damage or removal of the parathyroid gland after total thyroidectomy. Identification of such patients can be important for early institution of calcium supplementation therapy and sparing the patients from unpleasant symptoms. Such patients can be monitored till recovery of CSC, which is likely to occur in a few weeks' time.

Another important inference from this study is that patients who demonstrate a fall in iPTH at one hour after total thyroidectomy should have a very careful examination of their thyroidectomy specimen before it is dipped in formalin to look for inadvertently removed parathyroid glands. If found then auto transplantation of one or two of the identified glands can be done under local anesthesia in the brachioradialis muscle immediately thus sparing patient from potential permanent hypo-parathyroid state. For the purpose of intra-operative iPTH level estimation the rapid immunochemiluminesence assay should be performed. This can be completed within 8 to 20 minutes allowing the surgeon to make intra-operative decisions. The intra-operative iPTH assay can be performed in or close to the operating room to avoid delay caused by sample transport.

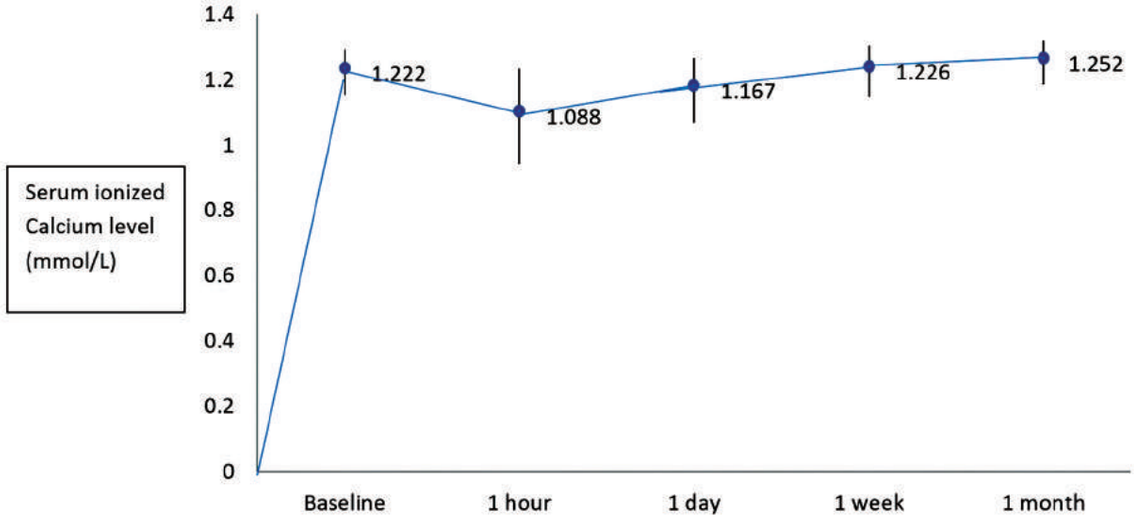

Fig. 3 Sequential values of ionic calcium after total thyroidectomy ($n = 36$) at baseline, 1hour, 1 day, 1 week and 1 month after surgery.

Conclusion

We observed a 9.3% drop in CSC, 40% drop in iPTH, and 10% drop in ionic calcium levels at 1 hour after total thyroidectomy. The values returned to near-normal levels at 1 month postoperatively in all patients. Although occult symptoms or signs of hypocalcemia could be evoked in first 4 days after surgery in six patients (16%) undergoing total thyroidectomy, obvious clinical features did not appear in any of our patients. As per expectations, drop in serum calcium or iPTH was not observed among hemithyroidectomy patients. Early institution of calcium replacement may be started on the basis of 1 hour post-thyroidectomy iPTH levels to spare the patients from unpleasant symptoms of hypocalcemia. Furthermore, effort should be made to identify the parathyroid glands in the thyroidectomy specimen and consider it for autotransplantation if the 1-hour iPTH falls to below defined hypoparathyroidism levels.

Ethical Approval Statement

The study was a part of MS (surgery) thesis protocol of Dr. Sweety Kumari and was duly approved by the institutional ethics committee and the PG medical board.

Conflict of Interest

None declared.

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Glaucoma the Silent Thief of Vision! A Study to Assess Current Trends on Awareness and Knowledge About Glaucoma

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Abstract

Introduction Worldwide nearly 70 million populations are affected by glaucoma, a leading cause of irreversible blindness. Awareness and knowledge about glaucoma is the key to early diagnosis and effective management to prevent debilitating blindness. In India, the literature has shown that the level of awareness ranges from 0.32 to 13.5%, much lower than developed countries. Previous studies found higher levels of education and socioeconomic status along with positive family history of ocular diseases were directly related to improved levels of awareness and knowledge about glaucoma. Considering the improvement in literacy standards, socioeconomic status, and better utilization of medical care in the past few years, we aimed to assess the current trends in levels of awareness and knowledge about glaucoma in the North Indian population.

Methodology This prospective cross-sectional questionnaire-based study included 1,536 participants enrolled from the outpatient department of a tertiary care teaching hospital. Data for the study were collected from the responses given by participants from two sets of questionnaires adopted and validated from previous similar work on awareness of glaucoma. Statistical analysis was done by applying the chi-square test and Fisher exact probability test using IBM SPSS Statistics version 20.

Results In total, 7.74% of study participants were aware of glaucoma and the newspaper was the most common source of (57.9%) information. A significant correlation ($p < 0.05$) was found for male sex, education status, and past medical history between aware and not aware participants; however, non-significant ($p = 0.182$) correlation was seen for upper and lower socioeconomic status among the same group participants. Only 16% of aware participants had a good knowledge of glaucoma.

Conclusion In current trends, the state of awareness and knowledge on glaucoma in the Indian population was poor compared to that in the Western world, although the levels of education status and the presence of past medical history had significant

Keywords
- Glaucoma
- awareness
- knowledge
- literacy
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correlation among aware and not aware population, but socioeconomic status had no significant correlation. Electronic media and health camps were among the least common source of information; hence, improvised awareness programs and opportunistic screening of glaucoma is the answer to control this silent thief of vision called glaucoma.

Introduction

Glaucoma is one of the major contributors to irreversible blindness; however, the progression of blindness can be slowed once diagnosed in the early stages of the disease. Globally, approximately 7 crore population is affected with glaucoma, wherein India has the second largest population affected with glaucoma.[1,2] Glaucoma is commonly called a silent thief of vision because, in the majority of cases, this condition is either asymptomatic or with subtle symptoms like floaters or frequent change of near glasses.[3] The disease is so silent in progression that a large percentage of the affected population presents to ophthalmologists in advanced stages of glaucoma with significant irreversible loss of visual fields.[3,4] Previous studies had concluded that nearly 50 to 90% of glaucoma cases remain undiagnosed; hence, a large number of cases are diagnosed at the advanced stage of the disease.[3,5] The most acceptable approach to prevent the glaucoma-induced blindness is the timely detection of glaucoma in its early stages of presentation.[2] Lack of awareness about glaucoma, literacy status, socioeconomic status, and insufficient healthcare facilities are important contributing factors to the late detection and early management of glaucoma.[3] Several previous studies found that either the lack of awareness or amount of patient's knowledge related to eye problems among population plays a significant role in timely detection and early management of glaucoma.[5–7]

The awareness and knowledge about glaucoma is directly or indirectly influenced by the factors, such as education status, socioeconomic status, family history of glaucoma, type of occupation, presence of chronic diseases like diabetes and hypertension, media attachment, and information by nongovernmental or governmental agencies.[6,8] The previous publications in Indian studies found that the awareness levels of glaucoma in the population were 13.5,[7] 10.2,[9] and 0.32%.[10] These findings suggest that the level of awareness about glaucoma had a vast range from a maximum of 13.5% to as low as 0.32%. In previous studies, the percentage of study participants aware about glaucoma having some knowledge was 10,[1] 18,[2] and 8.7%.[5] This wide range of awareness and low levels of knowledge about the glaucoma in Indian population is the major factors for delayed presentation and late management of glaucoma.

Over the past four decades, the Indian government has gradually adopted economy-related policies to improvise the economic status of the population in the country, similarly a huge growth in health care infrastructure, both in urban and rural sectors, has been noticed over past two decades. This infrastructure growth causes changes in perception, treatment-seeking behavior, and belief about health care in the Indian population.[11,12] The adult literacy rate showed a considerable improvement from 52.2% in 1991 to 74% in 2011 among the population of the age group of 7 years and above in India.[13] In the year 1976, the Indian government launched the National Programme for Control of Blindness (NPCB) where information, education, and communication were its important elements to spread awareness on eye care in the community.[14]

In lieu of the adoption of newer economy policies by the Indian government, shift in health care utilization among rural and poor population, and improvised literacy rate in India, this study was conducted to assess the current trends in the level of awareness and knowledge about glaucoma among rural north Indian population. We also analyzed the correlation of education status, literacy status, and past medical history with awareness and estimated the grades of knowledge among aware participants. Additional data were also analyzed to assess the commonest source of information about glaucoma in study participants.

Materials and Methods

This prospective cross-sectional questionnaire-based study was conducted in a rural tertiary care center in north India. Patients presenting to the outpatient department of the institute from November 2021 to April 2022 and those who were 18 years or older were included in the study. Informed consent was obtained from all participants, and the study was approved by institutional ethics committee. Participants with prior diagnosis of glaucoma were excluded from the study as they could have gained knowledge of the disease after diagnosis. At the confidence interval of 95%, prevalence (p) of 10%, absolute precision (d) of 1.5%, and at 90% power using formula $n = (Z\alpha + Z\beta)^2 \times pq/d^2$, where $Z\alpha = 1.96$, $Z\beta = 0$, and $q = 1 - p$, the sample size (n) calculated was 1,536.

Totally 1,536 participants fulfilling the inclusion criteria, who gave informed consent for study participation, were enrolled in this study. Demographic profiles of all participants including age, sex, occupation, literacy, and socioeconomic status were recorded. The subjects were classified as illiterate, middle school pass, high school pass, graduate, or professional according to the literacy status. Modified Kupuswamy classification[15] was used to determine the socioeconomic status of the subject based on education status, occupation of the head of the family, and per capita income per month. The study questionnaire (Annexure I) was initially designed in English

and then converted to Hindi, the local language. Patients giving affirmative response to the question "have you heard of glaucoma and know that it can cause blindness" were considered as being aware of the disease. Another set of study questionnaire (Annexure II) was designed to collect further information from those participants aware of glaucoma to know about their understanding of the disease in terms of source of information, knowledge of risk factors, symptoms, and treatment using structured questionnaire. These questionnaires were adopted from a similar study done on awareness of glaucoma in a tier 2 city by Prabhu et al[8] and validated by a group of ophthalmologists.

Glaucoma knowledge was graded as good, fair, and poor as per criteria using risk factors and treatment knowledge.

- Grade 1 (Good knowledge): Knows about two or more risk factors and has knowledge of medical and surgical treatment of glaucoma.
- Grade 2 (Fair knowledge): Knows at least one risk factor and one treatment modality of glaucoma.
- Grade 3 (Poor knowledge): No knowledge of risk factors or treatment.

Data were analyzed using IBM SPSS Statistics version 20 copyright IBM Corporation 2010, New York 10589, United States. Unpaired test, chi-square test, and Fisher exact probability test were used to look for the presence of a significant association of awareness and knowledge with other study variables in study participants. A probability (p) value less than 0.05 were considered statistically significant.

Results

The study included 1,536 participants of which 716 were males and 820 were females. In total, 119 (7.74%) participants were found to be aware of glaucoma. ►**Table 1** shows the comparison and correlation of various variables in glaucoma aware and unaware subjects.

Statistical correlation between male and female participants among aware and not aware groups was found to be significant ($p = 0.012$). The p-value was not significant between age group <50 years and >50 years participants ($p = 0.8386$) and also in upper and lower socioeconomic class participants ($p = 0.182$) among aware and not aware groups. However, a highly significant p-value (<0.001) was observed between aware and not aware groups when compared for literacy and past medical history (►**Table 2**).

The most common source of information about glaucoma among aware participants was found to be newspaper (57.9%) with the least common information source being health camp (19.3%) ►**Fig. 1**.

►**Table 3** shows the yes/no response for various questionnaires asked on risk factors, symptoms, and treatment of glaucoma to evaluate the knowledge about glaucoma among glaucoma-aware participants ($n = 119$). Nearly, one-fourth of aware participants answered yes for various risk factors (25.37%) and symptoms (27.17%) of glaucoma; however, two-third of aware participants said that treatment of glaucoma is possible (63.86%).

Among glaucoma-aware participants ($n = 119$), 16% had good knowledge, 30% had fair knowledge, whereas 54% participants had poor knowledge about glaucoma ►**Fig. 2**.

Discussion

This study assessed the current trends in the level of awareness and knowledge about glaucoma among patients attending the outpatient department of a rural tertiary care center in north India. In the study, we evaluated the current status of perception of the participants about the nature of glaucoma its subtle clinical course, irreversible loss of visual fields, and importance of screening for early diagnosis and management of the disease.

In our study, 7.74% (119/1,536) participants were found to be aware of glaucoma which falls in the range of awareness of glaucoma reported in India (0.32–13.5%) by the previous studies.[5,7,10,11] However, it is noteworthy that these Indian studies were population-based epidemiologic surveys on either rural or urban population. A hospital-based study in central India reported the awareness level as high as 27%[2] because they included both the undiagnosed patients presenting in the department of ophthalmology and the diagnosed patients of glaucoma, which may have accounted for the higher awareness level in their study. We, on the contrary, included only undiagnosed patients coming in the outpatient department of ophthalmology at our hospital and excluded diagnosed patients of glaucoma. In our study, the awareness level for glaucoma was low (7.74%) compared to the publication data from developed countries (70–93%); however, the level of awareness was higher compared to the developing countries such as Nepal (2.4%) and Ethiopia (2.4%).[7,16,17]

In the present study, the awareness about glaucoma was found to be more in participants with higher educational status. Those who were literate and educated above middle school were significantly ($p < 0.001$) more aware of glaucoma than those who were uneducated. Similar trends have been reported by other awareness studies.[7–9] In contrast to previous studies,[2,5,7,8] we observed no significant ($p = 0.182$) relationship for the awareness of glaucoma among the participants belonging to upper socioeconomic classes (UC and UMC) and lower socioeconomic classes (LMC and LC). These observations indicate that the awareness in the population is directly proportional to the education status and has no relation with the socioeconomic status of the society, probably in the individuals the awareness toward health care increases proportionally with their education level, which is not dependent on their socioeconomic status.

In this study, we found males to be significantly more ($p = 0.012$) aware than females about glaucoma, contrary to our findings in their studies Rewri and Kekkar[5] found no significant ($p = 0.99$) gender relationship and Ve Ramesh et al[7] found that females were more aware than males. Our finding may be contributed by the facts that a greater emphasis was given to male education as compared to female education in rural north India and also the existing difference in literacy rates both in rural (77.2 male vs. 57.9

Table 1 Demographic profile of glaucoma aware and not aware participants

Variable	Subcategory	Awareness about glaucoma		Total
		Yes ($n = 119$)	No ($n = 1,417$)	n = 1,536
Gender				
	Male	69 (57.98%)	647 (45.65%)	716
	Female	50 (42.01%)	770 (54.34%)	820
Age				
	<35	4 (3.36%)	73 (5.15%)	77
	36–45	31 (26.05%)	255 (17.99%)	286
	46–55	49 (41.11%)	622 (43.89%)	671
	56–65	34 (28.57%)	458 (32.32%)	492
	> 66	1 (0.84%)	9 (0.63%)	10
Mean age ± SD (years)		**50.63 ± 10.66**	**50.79 ± 10.41**	
Socioeconomic status (Modified Kupuswamy)				
	Upper class	12 (10.08%)	173 (12.2%)	185
	Upper middle class	28 (23.5%)	216 (15.24%)	244
	Lower middle class	47 (39.49%)	370 (26.11%)	417
	Upper lower class	24 (20.16%)	418 (29.4%)	442
	Lower class	8 (6.7%)	240 (16.93%)	248
Education status				
	Illiterate	5 (4.2%)	631 (44.5%)	636
	Middle school	60 (50.4%)	551 (38.8%)	611
	High school	48 (40.3%)	200 (14.11%)	248
	Graduate	2 (1.6%)	15 (1.05%)	17
	Professional	4 (3.3%)	20 (1.4%)	24
Past medical history				
	Diabetes	29 (24.3%)	30 (2.1%)	59
	Past ocular history	37 (31.2%)	36 (2.5%)	73
	Family history of glaucoma	8 (6.7%)	18 (1.2%)	26
	No past medical history	45 (37.8%)	1333 (94%)	1,378

Note: Bold data indicates the values which can easily be correlated with the text explained with results.
Abbreviation: SD, standard deviation.

female) and urban (88.8 male vs. 79.1 female) populations of India.[13]

Our study showed that the participants having any past medical history such as diabetes, positive family history of glaucoma, or ocular diseases were significantly ($p < 0.001$) more aware about glaucoma as compared to participants with no past medical history. Similar to our study, Prabhu et al[8] also found a higher awareness about glaucoma in diabetic patients. Patients with the family history of glaucoma and other ocular conditions were also found to be significantly more aware of the disease akin to previous studies.[10,18] These significant correlations with awareness about glaucoma may be accounted by regular visits of diabetic and ocular disease patients to the ophthalmologists.

The present study found newspapers to be the most common source of information in glaucoma-aware participants, followed by relatives and close acquaintances. Previous studies have, however, reported close acquaintances as the most common source of information.[2,5] These differences in the source may be attributed to an enhanced use of mass media to create awareness about glaucoma in the recent past.

We observed that 79 (66.3%) of the aware participants could not answer any risk factors or treatment modality of glaucoma. In total, 29 (24.3%) participants knew at least one risk factor and treatment modality of glaucoma, while only 20 (16.8%) participants knew about two risk factors and treatment modalities. These observations were similar to the study done by Prabhu et al[8] where among the aware patients 54.5% knew one/no risk factor or treatment modality, 22.7% knew at least one risk factor and treatment modality and 22.7% patients knew two or more risk factor and treatment modalities. These findings suggest that the awareness of disease can be improvised by using mass media and upgraded policies, but the detailed knowledge of the disease depends on the interaction with the specialist.

Table 2 Comparison and statistical correlation of different factors among glaucoma aware and not aware participants

Variable	Subcategory	Number of participants on basis of awareness		Statistical analysis	
		Aware $n = 119$	Not aware $n = 1,417$	*p*-Value	Odds ratio
					95% Confidence interval
Gender					
	Male	69	647	0.012[a]	1.642
	Female	50	770		1.124 to 2.399
Age (years)					
	<50	53	611	0.8386	1.059
	>50	66	806		0.7269 to 1.544
Education status					
	Illiterate	5	631	<0.0001[a]	0.0546
	Literate	114	786		0.2217 to 0.1346
Socioeconomic status					
	Upper class	40	389	0.1827	1.338
	Lower class	79	1,028		0.8988 to 1.992
Past medical history					
History of diabetes	Yes	29	30	<0.0001[a]	14.897
	No	90	1387		8.567 to 25.906
Family history of glaucoma	Present	8	18	<0.0001[a]	5.602
	Absent	111	1,399		2.382 to 13.174
Past history of ocular condition	Yes	37	36	<0.0001[a]	17.309
	No	82	1,381		10.392 to 28.830

[a]*p*-Value <0.05 significant.

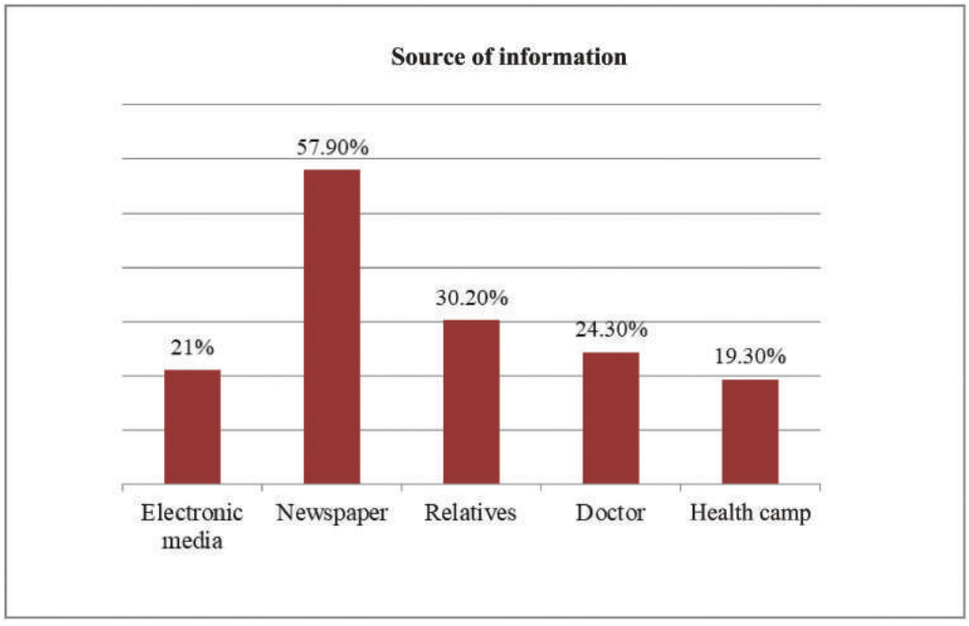

Fig. 1 Source of information in glaucoma aware participants.

Table 3 Response of glaucoma aware participants ($n = 119$) about glaucoma knowledge questionnaire

Questionnaire response		Yes	No
Risk factors			
	Diabetes	34 (28.57%)	85
	Family history	32 (26.89%)	87
	Steroid use	18 (15.12%)	101
	Obesity	35 (29.41%)	84
	Raised IOP	32 (26.89%)	87
		151 (25.37%)	444 (74.62%)
Symptoms			
	Asymptomatic	10 (8.40%)	109
	Field loss	43 (36.14%)	76
	Reading glasses change	44 (36.97%)	75
		97 (27.17%)	260 (72.82%)
Treatment			
	Possible	76 (63.86%)	43
	Eye drop	22 (18.48%)	97
	Laser	27 (22.68%)	92
	Surgery	44 (36.97%)	75
		169 (35.5%)	307 (64.5%)

Abbreviation: IOP, Intra Ocular Pressure.

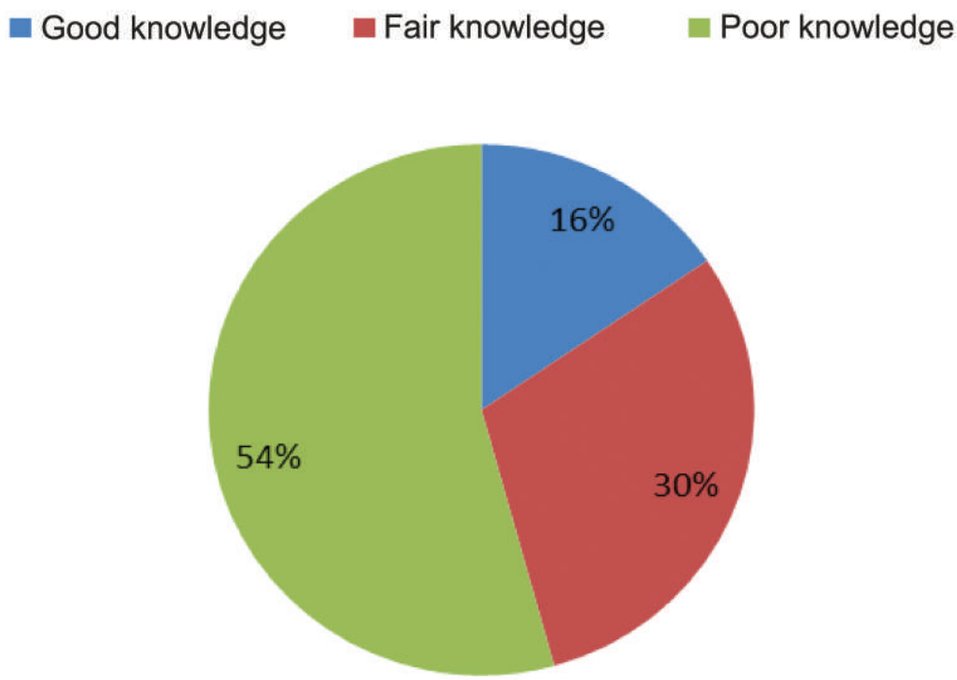

Fig. 2 Distribution of glaucoma aware participants ($n = 119$) in different grades of knowledge about glaucoma.

Our study indicated that awareness and knowledge in a rural population were poor despite improvised access to eye care services and the implementation of various health policies by the governmental agencies. Over past few years, many studies done in different sets of populations showed similar trends in levels of awareness and knowledge.[7–10]

Conclusion

The level of awareness among the Indian population was found to be poor compared with Western countries. This highlights the gaps in our health care system. The poor literacy rate accounts for such a poor level of awareness in the Indian population. A significant correlation was present between glaucoma-aware and not-aware population in terms of education status and past medical history because the patients with diabetes, ocular diseases, and positive family history of glaucoma regularly visit ophthalmologists. More than half of the study participants in glaucoma awareness group had poor knowledge about the disease, which indicates that disease details are independent of mere awareness. We found that the newspaper was the most common source of information and electronic media was the second least common source of information because the reach of newspaper in rural areas of India is still greater than the electronic media like TV or the internet.

The awareness programs should be planned in such a way that they are accessible to people belonging to all kinds of socioeconomic strata. The use of mass media such as TV/radio in glaucoma awareness programs should be enhanced. The unique concept of opportunistic screening by Vashist et al[19] can be used for early diagnosis of glaucoma. Opportunistic screening will allow for glaucoma screening in patients presenting to health care providers with other illnesses unrelated to glaucoma. Lastly, a person with some knowledge of glaucoma is more likely to seek earlier eye care intervention than those without any knowledge, and furthermore, they can become a source of information to others. Hence, eye care education programs should focus on increasing both specific knowledge on glaucoma and general awareness of the disease.

Authors' Contribution

A.K.J. did the review of the literature to formulate the study design, performed statistical analysis, and also wrote the manuscript. N.S. and N.K.S. prepared study questionnaire, master excel sheet of data, and also helped in statistical analysis. P.K.S. and S.R. collected the participant's data and filled it in the annexure form.

Conflict of Interest

None declared.

Acknowledgment

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A Scientometric Analysis of the most Highly Cited Publications on Fracture Research from India: 1989–2022

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Abstract

Background India bears a huge burden of accidents and fractures. This study aimed to study the bibliometric characteristics of India's fracture research output during last three decades. The most highly cited publications (HCPs; with 20 or more citations) on orthopaedic fracture research from India were analyzed on various parameters.

Methodology The Scopus database was used to identify publications on fractures that originated from India, between 1989 to 2022. The top HCPs were retrieved. A bibliometric and network analysis was used to identify the key players, such as organizations, authors and journals, and important keywords besides identifying their collaborative interactions and visual co-occurrences of significant keywords using VOSviewer and Biblioshiny software.

Results Of the total 1,048 Indian publications, 126 (10.02%) were HCPs (cited 4,695 times). External funding was received in 1.59%, and international collaboration in 15.08%. The most productive organizations were All India Institute of Medical Sciences (AIIMS), New Delhi, followed by Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, and Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences (PGIMS), Rohtak. The most impactful organizations were Maulana Azad Medical College (MAMC), Delhi, followed by the Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Pondicherry, and Sancheti Institute of Orthopaedic Research & Rehabilitation, Pune. The most productive authors were R. Malhotra, M.S. Dhillon, and N.K. Magu, and the most impactful authors were U. K. Meena, A.P. Singh, and P. Sancheti. Delhi was the epic of research, followed by Karnataka, Tamil Nadu, and Chandigarh.

Conclusion This study provides an insight into the research trends, the most influential contributions, and the performance of Indian organizations and authors. It gives some ideas about the past, present, and future hotspots in research.

Keywords
- bone
- fracture
- orthopaedics
- high-cited publications
- India
- scientometrics
- bibliometrics
- journals
- institution
- author

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Introduction

India bears a heavy burden of accidents and fractures, due to rising road traffic accidents (RTAs),[1,2] causing increased morbidity and disability, and impacting the quality of life, besides imposing a significant economic burden on the health system.[3] Osteoporotic fractures are also on the rise.[4–7]

There has been an increased interest in fractures as is evidenced by numerous published research, research grants, and the development of fracture-related activities. As the body of literature regarding fractures continues to grow, an analysis of the most impactful literature is justified to direct future research and pay tribute to the highest contributing work within the field.

Several publications have investigated the most influential articles on various bone fractures like calcaneus,[8] hip,[9–11] proximal humerus,[12] scaphoid,[13] and spine.[14–16] However, fracture research at the global and national levels has been rarely studied from a bibliometric perspective. Among global studies, Baldwin et al[17] studied the 100 most cited articles in fracture surgery and identified their characteristics to determine the qualities that make an article highly cited in this field. At the national level, Dong et al[18] studied the characteristics of the most-cited articles on fracture surgery by Chinese authors.

No bibliometric study has investigated, so far, the most influential articles relating to Indian fracture research. We believe that the most highly cited publications (HCPs) of fracture research will have the most historically influential impact and will also play a significant role as the basis for recent studies to build on. Therefore, we decided to undertake a comprehensive review of the most influential articles related to fracture research from India. The study aims to identify India's HCPs, examine their trends, and identify the various characteristics of fracture research between 1989 and 2022, using bibliometric methods.

Methods

The terms related to fractures were searched in the Scopus database for articles published between 1989 and 2022 for retrieving relevant output on fracture research from Indian on December 2, 2022 using the retrieval search strategy highlighted in ►**Fig. 1**. In all, 1,408 records on India's fracture research were retrieved, of which 126 HCPs with a total

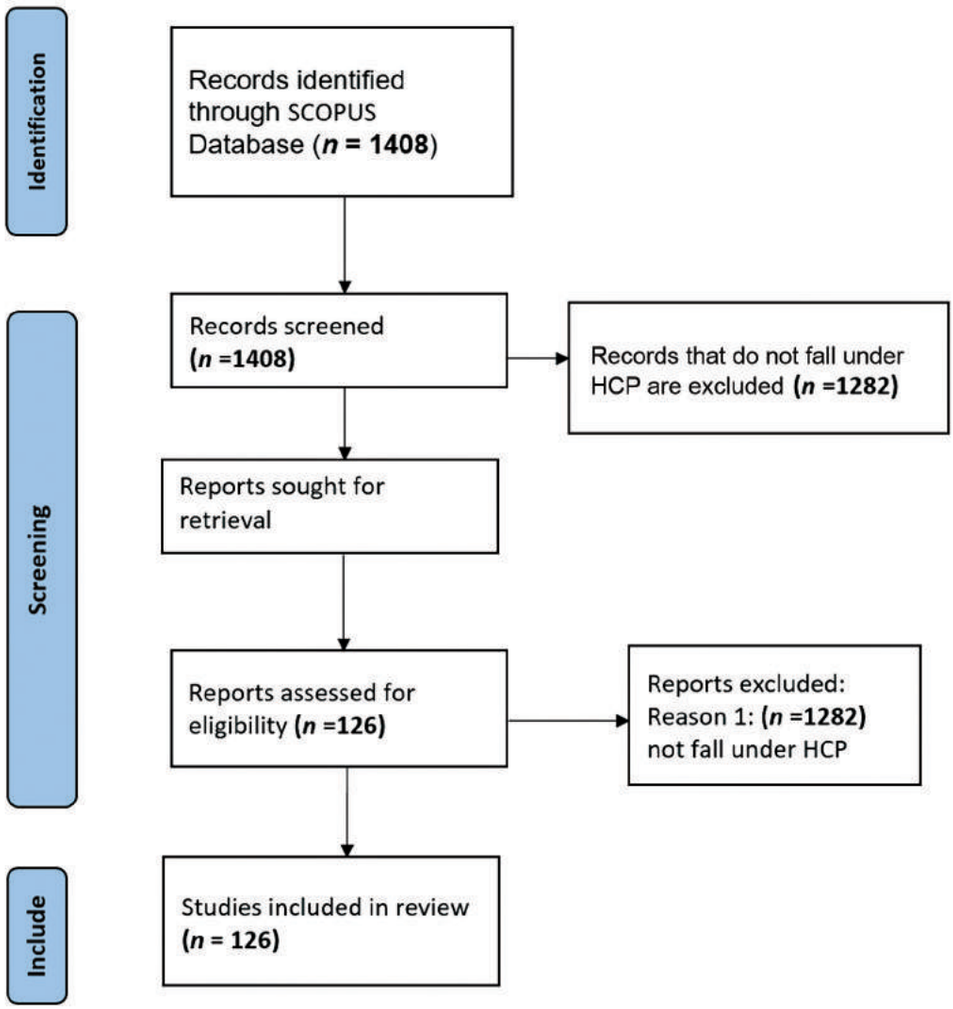

Fig. 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) chart showing the inclusion and exclusion details of the study.

citation [TC] of ≥20 were isolated, after excluding 1,282 articles that were not HCPs.

Detailed ranking and analysis of these HCPs are useful for identifying the most influential articles in guiding our decision-making. Bibliometric tools were used for this study. The main analyses include publication and citation counts, the contribution of countries, institutions, authors, funding agencies and journals, and the clustering of keywords. This study used VOSviewer for visualizing the co-occurrence analysis of keywords. (N.B.: Co-occurrence is a method to analyze text that includes a graphic visualization of potential relationships between people, organizations, countries, or other entities represented within written material.)

The most productive authors were defined as those who have contributed more than the average productivity of all the authors. The most impactful organizations were defined as organizations that have registered a higher citation per paper (CPP) than the average CPP of all the organizations. India's authors and institutions were included in this study when one of the authors or institutions in the publication were from India.

((TITLE(fracture) AND TITLE(orthoped* or orthopaed*)) AND PUBYEAR > 1988 AND PUBYEAR < 2023) OR ((TITLE (fracture) AND SRCTITLE(orthoped* or orthopaed*)) AND PUBYEAR > 1988 AND PUBYEAR < 2023) AND (LIMIT-TO (AFFILCOUNTRY,"India"))

Bibliometrics is a method of statistical analysis used to assess a particular subject's characteristics and major developmental trends based on published research publications. It is a validated method for collecting and identifying impactful studies across scientific and medical fields. Because TC count is thought to be predictive of an article's overall impact, focusing on TC count through careful analysis allows scholars to present both empirical and subjective findings related to the most influential works within a field.[19]

Results

Overall Picture

The search on India's fracture research in the Scopus database for articles published between 1989 and 2022 yielded 1,408 records. Of these, 126 (10.02%) were HCPs, having received ≥20 TCs. These HCPs received 4,695 citations, averaging 37.26 citations per paper (CPP). The HCPs increased from 1 in 1989 to 17 in 2012 and then decreased to 0 in 2022. The highest number of HCPs were published in 2012 ($n = 17$), followed by 2011 and 2014 at 12 each. Of all of the years examined, 2011 had the highest number of citations ($n = 695$). Of the 126 HCPs, 110 articles were in the citation range of 20 to 50, 12 in the citation range of 51 to 98, and 4 in the citation range of 105 to 347.

Top 10 High-Cited Publications

The top 10 HCPs in India's fracture research are listed in ►**Table 1**.[20–29] These 10 HCPs have received a combined 1,141 citations, averaging 114.1 CPP. Of these top 10 HCPs, 6 and 4 were in the citation range of 65 to 98 and 105 to 347, respectively. The 10 HCPs comprise eight articles and two reviews, and involve the participation of a single organization (zero collaboratives) in four articles and the participation of ≥2 organizations (international collaborative) in six articles. Four foreign countries were involved in the publication of seven international collaborative HCPs included in the study, three from the United Kingdom, two from the United States, and one each from Australia and Switzerland. The 10 HCPs involve the participation of 23 organizations and 39 authors, of which 10 organizations and 22 authors are Indians. The 10 Indian organizations involved in the publication of one article each include Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh; All India Institute of Medical Sciences (AIIMS), New Delhi; Maulana Azad Medical College (MAMC), New Delhi; Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Pondicherry; and Indian Institute of Science (IISc), Bangalore. Articles from eight journals were in the 10 HCPs: 3 articles from *International Orthopaedics* (IF = 3.479) and one article each from *Acta Orthopaedics* (IF = 3.717), *Indian Journal of Orthopaedics* (IF = 1.0303), *Journal of Orthopaedic Trauma* (IF = 2.512), *Hong Kong Journal of Orthopaedic Surgery* (IF = 1.482), *Journal of Pediatric Orthopaedics: Part B* (IF = 1.306), *Orthopaedics & Traumatology: Surgery & Research* (IF = 2.256), and *Proceeding of the Nation Academy of Sciences of United States* (IF = 12.799).

Citation Life Cycle Pattern of Top 10 HCPs

In the initial years after publication, articles generally receive a small but growing number of citations until, eventually, they reach a peak from where they tend to decline (►**Fig. 2**). Among the top 10 HCPs, the article authored by Dhanwal et al in 2011[20] received the highest number of 347 citations during the study period. However, the article by Garg et al in 1993[21] is cited 135 times.

Funding and Collaboration

Only two (1.59%) HCPs received external funding and they registered 452 citations, averaging 226.0 CPP. Nineteen (15.08%) publications were international collaborations, and these received 1,249 citations, averaging 65.74 CPP. Authors from 11 foreign countries collaborated with Indian authors on fracture research. The United Kingdom ($n = 7$) and United States ($n = 6$) contributed the maximum number of articles, followed by Canada and Netherlands ($n = 2$ each), and Australia, Denmark, Germany, Ireland, Nepal, Switzerland, and U.A.E. ($n = 1$ each).

Publication Pattern

Of the 126 HCPs, original research articles accounted for the highest number of publications (110/126 [97.30%]), followed by reviews (11/126 [8.73%]) and conference papers (5/126 [3.97%]). Age or osteoporosis (10 articles) and vitamin D deficiency (5 articles) were the risk factors for the majority of fracture cases in most HCPs. The main causes of fracture were reported to be injury in 18 HCPs, RTAs in 6 HCPs, and falls in 6 HCPs.

Table 1 Details of the top 10 high-cited publications

Sl. no.	Study	Title	Source	Total citations
1	Dhanwal et al[20]	Epidemiology of hip fracture: worldwide geographic variation	Indian J Orthop 2011;45(1):15–22	347
2	Garg et al[21]	Percutaneous autogenous bone marrow grafting in 20 cases of ununited fracture	Acta Orthop 1993;64(6):671–672	135
3	Changulani et al[22]	Comparison of the use of the humerus intramedullary nail and dynamic compression plate for the management of diaphyseal fractures of the humerus. A randomised controlled study	Int Orthop 2007;31(3):391–395	120
4	Johnson et al[23]	Hydrogel delivery of lysostaphin eliminates orthopedic implant infection by *Staphylococcus aureus* and supports fracture healing	Proc Natl Acad Sci U S A 2018;115(22): E4960–E4969	105
5	Kulshrestha et al[24]	Operative versus nonoperative management of displaced midshaft clavicle fractures: a prospective cohort study	J Orthop Trauma 2011;25(1):31–38	98
6	Meena et al[25]	Predictors of postoperative outcome for acetabular fractures	Orthop Traumatol Surg Res 2013;99(8):929–935	69
7	Putti et al[26]	Locked intramedullary nailing versus dynamic compression plating for humeral shaft fractures	J Orthop Surg (Hong Kong) 2009;17(2):139–141	68
8	Kannan et al[27]	Arthroplasty options in femoral-neck fracture: answers from the national registries	Int Orthop 2012;36(1):1–8	67
9	Singisetti and Ambedkar[28]	Nailing versus plating in humerus shaft fractures: a prospective comparative study	Int Orthop 2010;34(4):571–576	66
10	Johari and Sinha[29]	Remodeling of forearm fractures in children	J Ped Orthop B 1999;8(2):84-87	65

Geographical Distribution by Indian States

Delhi had the largest share of publication (30.95% and 39 articles) in Indian HCPs, followed by Karnataka and Tamil Nadu (at 11.90% and 15 articles each) and Chandigarh (10.32% and 13 articles).

Type and Subtype of Fractures

By anatomical location, the major publication focus was on the femur ($n = 42$ and 33.33% share), followed by the tibia ($n = 18$ and 14.29% share), humerus/humeral and hip ($n = 16$ and 12.7% share each), acetabulum and spine ($n = 7$ and 5.56% share each), radius ($n = 6$ and 4.76% share), clavicle ($n = 4$ and 3.17% share), etc.

Ulnar and clavicular fractures registered comparatively higher CPPs (81.33 and 66.0, respectively) than all the fractures identified in this study, followed by forearm fractures ($n = 65.0$ CPP) and hip fractures ($n = 48.69$ CPPs).

By sex distribution: These HCPs were focused on males in 84 articles and on females in 81 articles. (N.B.: There is overlapping of articles among these two population categories, as each article may report more than one category.)

By population age groups: Among the 126 HCPs, 62 focused on the adult population, 48 on the middle-aged, 36 on the aged, and 43 on children and adolescents. (N.B.: There is an overlapping of articles among these population age groups, as more than one fracture can be reported in each article.) The major focus in fracture type in the 126 HCPs by population age groups was as follows:

- *Adults:* Femur/femoral, tibia/tibial, and humeral/humerus fractures (10 articles and 16.13% share each); femur/femoral neck fractures (8 articles and 12.9% share); hip and acetabulum fractures (6 articles and 9.68% share each); femur shaft fracture (3 articles and 4.84% share); clavicle, femur/femoral intertrochanteric, pelvis, and radius/radial fractures (2 articles and 3.23% share each), etc.
- *Middle aged:* Tibia/tibial fractures (9 articles and 18.75% share), femur/femoral fractures (8 articles and 16.67% share), humerus/humeral fractures (7 articles and 14.58% share), hip fractures (6 articles and 12.5% share), femoral neck fractures (5 articles and 10.42% share), spine, acetabulum, and radial/radius fractures (4 articles and 8.33% share each), etc.
- *Elderly:* Hip fractures (9 articles and 25.0% share); humerus/humeral fractures (7 articles and 19.44% share); femur/femoral fractures (6 articles and 16.67% share); femur intertrochanteric, tibia/tibial, and femur/femoral neck fractures (4 articles, and 11.11% share each); acetabulum and femur trochanteric fractures (2 articles and 5.56% share each), etc.
- *Children and adolescents:* Humeral/humerus fractures (13 articles and 30.23% share); femur/femoral fractures

(12 articles and 27.91% share); femur/femoral neck fractures (9 articles and 20.93% share); acetabulum, spine, femur shaft, ulna, and tibia/tibial fractures (2 articles and 4.65% share each); radius/radial and hip fractures (1 article and 2.33% share each), etc.

Significant Keywords

A total of 587 author keywords that appeared in 128 HCPs on India's fracture research were identified. Some of the important keywords with the comparatively largest frequency of occurrence were "osteosynthesis" and "fracture healing" ($n = 40$ each), "fracture fixation, internal" ($n = 37$), "fracture fixation" ($n = 24$), etc. (►**Fig. 2**). A total of 47 keywords with a frequency of more than two were chosen for the co-occurrence network. The co-occurrence network map was constructed with the help of VOSviewer, which revealed that these 47 keywords were spread over four clusters. The 48 keywords have 636 links with total link strength of 1,777.

Most Productive and Most Impactful Organizations

In all, 151 organizations participated in 126 HCPs, of which 122 organizations published 1 article each, 69 organizations 2 to 5 articles each, and 2 organizations 8 to 12 articles each. The top 26 organizations contributed 2 to 16 articles each and together contributed 104 articles and 3,796 citations, accounting for 82.54 and 81.48% share in total publications and total citations. It was also observed that the top 10 organizations contributed more than the average group publication productivity (4.0) of 26 organizations, and 7 organizations registered CPP and relative citation index (RCI) more than the group average (36.5 and 0.99, respectively) of the top 26 organizations. ►**Table 2** presents the profile of the top 8 most productive and top 8 most impactful organizations. The collaboration links among the top 26 organizations were observed to be weak, as there only were 38 institutional pairs having 1 collaboration link and 2 institutional pairs having 2 collaboration links (PGIMER, Chandigarh–Dr Ram Manohar Lohia Hospital, New Delhi and Indraprastha Apollo Hospital, Delhi–Vardhman Mahavir Medical College [VMMC] & Safdarjung Hospital, New Delhi).

Most Productive and Most Impactful Authors

In all, 470 Indian authors participated in 126 HCPs, of which 411 authors published 1 article each, 43 authors published 2 articles each, 7 authors published 3 articles each, 5 authors published 4 articles each, 3 authors published 5 articles each, and 1 author published 6 articles. The top 46 authors contributed 2 to 6 articles each and together contributed 122 articles and 3,527 citations, accounting for a share of 96.83 and 75.70% in total publications and total citations, respectively. It was also observed that the top 16 authors contributed more than the average group publication productivity (2.65) of 46 authors, and 19 authors registered CPP and RCI of more than the group average (28.91 and 0.78, respectively) of the top 46 organizations. ►**Table 3** presents the profile of the top 8 most productive and 8 most impactful authors. The details of the HCPs of the most productive authors (with references) are presented in ►**Supplementary Table S1** (available in the online version only).

The collaboration links among the top 46 authors were considered to be stronger compared to institutional collaboration. Among author collaboration pairs, 206 pairs have 1 collaboration link, 20 pairs have 2 collaboration links, 4 author pairs have 3 collaboration links, and 2 author pairs

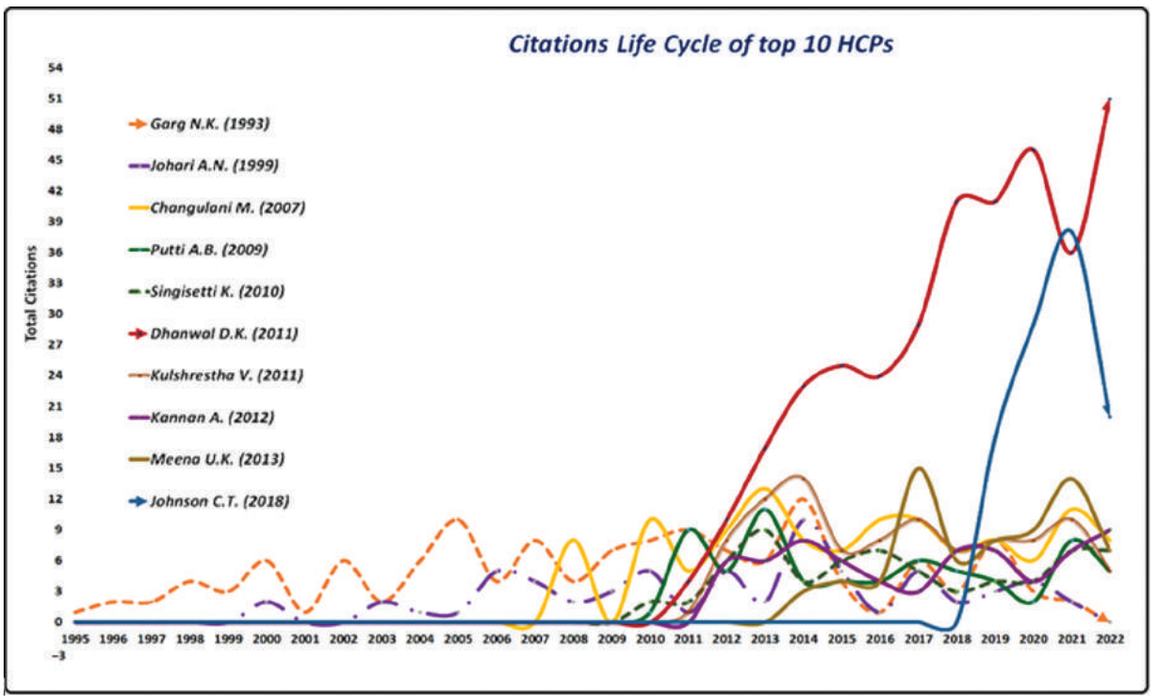

Fig. 2 Citation life cycle of top 10 high-cited publications.

Table 2 Profile of top 8 most productive and 8 most impactful organizations

Sl. no.	Organizations	Total papers	Total citations	Citations per paper	Relative citation index	Total link strength
Top 8 most productive organizations						
1	AIIMS, New Delhi	16	568	35.50	0.96	11
2	PGIMER, Chandigarh	13	428	32.92	0.89	13
3	Pt. BD Sharma PGIMS, Rohtak	9	279	31.00	0.84	0
4	UCMS, New Delhi	5	173	34.60	0.94	4
5	MAMC, Delhi	5	505	101.00	2.73	6
6	Indraprastha Apollo Hospital, Delhi	5	189	37.80	1.02	7
7	VMMC and Safdarjung Hospital, New Delhi	4	116	29.00	0.78	1
8	Dr. Ram Manohar Lohia Hospital, Delhi	4	121	30.25	0.82	4
Top 8 most impactful organizations						
1	MAMC, Delhi	5	505	101.00	2.73	6
2	JIPMER, Pondicherry	2	165	82.50	2.23	0
3	Sancheti Institute of Orthopaedic Research & Rehabilitation, Pune	2	94	47.00	1.27	13
4	Lok Nayak Hospital, Delhi	2	89	44.50	1.20	1
5	Parvathy Hospital, Chennai	2	78	39.00	1.05	4
6	Guru Teg Bahadur Hospital, Delhi	4	153	38.25	1.03	2
7	Indraprastha Apollo Hospital, New Delhi	5	189	37.80	1.02	7
8	Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh	3	107	35.67	0.96	4

Abbreviations: AIIMS, All India Institute of Medical Sciences; MAMC, Maulana Azad Medical College; PGIMER, Postgraduate Institute of Medical Education and Research; Pt. BD Sharma PGIMS, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences; UCMS, University College of Medical Sciences; VMMC, Vardhaman Mahavir Medical College."

Table 3 Profile of top 8 most productive and 8 most impactful authors

Sl. no.	Name of the author	Affiliation of the author	Total papers	Total citations	Citations per paper	Relative citation index	Total link strength
Top 8 most productive authors							
1	R. Malhotra	AIIMS, New Delhi	6	216	36.00	0.97	21
2	M.S. Dhillon	PGIMER, Chandigarh	5	181	36.20	0.98	17
3	N.K. Magu	Pt. BDS PGIMS, Rohtak	5	134	26.80	0.72	19
4	R. Singh	Pt. BDS PGIMS, Rohtak	5	139	27.80	0.75	16
5	S. Aggarwal	PGIMER, Chandigarh	4	141	35.25	0.95	12
6	S.K. Tripathi	PGIMER, Chandigarh	4	136	34.00	0.92	14
7	R.K. Sen	PGIMER, Chandigarh	4	136	34.00	0.92	14
8	R. Vaishya	Indraprastha Apollo Hospital, Delhi	4	78	19.50	0.53	12
Top 8 most impactful authors							
1	U.K. Meena	SMS Medical College, Jaipur	2	102	51.00	1.38	5
2	A.P. Singh	UCMS, Delhi	2	96	48.00	1.30	6
3	P. Sancheti	Sancheti Institute of Orthopaedic Research & Rehabilitation, Pune	2	94	47.00	1.27	76
4	L. Maini	MAMC, Delhi	3	129	43.00	1.16	7
5	D. Gulati	UCMS, Delhi	2	73	36.50	0.99	6
6	M.S. Dhillon	PGIMER, Chandigarh	5	181	36.20	0.98	17
7	R. Malhotra	AIIMS, New Delhi	6	216	36.00	0.97	21
8	A.K. Singh	Mayo Institute of Medical Sciences	3	107	35.67	0.96	3

Abbreviations: AIIMS, All India Institute of Medical Sciences; BDS PGIMS, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences; MAMC, Maulana Azad Medical College; PGIMER, Postgraduate Institute of Medical Education and Research; SMS, Sawai Madho Singh; UCMS, University College of Medical Sciences.

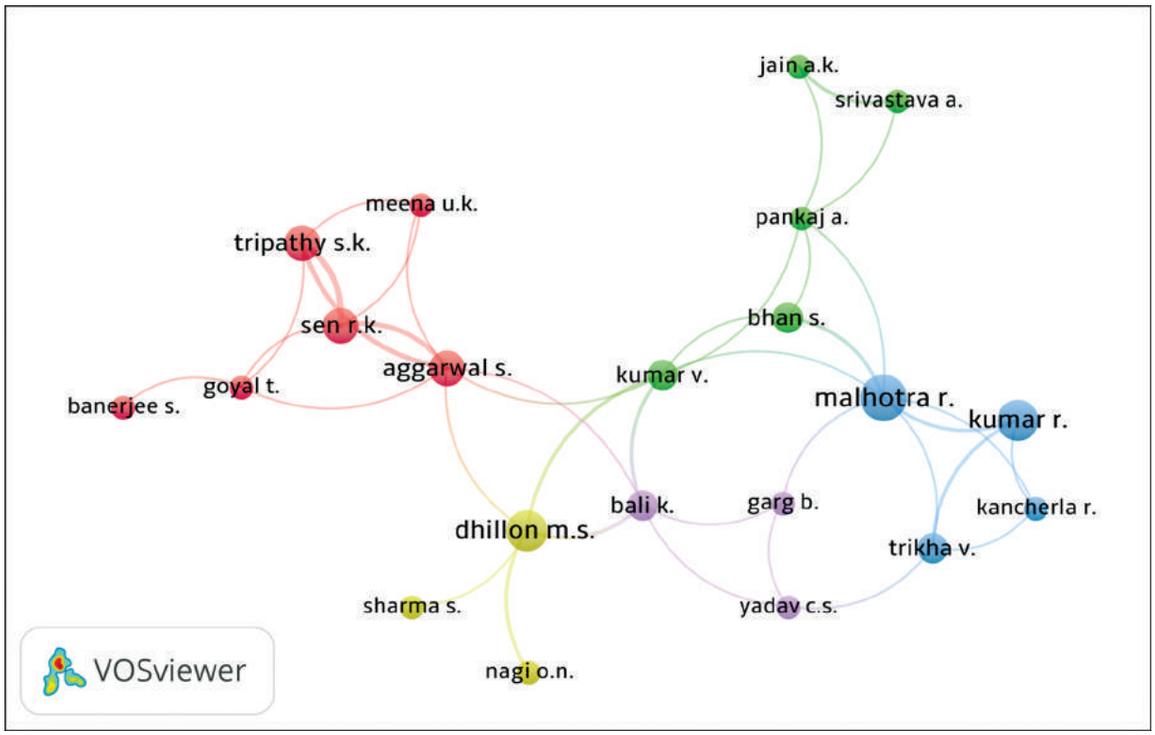

Fig. 3 Authors' co-authorship network.

have 4 collaboration links. The author network collaborative map among top authors is shown in ►**Fig. 3**.

Most Productive and Impactful Journals

The 126 HCPs were published in 27 journals: 21 journals published 1 to 5 articles, 2 journals 6 to 10 articles, and 4 journals 11 to 122 articles. The details of the top 8 most productive journals in publication output are presented in ►**Supplementary Table S2** (available in the online version only). The *Indian Journal of Orthopaedics* and *International Orthopaedics* ($n = 22$ each) were the most productive journals, whereas the *Proceedings of the National Academy of Sciences of USA* and *The Lancet* were the most impactful journals.

Discussion

Despite a large burden of fractures in the Indian population, due to road accidents[1,2] and other causes, not much research has been done so far, especially on the prevalence of these fractures and the published literature from Indian authors on this topic. However, the research output of the Indian authors in other fields like orthopaedics[30–32] and arthroplasty[33] have been studied. Rupp et al[34] found an increase in the incidence of fractures by 14% in Germany between 2009 and 2019. They observed that the most common fractures were femoral neck fractures (120 per 100,000 persons per year), pertrochanteric femoral fractures (109 per 100,000 persons/y), and distal radius fractures (106 per 100,000 persons/y). We also noted a significantly higher incidence of fractures in the lower limb, accounting for two-thirds of fractures (65.98%), compared to the upper limb fractures. However, the publications on upper limb fractures were more impactful and received a higher CPP, as compared to the publications on lower limb fractures. In our study, the HCPs related to fractures in adults and older people were substantially more (83/126) as compared to those of adolescents and children (43/126). In a systematic analysis of global, regional, and national burden of bone fractures in 204 countries and territories between 1990 and 2019, it was suggested that strategies should be focused on decreasing the incidence and burden of fractures by screening for osteoporosis in older people, promoting diet to improve bone health, reducing the risks of falls, providing a safe environment at work, and reducing the RTA by enforcing policy reforms and road safety measures.[35]

Using a bibliometric approach, the present study has identified and retrieved the most relevant and comparative HCPs in India's fracture research. We studied mainly the present trends and characteristics of India's fracture research by identifying the main types and subtypes of fractures and their distribution by anatomical location, sex, and population age groups. We identified the prominent collaborating countries, organizations, authors, and journals, besides significant keywords.

Vaishya et al reported that India's publications in orthopaedics grew at a rate of 20.8% annually in the last two decades, and 10.4% of Indian studies received external funding and 16.3% were international collaborations.[30] Karlapudi et al[31] found New Delhi to be the epicenter of publications related to orthopaedics, similar to our findings related to fractures. Vaish et al[32] found that in Indian HCPs related to

orthopaedics 24.58% publications received external funding and 36.87% publications were an international collaboration. This is higher than our findings according to which there was external funding in 1.59% publications and international collaboration in 15.08% publications, signifying a lower interest in the fracture research from India. We concur with the views that national and international collaboration in research helps in exchanging ideas, provides better-quality results, and may provide access to external funding for research.[36,37]

The leading teaching government institutions of India were the most productive and impactful organizations in fracture research, with AIIMS, New Delhi, PGIMER, Chandigarh, and Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences (Pt. BD Sharma PGIMS), Rohtak, being the most productive organizations. The MAMC, Delhi, JIPMER, Pondicherry, and Sancheti Institute of Orthopaedic Research & Rehabilitation, Pune, were the most impactful organizations, receiving the highest CPP. The top three organizations reporting the highest collaboration linkages and intensity with other Indian and foreign organizations were the following: PGIMER, Chandigarh, and Sancheti Institute of Orthopaedic Research & Rehabilitation, Pune ($n = 13$ linkages each) and AIIMS, New Delhi ($n = 11$ linkages).

Among the authors, R. Malhotra, M.S. Dhillon, and N.K. Magu were the most productive authors with 6, 5, and 5 articles, respectively, and U.K. Meena, A.P. Singh, and P. Sancheti registered the highest CPP (►**Supplementary Table S1**, available in the online version only). The authors reporting the highest collaborative linkages were P. Sancheti (76 linkages), R. Malhotra (21 linkages), M.S. Dhillon (17 linkages), and R. Singh (17 linkages). Among journals, the *Indian Journal of Orthopaedics*, *International Orthopaedics*, *Hong Kong Journal of Orthopaedic Surgery*, and *Journal of Orthopaedics & Traumatology* were the most productive journals (with 11–22 articles). The *Proceedings of the National Academy of Sciences of USA*, *The Lancet*, *Acta Orthopaedica*, and *Journal of Pediatric Orthopaedics: Part B* registered a comparatively higher CPP, and all these journals have a higher impact factor.

There are a few limitations to the present study. Only a single database of Scopus was searched, and other databases and sources (e.g., Web of Science) were not included in this bibliometric analysis. Therefore, some potential information may have been missed due to the noninclusion of some of the publications in the Scopus database. However, the use of multiple databases may lead to other difficulties in merging existing data in different databases available in different formats. In addition, there are chances that funding-related information may not be complete, an author's name may be similar to some other author's name, etc. We acknowledge that bibliometric studies do not involve clinical data of the patients; however, this is a well-established research method of evaluation of scientific contents. These studies are also valuable and useful as supporting tools for decision-making in setting research priorities, tracking the evolution of science and technology, funding allocation, and rewarding scientific excellence, among others.[38] We believe that bibliometrics is an objective and quantitative way of measuring research impact. The methodology is reproducible, transparent, and scalable, and one can assess the bibliometrics on an individual, institutional, national, or international level. On the negative side, the metrics can be exploited by researchers and journals to artificially boost bibliometric scores.

Conclusion

In this bibliometric study, we identified from the Scopus database 126 HCPs (≥20 citations) on Indian fracture research published during 1989 to 2022. Delhi was the epicenter of research and publication activities on the topic. The most productive organization were AIIMS, New Delhi, and PGIMER, Chandigarh, whereas the most impactful organizations were MAMC, New Delhi, and JIPMER, Pondicherry. The most productive authors were R. Malhotra and M.S. Dhillon, and the most impactful authors were U.K. Meena and A.P. Singh. *Indian Journal of Orthopaedics* and *International Orthopaedics* published the maximum number of HCPs, but the most impactful publications were from *Proceedings of the National Academy of Sciences of USA* and *The Lancet*, with an impact factor of 12.799 and 202.7, respectively.

To diversify India's research on fractures, there is an urgent need to develop a national registry and expand international collaboration, which will help improve both research output and research impact and quality. These research topics are expected to continue to be the hotspots and focus of research. Citation number–based identification of important articles will help current practitioners gain insight into the past and current trends in their respective fields and provide the foundation for further investigations.

Authors' Contribution

R.V. was responsible for conception of the study, literature search, manuscript writing, editing, and final reading of the manuscript. B.M.G. contributed to conception of the study, literature search, data collection and analysis, manuscript writing, and final reading of the manuscript. M.K. contributed to literature search, data collection and analysis, manuscript writing, and final reading of the manuscript. A.V. contributed to literature search, manuscript writing, editing, final reading, and submission of the manuscript.

Conflict of Interest

None declared.

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Molecular Docking Insights of Newly Synthesized Schiff Base Monomers and Evaluating the Anticancer Activity of Their Polymers

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Abstract

Introduction The molecular docking technique has shown efficacy with small molecules but faces challenges when applied to macromolecules. To overcome this limitation, a focused approach targeting the active repeat units (monomers) of macromolecules was adopted. This study synthesized ten new dihydroxy Schiff base monomers (SBM1-SBM10) featuring azo moieties and alkoxy side groups. These were attached to human 3-alpha-hydroxysteroid dehydrogenase type 3 (4XO6), a protein linked to breast cancer, using molecular docking via the AutoDock tool.

Materials and Methods The synthesis of dihydroxy Schiff base monomers SBM1-SBM10 with azo moieties and alkoxy side groups was carried out. These synthesized monomers were then docked with human 3-alpha-hydroxysteroid dehydrogenase type 3 (4XO6) utilizing AutoDock. Among these, the most promisingly docked monomer, SBM8, was selected for further experimentation. SBM8 was polymerized with terephthaloyl chloride to produce a novel polyester termed PolySyringaldehydeDiaminodiphenylSulfone (PSDS). The anticancer activity of PSDS was assessed using the MCF7 human breast cancer cell line. Concurrently, its cytotoxicity was evaluated via the MTT assay employing a normal VERO cell line.

Results The molecular docking analysis revealed the best-docked monomer, SBM8, which was subsequently used for the synthesis of PSDS. The newly formed polyester, PSDS, demonstrated significant anticancer properties against the MCF7 human breast cancer cell line. Simultaneously, the cytotoxicity evaluation on the normal VERO cell line indicated a favorable safety profile for PSDS.

Conclusion The study's findings highlight the successful synthesis and docking of dihydroxy Schiff base monomers with 4XO6, resulting in the creation of PSDS. This newly synthesized polyester, PSDS, exhibited promising anticancer activity against the MCF7 cell line while demonstrating minimal cytotoxicity towards normal VERO cells. These results suggest the potential of PSDS as a targeted therapeutic agent against breast cancer, warranting further investigation and development.

Keywords
- docking
- monomers
- anticancer activity
- polymers
- breast cancer

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Introduction

Schiff bases are compounds that contain an azomethine (–C = N–) linkage formed through the condensation of an aldehyde and an amine. They have various applications in fields such as pigments, dyes, catalysts, intermediates in organic synthesis, chemical sensors, and pharmacy. Schiff base aromatic polyester is also reported to exhibit good biological activities. Therefore, polyazomethine esters have been evaluated as anti-inflammatory, antioxidant, and antitumor agents through molecular docking studies.[1–5]

Breast cancer is the most common malignancy in women, and MCF7 cell lines are commonly used for in vitro analysis.[6] Molecular docking analysis can be employed to model the interaction between a small molecule and a protein at the atomic level, enabling us to characterize the behavior of small molecules at the binding site of target proteins and elucidate fundamental biochemical processes.[7]

Molecular docking involves generating multiple possible conformations of the ligand within the protein binding site. Therefore, the availability of the three-dimensional structure of the molecular target is a necessary condition. This structure can be experimentally solved (e.g., by X-ray crystallography or nuclear magnetic resonance [NMR]) or obtained through computational techniques (e.g., homology modeling).[8] Accurate prediction of ligand-target complexes is crucial in modern structure-based drug design.[9] Additionally, ligand matching and corresponding scoring can confirm the binding efficiency of each molecule at the binding site.[10] The docking process comprises two basic steps: (1) predicting the ligand's conformation, position, and orientation within the binding site (commonly referred to as a pose) and (2) assessing the binding affinity. These steps involve sampling methods and evaluation schemes.[11]

In the present study, 10 synthetic compounds were synthesized using previously reported methods and evaluated for anticancer activity using human 3-alpha-hydroxysteroid dehydrogenase type 3. The best-docked monomer, SBM8, was polymerized into polyester (PSDS) using terephthaloyl chloride, and its anticancer activity was evaluated using the MTT assay.

The 10 monomers, SBM1-SBM10 (►**Fig. 1**), were synthesized using the previously described method[12] and docked to human 3-alpha-hydroxysteroid dehydrogenase type 3 using the AutoDock tool. The Lamarckian genetic algorithm was employed, utilizing the conformational changes of molecules after in situ optimizations. A quasi-blind docking method was used to define the potential inhibitor binding sites on the surface of human 3-alpha-hydroxysteroid dehydrogenase type 3. It has been demonstrated that the B-chain of human 3-alpha-hydroxysteroid dehydrogenase type 3 interacts with 5-alpha-dihydrotestosterone (5-alpha-DHT) and reduces MCF7 cell growth.[13]

Therefore, the target protein, human 3-alpha-hydroxysteroid dehydrogenase type 3, was used in molecular docking analysis to search for potential inhibitors with the lowest possible binding energy. The crystal structure of human 3-alpha-hydroxysteroid dehydrogenase type 3 (PDB entry 4XO6) was derived and employed for the docking experiments. The target protein was generated by editing the PDB file and removing the heteroatoms (nicotinamide adenine dinucleotide phosphate [NADP]), 1,2-ethanediol, (3beta, 5alpha)-3-hydroxyandrostan-17-one, and all water molecules from the complex structure.

Gasteiger charges were assigned to the structure of human 3-alpha-hydroxysteroid dehydrogenase type 3 using tools from the AutoDock suite. The induced fit docking interface was utilized for docking purposes, and the ligands were treated as flexible. A grid box for ligand docking experiments was centered on the protein molecule, with a spacing of 0.375 and x, y, and z coordinates of 12.077, 13.052, and –30.711, respectively, resulting in a total of 126,126,126 grid points. Additional experimental parameters employed in AutoDock included a population size of 300, the number of generations was set to 27,000, and the number of evaluations was set to 20,000,000. The docking runs were performed 50 times with a mean square deviation cutoff of 1.

Cell Lines and Culture

The MCF-7 human breast cancer cell line was obtained from the King Institute of Preventive Medicine and Research, Chennai, India. The cells were cultured in a culture flask using Minimum Essential Medium supplemented with 3% L-glutamine, 10% fetal bovine serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL), amphotericin B, and 7.5% sodium bicarbonate. The cells were grown in a vented T25 mL culture flask and incubated at 37°C in a 5% CO_2 incubator.

After 3 days, the formation of an approximately 80 to 90% confluent monolayer (adherent) was confirmed using a 40× inverted microscope. Subsequently, the cells were subcultured using Trypsin Phosphate Versene Glucose (TPVG) solution in combination with minimal essential medium for further investigations.

Preparation of Stock Solution

Preparation of Stock Solution

The polymer PSDS was dissolved in 1 mL of dimethyl sulfoxide (DMSO) at a concentration of 0.1% (v/v). The volume was then made up to 10 mL using complete medium (MEM) to obtain the extract stock solution at a concentration of 10 mg/mL.

Extract Dilution

The stock solution was further diluted with complete medium to obtain concentrations of 31.25, 62.5, 125, 250, 500, and 1,000 µg/mL. All dilutions were stored in an airtight container until tested.

Cytotoxicity Study: MTT Assay:

The growth inhibition of MCF-7 cells and VERO cells by polyester PSDS was determined using the MTT assay.[14] This well-accepted in vitro method is used for screening

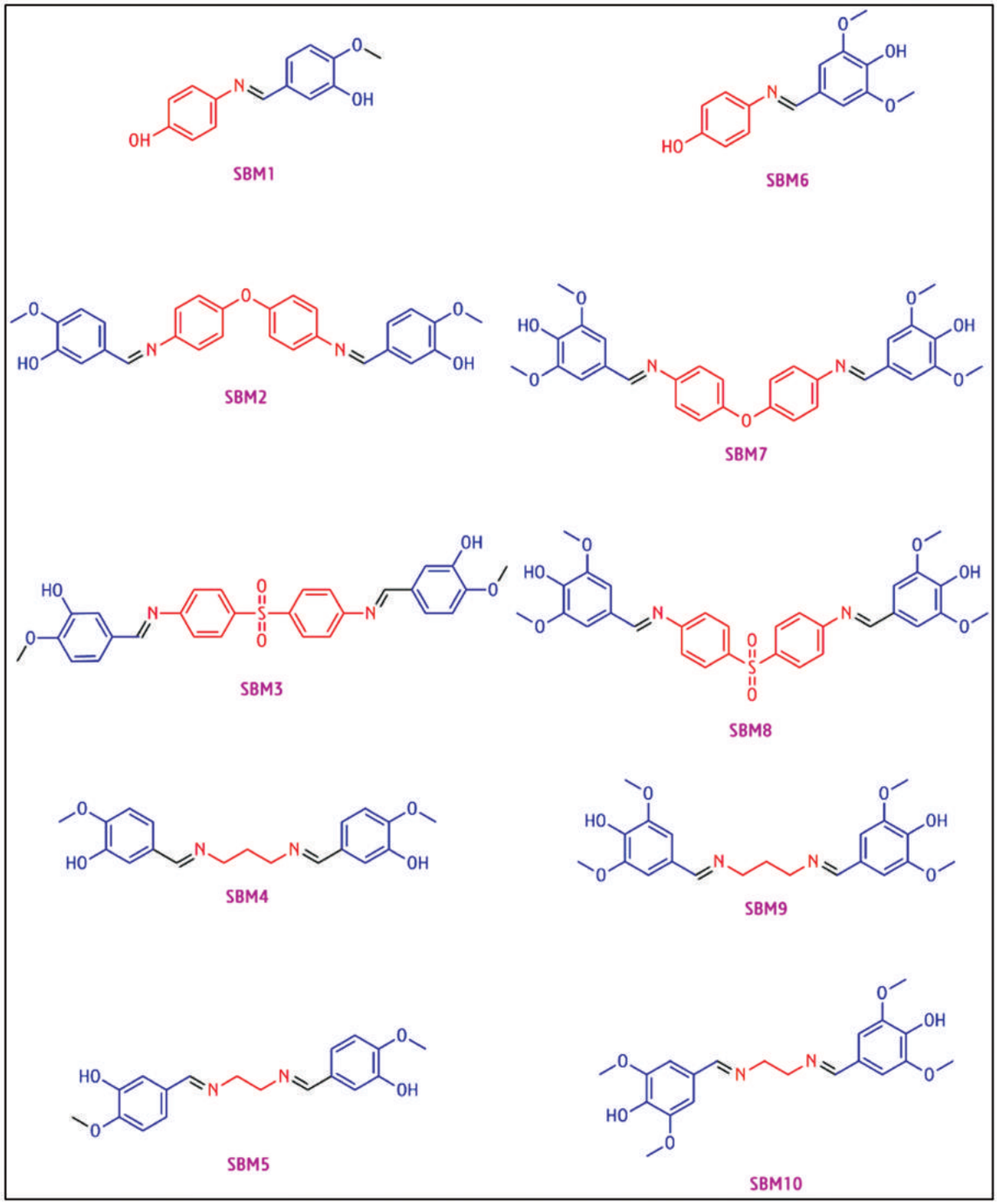

Fig. 1 Structure of two new series of Schiff base monomers (SBM1-SBM10).

drugs with cytotoxic activity and helps determine the IC_{50} concentration of the polymer.

For the assay, cells were harvested and seeded at a density of 10^5 cells/well in 96-well plates. The plates were then incubated for 72 hours at 37°C with 5% CO_2 to allow cell attachment. After 24 hours, different concentrations of the polyester (1, 10, 25, 50, 100 µg/mL), DMSO as vehicle, and a standard 5-fluorouracil (5FU) were added to the cells, followed by incubation for 48 hours. Each concentration was tested in triplicate.

After the incubation period, the medium was replaced with phenol red- and FBS-free medium. Then, 20 µL of MTT dye was added per well, and the plate was wrapped with aluminum foil and incubated for an additional 4 hours. Following incubation, the medium was carefully removed, and 100 µL of DMSO was added to each well to solubilize the

formazan crystals. The optical density (OD) was measured at a wavelength of 540 nm.

The percentage of cell inhibition was determined using the following formula:

$$\% \text{ of cell viability} = 1 - \text{OD of test/OD of control} \times 100$$

Results and Discussion

The evaluation of the binding affinity of synthetic compounds with human 3-alpha-hydroxysteroid dehydrogenase type 3 was conducted. Among the 10 docked compounds, compound SBM8 exhibited a higher binding affinity, and thus was selected for further studies. The positive control, 5 alpha-DHT (AOX), was used for comparison of binding affinity.

Compound SBM8 was docked with a predicted potential of 24 aromatic carbons and a rotatable bond count of 12. Additionally, SBM10 and SBM6 demonstrated better binding energies of –8.881 and –8.111 kcal/mol, respectively. The binding energy between the ligand–protein complex was –10.94 kcal/mol, with a ligand efficiency of 0.27. The inhibitory potency was determined as 9.56 µM. The intermolecular energy was –14.52 kcal/mol, the van der Waals hydrogen bond dissolution energy was –14.32 kcal/mol, and the electrostatic energy was –0.2 kcal/mol. The total internal energy was –3.25 kcal/mol, while the torsional energy was 3.58 kcal/mol. The unbound or free energy was determined as –3.25 kcal/mol.

Compound SBM8 formed a hydrogen bond between the protein and the ligand molecule at the amino acid LYS270: HZ1, with a bond length of 3.06 Å (►**Fig. 2**). The positive control AOX-coupled complex formed a hydrogen bond with the amino acid VAL54:OH, with a bond length of 2.04 Å, as reported.

From the docking studies, it was observed that the SBM8 monomer exhibits superior ligand characteristics compared to the previously studied positive control drug, AOX. SBM8 demonstrates a higher binding affinity and forms stronger bonds with the target macromolecule. The macromolecule plays a crucial role in deactivating the most potent androgen, 5-DHT (AOX control), which subsequently leads to the downregulation of enzyme production and a reduction in MCF7 cell growth.

Previous studies have reported that 5-DHT inhibits proliferation of cancer cell lines, including MCF-7, MDA-MB435S, BT-20, and T47-D. Furthermore, recent research has indicated that 5-DHT can decrease the expression of the efflux transporter, adenosine triphosphate (ATP) binding cassette subfamily G isoform 2 (ABCG2), on the plasma membrane of MCF7 cancer cells, leading to a decline in breast cancer cell growth.[15]

The SBM8 monomer demonstrates potent inhibitory effects on MCF7 cancer cells and was further tested in vitro using the MTT assay with the MCF7 cell line.

Preparation of polyester PSDS: The polyester PSDS was prepared using a previously reported method[12] and the characterization results are the following: yield—72%, FTIR (/cm): 2,915 and 2,846 (–CH_2, stretching), 1,734 (C = O-ester, stretching), 1,655 (C = N, extension), 1,612 and 1,547 (C = C-aromatic, extension), and 1,125 (C–O-ester, stretching). ^{1}H NMR (ppm) (DMSO): 8.74 (s, –CH = N), 7.69 (d, 2H), 7.58 (d, ^{1}H), 7.40 (d, ^{1}H), 7.03 (d, ^{1}H), 6.93 (s, ^{1}H), 2.45 (t, ^{2}H), 1.92 (q, ^{4}H), 1.27 (q, ^{8}H), and 3.96 (s, ^{3}H). 13C NMR (ppm) (DMSO): 172.35 (ester carbon); 160.80 (C = N carbon); 149.62, 141.26, 132.12, 125.69, 122.13, 115.62, 111.30, 61.91 (methylene

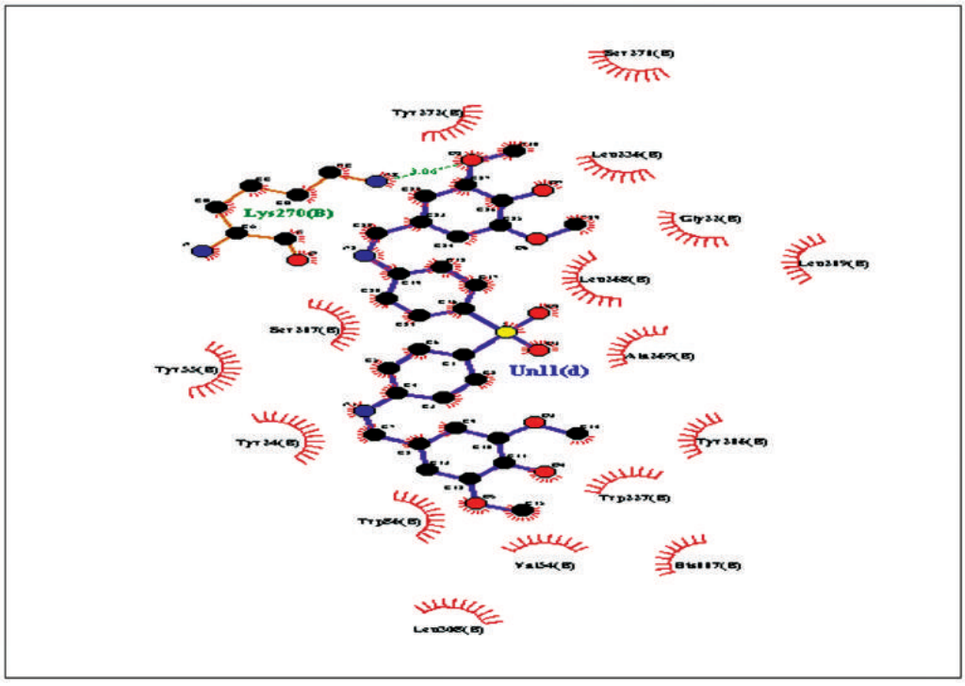

Fig. 2 Interactions from docking performance of the most potent SBM8 with human 3-alpha hydroxysteroid dehydrogenase type 3 (PDB entry 4XO6) visualized in PyMOL.

carbon); 56.33 (methoxy carbon); and 42.5, 33.4, 23.17, 23.9, and 21.5 (aliphatic methylene carbons).

Based on the docking results, the SBM8 monomer with the best score was successfully polymerized using terephthaloyl chloride following the described procedure. The cytotoxic activity of the resulting polymer, PSDS, was evaluated against the MCF7 human breast carcinoma cell line and the VERO African green monkey kidney cell line. The IC_{50} values obtained were 31.2 µg/mL for MCF7 cells and 275 µg/mL for VERO cells (►**Figs. 3** and **4**). These results indicate

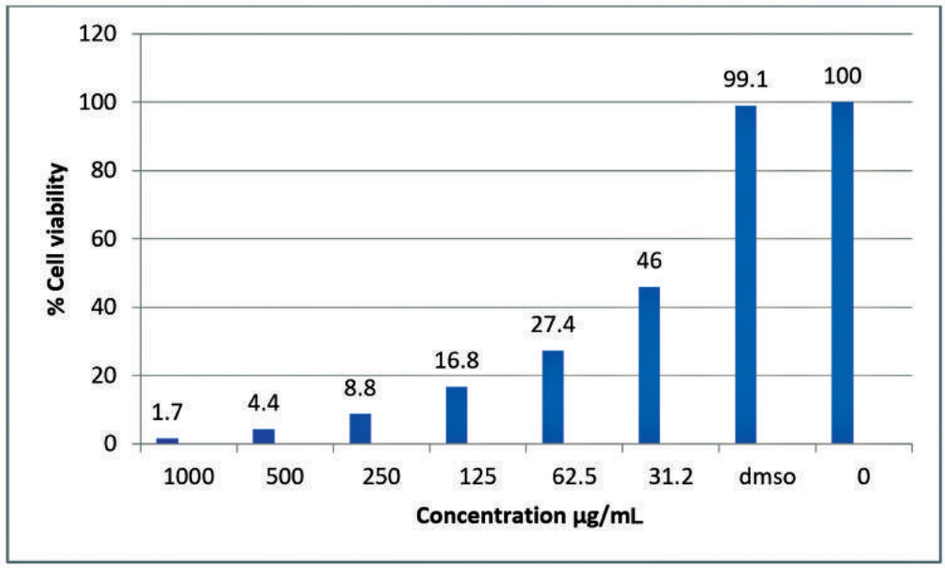

Fig. 3 Graphical representation of percentage of cell viability on the variation of concentration of PSDS.

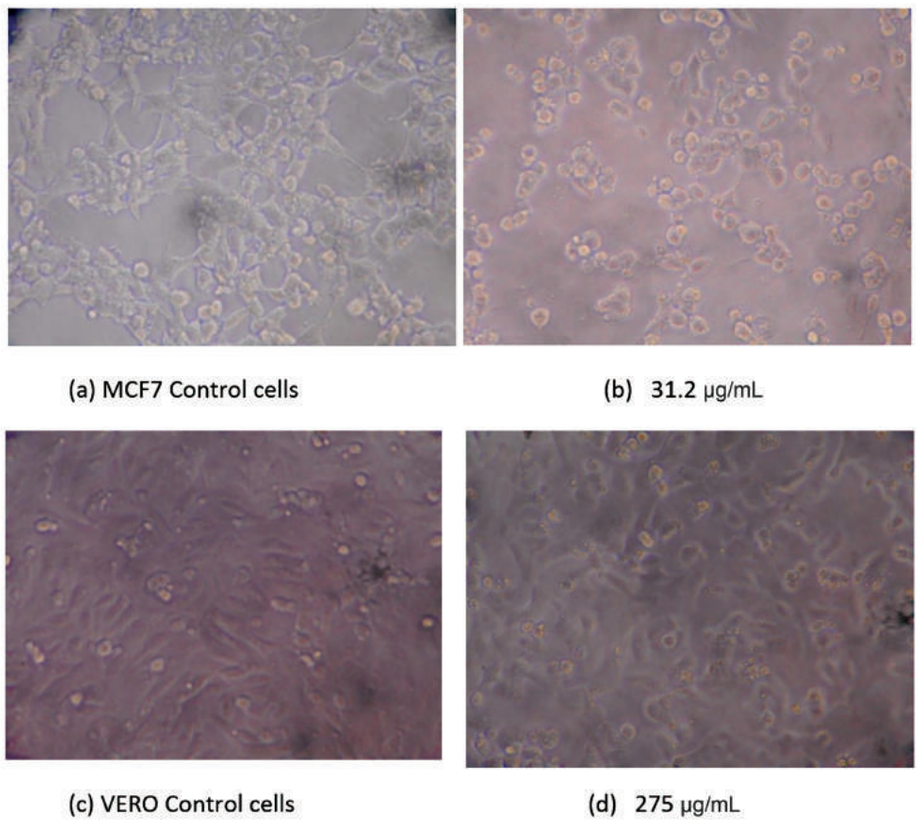

Fig. 4 Cytotoxic potential of Schiff base PSDS in MCF-7 breast cancer and VERO cell line. (**a**) Untreated MCF7 control cells, (**b**) cells treated with 31.2 µg/mL, (**c**) untreated VERO control cells, and (**d**) cells treated with 275 µg/mL.

that PSDS exhibits cytotoxic effects on cancer cells at relatively low concentrations, while being less toxic to normal cells at higher concentrations, highlighting its potential as an anticancer agent.

Conclusion

In conclusion, novel Schiff base monomers were synthesized using a previously established method, and docking studies revealed that the SBM8 monomer displayed strong binding affinity with human 3-alpha-hydroxysteroid dehydrogenase type 3. The subsequent in vitro analysis using the MTT assay confirmed the cytotoxic activity of the polymer PSDS derived from SBM8. The in silico docking results align well with the in vitro analysis, demonstrating the potential of using monomers to screen and identify polymers with cytotoxic properties. This research opens up new avenues for screening of polymers based on their monomers for their cytotoxic potential.

Conflict of Interest

None declared.

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"Brain on Fire": Hyperperfusion as a Hallmark of Hyperammonemic Encephalopathy

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Abstract

We describe a very rare case of acute fulminant hepatic failure as a complication of acute viral hepatitis caused by hepatitis A virus, complicated by hyperammonemic encephalopathy. The brain magnetic resonance imaging (MRI) findings were suggestive of cytotoxic edema involving bilateral cerebral hemispheres. The novel findings of hyperperfusion on arterial spin labeling perfusion MRI and hyperemic hypoxia on susceptibility weighted imaging are discussed. The patient had a rapid progression of cerebral edema and succumbed to the illness despite supportive care. Characteristic neuroimaging findings may help in the diagnosis of acute hyperammonemic encephalopathy of brain MRI, which may be useful in leading to appropriate clinical workup and diagnosis of the underlying cause of hyperammonemia. In our case, hyperammonemic encephalopathy was precipitated by fulminant hepatic failure caused by hepatitis A virus, which is a rare occurrence.

Keywords

- hepatic encephalopathy
- hyperammonemia
- hepatitis

Introduction

Fulminant acute hepatic failure (FAHF) is a rare clinical syndrome characterized by rapidly progressive hepatic dysfunction that has a very high case fatality rate of the order of 50 to 70%.[1,2] Drug overdose (such as acetaminophen), viral hepatitis, alcohol abuse, and toxin exposure account for most of the cases of fulminant hepatic failure reported in the published literature. FAHF is an extremely rare complication of viral hepatitis caused by hepatitis A virus (HAV).[1] HAV, a picornavirus, is spread by the fecal–oral route and usually leads to a self-limiting acute hepatitis, which confers a lifelong immunity to the infected person. FAHF has been reported in less than 1% of patients infected with HAV. Patients with FAHF develop acute hepatic encephalopathy, markedly raised serum bilirubin levels, coagulopathy, and transaminitis (of the order of >1,000 U/L). Development of acute hepatic encephalopathy has been postulated to be due to accumulation of toxins within the blood, which are normally handled by metabolism in the liver.[3] Hyperammonemia has been reported to be the most implicated cause for acute hepatic encephalopathy. Hyperammonemic encephalopathy has a characteristic neuroimaging phenotype, which is distinct from hypoxic and other toxic encephalopathies and, in relevant clinical setting, may be pathognomonic of acute hyperammonemic encephalopathy.[4] Herein we report of case of acute encephalopathy in which neuroimaging findings led to a diagnosis of acute hyperammonemia, which was subsequently found to be due to HAV-associated FAHF. Our case is unique as we report the findings of arterial spin labeling (ASL) magnetic resonance imaging (MRI) perfusion

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in acute hyperammonemic encephalopathy due to HAV-associated FAHF. To the best of our knowledge, such findings have not been reported in the published literature.

Case Description

A 17-year-old adolescent girl was brought to the emergency department of our institution after she was found unconscious after a brief febrile illness of 2 days' duration. There was no history suggestive of any previous comorbid illness. The bystanders did not report any seizure or trauma. There was no fecal or urinary incontinence and no tongue bite. On examination, the patient was found to be in altered sensorium with a Glasgow Coma Scale (GCS) score of 9/15. The clinical examination was remarkable for icterus. The abdominal examination was within normal limits. Review of systems did not reveal any significant abnormality. There was no history suggestive of drug overdose or toxin intake. The following vitals were recorded at presentation: heart rate of 110 beats per minute (regular), respiratory rate of 22/min (irregular), SPO_2 of 93% on room air, and blood pressure of 140/90 mm Hg.

Considering the patient was in acute encephalopathy with reduced GCS, MRI of the brain was done, which revealed diffuse symmetric edema involving the cerebral cortex in both the cerebral hemispheres, manifesting as a cortical swelling and hyperintense signal on T2-weighted (T2W) image (►**Fig. 1a**) and fluid attenuated inversion recovery (FLAIR) images (►**Fig. 1b**). Effacement of the sulcal spaces and basal cisterns was noted (►**Fig. 1c**). Bilateral uncal herniation was evident (►**Fig. 1d**). Diffusion weighted images revealed

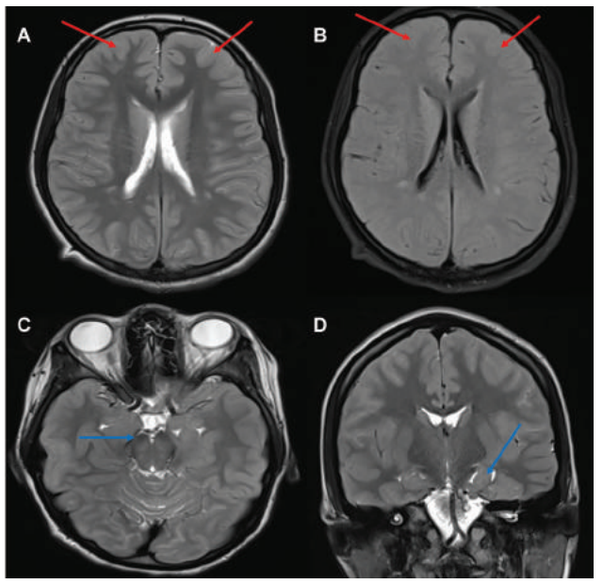

Fig. 1 Magnetic resonance imaging (MRI) of the brain. (**A**) Axial T2-weighted (T2W) image shows diffuse cortical edema in bilateral frontal lobes (*red arrows*). (**B**) Axial fluid attenuated inversion recovery (FLAIR) image shows hyperintense signal in the involved cortex with loss of gray–white differentiation (*red arrows*). (**C**) Axial T2W image shows effacement of the basal cisterns (*blue arrow*). (**d**) Coronal T2W image shows left-sided uncal herniation (*blue arrow*).

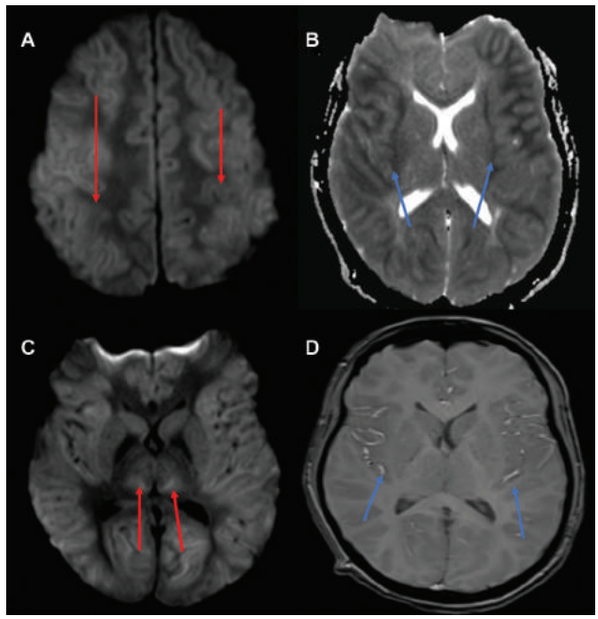

Fig. 2 Magnetic resonance imaging (MRI) of the brain. (**A**) Axial diffusion weighted image shows cortical diffusion restriction with relative sparing of bilateral peri-Rolandic regions (*red arrows*). (**B**) Axial apparent diffusion coefficient (ADC) map shows symmetric cortical and juxtacortical cytotoxic edema involving the insular region (*blue arrows*). (**C**) Symmetric diffusion restriction noted in bilateral thalami on axial diffusion weighted image (*red arrows*). (**D**) Axial susceptibility weighted image (SWI) shows hyperintense signal in cortical veins (*blue arrows*), suggestive of hyperemic hypoxia in the setting of cytotoxic edema.

cortical diffusion restriction, characterized by involvement of the bilateral insula and cingulate gyri with relative sparing of bilateral peri-Rolandic cortex and parieto-occipital regions (►**Fig. 2a**). On apparent diffusion coefficient (ADC) maps, symmetric hypointense signal involving the cortex and juxtacortical white matter was noted corresponding to the areas of diffusion restriction (►**Fig. 2b**). These findings suggested diffuse cytotoxic edema. Symmetric cytotoxic edema was also noted in bilateral thalami (►**Fig. 2c**). Sparing of the cerebellum, brainstem, and basal ganglia was noted (►**Fig. 2d**). On susceptibility weighted images (SWI), relative hyperintense signal was noted in cerebral arteries as well as cortical veins (►**Fig. 2e**), indicating reduced cerebral oxygen extraction due to cytotoxic edema and subsequent increased concentration of oxyhemoglobin in the cortical veins. No hemorrhages were noted. ASL MRI perfusion revealed diffusely increased perfusion in the bilateral cerebral cortices, corresponding to the areas involved on T2W, FLAIR, and DWI sequences (►**Fig. 3**). Considering the typical neuroimaging findings and the pattern of involvement, a diagnosis of acute hyperammonemic encephalopathy was considered. The patient was intubated and managed with intravenous (IV) mannitol and lactulose administered through a nasogastric tube.

Laboratory investigations showed total serum bilirubin level of 10.8 mg/dL, alkaline phosphatase level of 320 U/L, alanine aminotransferase (ALT) level of 1,400 U/L, and aspartate aminotransferase level of 1,700 U/L. Prothrombin time was noted to

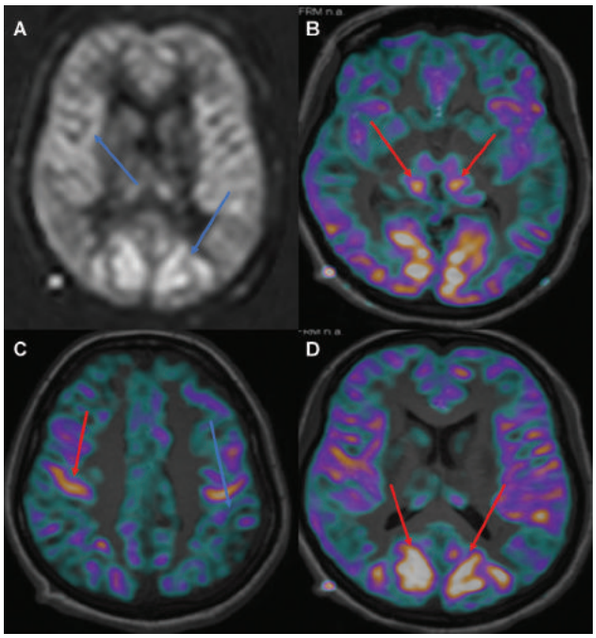

Fig. 3 Perfusion weighted images using arterial spin labeling. (**A**) Grayscale arterial spin labeling (ASL) image shows bilateral symmetric cortical hyperperfusion (*blue arrows*). (**B**) Fused ASL T1-weighted (T1W) image shows symmetric hyperperfusion in bilateral thalami (*red arrows*). (**C**) Fused ASL-T1W image shows hyperperfusion in precentral gyrus (*red arrow*) with relatively reduced perfusion in postcentral gyrus (*blue arrow*). (**D**) Axial fused ASL-T1W image shows bilaterally symmetric hyperperfusion in bilateral occipital lobes (*red arrows*).

be 65 seconds and international normalized ratio (INR) of 4.5. Blood sugars and renal functions were within normal range. Serum immunoglobulin M (IgM) for HAV was found to show significantly high titers. Serum ammonia level was noted to be 114 µmol/L. Arterial blood gas analysis revealed the presence of metabolic acidosis with pH of 7.2 (normal range: 7.35–7.45), $PaCO_2$ of 33 mm Hg (normal range: 35–45 mm Hg), bicarbonate level of 18 mmol/L (21–28 mmol/L), and PaO_2 of 92 mm Hg (normal range of 80–100 mm Hg). Thus, a final diagnosis of FAHF caused by acute viral hepatitis complicated by acute hepatic encephalopathy was made. The patient had a rapid deterioration of symptoms. She did not respond to supportive measures and ultimately succumbed to the illness within 3 days of hospital admission.

Discussion

Hyperammonemia leads to encephalopathy by various mechanisms. In the cases with acute liver failure, serum ammonia levels rise. The increased ammonia levels result in increased influx of ammonia into the astrocytes, both the active transport of quaternary ammonium ions and diffusion of ammonia through the blood–brain barrier. Within the astrocytes, the elevated levels of ammonia and ammonium ions lead to osmotic derangements resulting in swelling of the astrocytes.[5] Another proposed mechanism includes alteration in sodium-potassium pump function, uptake of glutamine, potassium, pyruvate, and lactate within the astrocytes and excitotoxicity mediated with increased extracellular glutamate levels, which activate the N-methyl-D-aspartate (NMDA) receptors.[6] Hyperammonemia may result from hepatic causes (such as acute or chronic liver failure), portosystemic shunting (such as after transjugular intrahepatic portosystemic shunt procedure), and urea cycle disorders.[7] Hyperammonemic encephalopathy in the setting of FAHF secondary to HAV infection is extremely rare.[1] The alterations in cerebral blood flow in hyperammonemic encephalopathy have been investigated in various publications with varying results. While most of the previous publications suggest a reduction in the cerebral blood flow (CBF) in cases of hyperammonemic encephalopathy, few publications have shown elevated CBF, particularly in type B hepatic encephalopathy, which results from portosystemic shunting.[8,9] In one of the studies reporting ASL findings in hyperammonemic encephalopathy due to urea cycle disorders, initial hypoperfusion was followed by hyperperfusion.[10] In the index case, profound increase in CBF was noted on ASL in the acute phase. These findings are in correspondence with the presence of cytotoxic cerebral edema. The cytotoxic state results in reduced oxygen utilization in the brain, which probably results in compensatory increase in CBF.[11] In our case, these findings are corroborated by the findings on SWI, which shows an increase in intracranial venous signal due to reduced utilization of oxygen by brain and thus increase in oxyhemoglobin concentration in the venous blood, a state that may be referred to as hyperemic hypoxia.[12]

Although the neuroimaging findings were characteristic of hyperammonemic encephalopathy in the index case, few differential diagnoses need to be considered in such cases presenting with encephalopathy. Status epilepticus may result in similar findings of diffuse cytotoxic edema and hyperperfusion with hyperemic hypoxia.[13] However, the clinical scenario excluded convulsive seizure. Still, nonconvulsive status epilepticus needs to be kept in the differential diagnosis. Hypoxic encephalopathy in adults could result in diffuse cortical cytotoxic edema; however, peri-Rolandic involvement and cerebellar and basal ganglia involvement are usually seen in hypoxic–ischemic encephalopathy.[14] Osmotic demyelination may show changes of cytotoxic edema in pontine and extrapontine distribution; however, basal ganglia involvement is characteristic in extrapontine forms of osmotic demyelination.[15] Also, clinical scenario of rapid correction of hyponatremia is suggestive of the diagnosis in such cases. Hypoglycemic encephalopathy in adults is suggested by cytotoxic edema involving the basal ganglia and cerebral cortex, in particular, parieto-occipital and insular involvement. In adults with hypoglycemic encephalopathy, the cerebellum and brainstem are spared. Diffusion restriction in bilateral corona radiata has also been described in hypoglycemic encephalopathy.[16] On the other hand, hyperglycemic encephalopathy may be encountered in diabetic ketoacidosis and is characterized by diffuse cytotoxic cerebral edema. However, the diagnosis is usually evident by clinical and laboratory parameters.[17] Posterior reversible encephalopathy syndrome (PRES) is characterized by bilaterally symmetrical edema involving the parieto-occipital

region and may involve the cerebellum and brainstem. In typical cases, no diffusion restriction is seen. Pattern of hypoperfusion is commonly encountered in PRES, although some cases of hyperperfusion have also been described.[18]

Conclusion

We have described an exceedingly rare complication of HAV infection wherein the patient developed rapidly progressive hyperammonemic encephalopathy following FAHF. Characteristic neuroimaging findings have been described. We emphasize on diffuse hyperperfusion in the cerebral cortex in the setting of cytotoxic edema, exquisitely depicted by ASL perfusion. Also, the findings of hyperemic hypoxia on SWI reinforce the hypothesis of reduced cerebral oxygen utilization in the setting of cytotoxic edema in this case. To the best of our knowledge, such findings are novel and have not been described previously in a case of HAV-associated FAHF and hyperammonemic encephalopathy.

Ethics Statement

The authors declare that the manuscript conforms to the Declarations of Helsinki. Informed written consent was taken from the guardians of the patient for data acquisition and publication.

Authors' Contribution

S.P. contributed to drafting of the manuscript and data acquisition. P.S. contributed to data acquisition, and approval of the final draft of the manuscript. R.G. contributed to drafting of the manuscript and approval of the final draft of the manuscript. P.B. contributed to data collection and approval of the final draft of the manuscript.

Conflict of Interest

None declared.

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Recurrent Hypokalemic Paresis—A Possibility of Liddle-Gitelman Overlap

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Abstract

Hypokalemia can occur due to various causes that have diverse manifestations varying from mild asthenias to acute onset paraparesis to life-threatening cardiac arrhythmias. A proper algorithmic evaluation for hypokalemia can help us identify various correctable causes and can be life-saving. Here, we discuss a case of 35-year-old hypertensive female who had presented as a case of acute onset flaccid paraparesis. We have further discussed our approach in reaching her diagnosis and stressed upon importance of algorithmic approach in the evaluation of patients presenting with recurrent hypokalemia. It may help in unmasking a rarer cause.

Keywords

- Gitelman syndrome
- hypokalemia
- Liddle syndrome

Introduction

Hypokalemic paresis is a type of metabolic channelopathy that manifests as acute onset flaccid paresis which if untreated can lead to involvement of respiratory muscles and cardiac arrhythmias. It can either be due to primary or secondary causes. Secondary causes include thyrotoxic periodic paralysis, renal tubular acidosis, Gitelman syndrome, Liddle syndrome, diarrhea, barium poisoning, and primary hyperaldosteronism.[1] The clinical manifestations of primary hypokalemia are similar to that of secondary hypokalemia but severity and management vary and therefore it is imperative to identify the cause. Here, we will discuss a case of hypokalemic periodic paresis with the possibility of one of the rarer causes.

Case Report

A 35-year-old married female presented with 1-day history of sudden onset quadriparesis, predominantly involving proximal part of lower limb. It was nonprogressive, not associated with any sensory disturbance, no bowel or bladder involvement. The patient was on a pilgrimage, climbing down a flight of stairs when she noticed weakness in bilateral lower limbs. It was associated with generalized body weakness and decreased grip strength. There was no associated fever, no associated abnormal body movements, no history of trauma, and no girdle-like sensation. No history of excessive meal intake, alcohol intake, or fasting prior to the occurrence of the weakness was reported. The patient provided history of similar episodes, first one occurring 13 years back at the age of 22 years, 6 months after the delivery of her first child; subsequent episode occurred 3 and 1 years back, respectively. Each event was associated with a hospital visit, where serum potassium levels were found to be low and the patient was supplemented with oral and intravenous potassium that relieved patient's symptoms; no subsequent recording of potassium levels post-correction was available. Patient was not on any supplementation or treatment in the intervening periods between the episodes. The patient was labeled as hypokalemic periodic paralysis in one of the hospital visits. No additional investigations were available with the patient. The patient was a known hypertensive and hypothyroid for the past 4 years on

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amlodipine 5 mg and euthyroid on 25 µg of levothyroxine. No other medications were being taken by the patient. No family history of similar weakness episodes in parents or siblings was present. On initial examination, the patient had a blood pressure of 171/100 mm Hg with pulse rate of 65/min and random blood sugar value of 201 mg/dL. She was conscious, oriented to time place person. Her power in the lower limb was ⅖ bilaterally and upper limb ⅗. There was areflexia and the tone was flaccid. There was no sensory deficit on examination. The patient's electrocardiography showed prominent U waves and her potassium levels recorded were 2.0 mEq/L. Magnesium levels were recorded to be 1.1 mEq/L, which were also low. Other electrolytes were within normal limits, and kidney function was normal. The patient was persistently in metabolic alkalosis. Further urinary studies of the patient were done, including urine spot potassium-creatinine ratio that was 57.5 mmol/g of creatinine (57.5 mEq/g of creatinine), indicating renal excretion of potassium, urinary sodium 76.1 mmol/L (76.1 mEq/L), and urine osmolality of 163 mOsm/kg H20. Transtubular potassium gradient calculated was 10.7 that confirmed renal loss of potassium. Urine calcium/creatinine ratio was found to be 0.17. Urinary chloride was increased to 99.8 mmol/L (99.8 mEq/L). Spot urine calcium and magnesium were 5 (2.5 mEq/L) and 4 mg/dL (3.2 mEq/L), respectively. Thyroid profile was suggestive of normal T3 T4 levels and thyroid-stimulating hormone of 5.64. Serum cortisol levels were found to be normal. Antinuclear antibodies of the patient were found to be negative using immunofluorescence assay. Further investigations revealed the patient to have grade 3 hypertensive retinopathy with Salu's and Gunn's sign positive. Ultrasound whole abdomen was suggestive of normal studies, and adrenal glands visualized to be normal.

During her hospital stay, she was initially supplemented with 160 mEq per day of potassium cumulatively through oral and central venous route. The symptoms of the patient improved gradually over a period of 3 days, but the potassium levels remained below 2.5 mEq/L, despite concomitant correction of serum magnesium. The potassium supplementation was further increased to 200 mEq/day with no subsequent improvement in the serum potassium levels. Her random blood glucose levels during stay were between 100 and 160 mg/dL with only one initial high recording and systolic blood pressure levels 140 to 149 mm Hg on amlodipine 10 mg daily. Patient was given spironolactone 50 mg once daily (od) for 3 days, but no improvement in the potassium levels was observed, despite daily supplementation. Spironolactone supplementation was stopped and the patient was started on amiloride 5 mg od with the continuation of potassium supplementation. Potassium levels were subsequently found to be in the range 3.5 to 4.5 mEq/L. Post initiation of the potassium-sparing diuretics, the 24-hour urinary potassium was found to be in the normal range. The patient was then discharged on amiloride 5 mg od and daily oral supplementation of 60 mEq of potassium. The patient was diagnosed as a case of salt-losing nephropathy with possibility of Liddle syndrome or Gitelman syndrome as overlapping features of both were found. Patient was followed 2 weeks post-discharge and had potassium levels of 3.8 mEq/L and blood pressure of 128/76 mm Hg. Patient is on regular follow-up and has had no episodes of hypokalemia after 4 months.

Discussion

Case report discusses a patient of hypokalemia paresis and her evaluation for the same. Our patient presented with flaccid areflexic paraparesis. On further evaluation, we found the cause for hypokalemia to be renal. She was further evaluated to look for exact cause of hypokalemia. We narrowed down to a possibility of Liddle syndrome in view of secondary hypertension and response to amiloride. The possibility of Gitelman was also considered in our case due to hypokalemia with normal calcium levels and hypomagnesemia.

Boorugu et al described a case of 53-year-old male with Liddle syndrome who presented with hypokalemic periodic paralysis.[2] Liddle syndrome is characterized by hypertension, hypokalemia, and metabolic acidosis. Patient has mutations leading to constitutive activation of epithelial sodium channels that leads to sodium and water retention. This causes a low level of renin and angiotensin. Patients are usually diagnosed at younger ages as a cause of secondary hypertension. It rarely presents as hypokalemic paresis. Patients respond to potassium-sparing diuretics, similar to that seen in our patient. But to prove it to be Liddle, we need renin and angiotensin measurements. Kayal et al studied 56 cases of hypokalemic periodic paresis over a period of 24 months in a single-center prospective study.[1] They tried to find out the causes of hypokalemia in the patients. About 42.9% of the reported cases had secondary causes of hypokalemia. Of this, 7.14% cases were due to Gitelman syndrome. It is characterized by metabolic alkalosis with hypokalemia, hypocalciuria, and hypomagnesemia. The reported incidence of Gitelman syndrome in Indian studies varies from 3.2 to 13.3%. Only two cases of Liddle syndrome presented as hypokalemic paralysis. This is a very rare presentation of Liddle as they usually present with muscle weakness due to hypokalemia rather than paralysis. They also reported that hypokalemia was more severe and required a longer duration of treatment to bring to normal levels in various secondary causes of hypokalemia.

Patra et al analyzed 200 patients presenting with hypokalemic periodic paralysis to determine the cause of hypokalemia.[3] Gitelman syndrome was reported as the most important secondary cause of hypokalemia paralysis found in 28% of the studied population. The lack of genetic studies to determine Gitelman syndrome as the cause was a limitation, though no other cause could be determined as per the clinical parameters. Gitelman syndrome occurs to loss of function mutation of thiazide-specific sodium chloride channels in the distal convoluted tubules. Determining the cause of hypokalemia is important as it helps in guiding the treatment. Chandramohan et al in another study followed up patients of hypokalemic paresis to ascertain the cause in tertiary care center in India.[4] They reported Gitelman syndrome as the second most common cause of hypokalemia in these patients. Another interesting finding reported by them was that patients with secondary causes of

hypokalemia paresis had higher degree of renal potassium losses and took longer to treat.

In ►**Figs. 1** and **2**, we represent the algorithmic approach to evaluation of hypokalemia with simple laboratory tests.[5]

This case may be a rare case of hypokalemic paresis. Simple laboratory evaluation can lead to a fairly acceptable diagnostic possibility for the patient even if all tests are not feasible. Such detailed evaluation will help in long-term alleviation of the symptoms and prevention of further such episodes. Due to nonavailability of genetic profiling and renin angiotensin aldosterone system testing at our center, we were unable to confirm a final diagnosis but did narrow down to two possibilities by following the algorithmic approach detailed above. We must always have a high index suspicion and should thoroughly evaluate patients coming to us with hypokalemia.

Conclusion

Patients presenting with hypokalemia should be evaluated for causes of hypokalemia. Simple tests like urinary electrolytes, urinary creatinine, and urine osmolality can be used to evaluate for secondary causes of hypokalemia. This can aid in establishing unusual causes of hypokalemia, missing out on which can be life threatening for the patient, who may end up

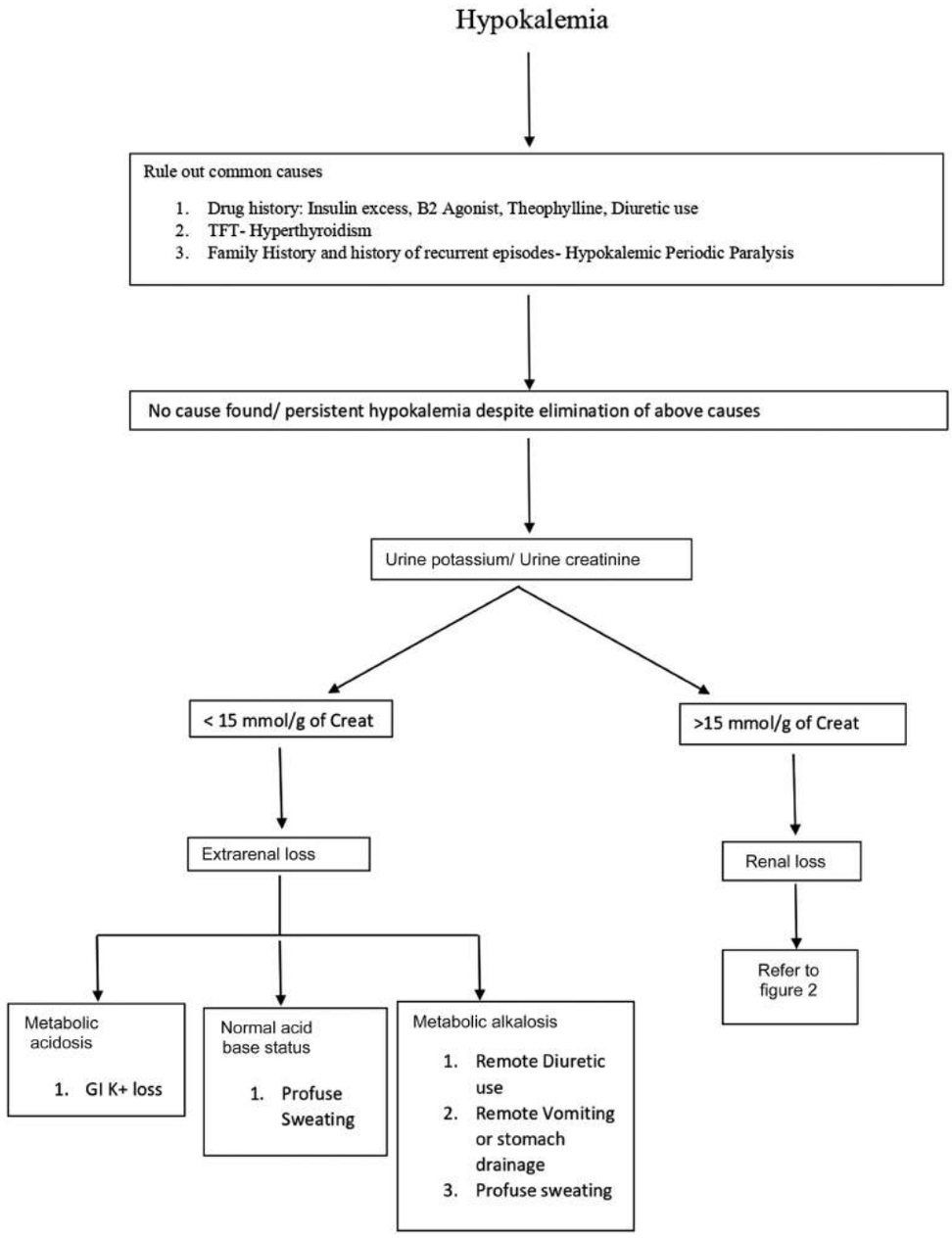

Fig. 1 Evaluation of hypokalemia. TFT, thyroid function test.

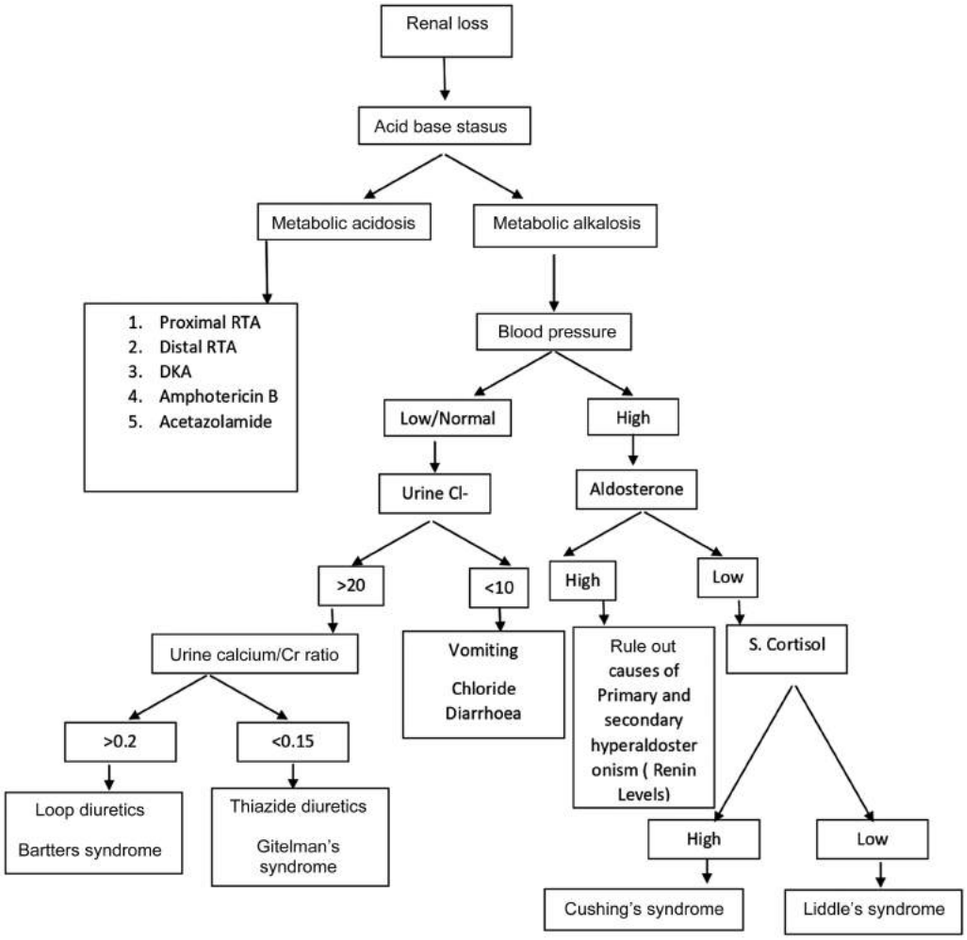

Fig. 2 Evaluation of renal causes of hypokalemia. DKA, Diabetic Ketoacidosis; RTA, renal tubular acidosis.

developing life-threatening arrhythmia owing to recurring episodes of hypokalemia.

Here, we propose a possibility of one of the rare causes of hypokalemia, a possibility of Liddle-Gitelman overlap syndrome in a patient having features common to both. Further case reports and genetic studies are required to throw light on such possibility.

Authors' Contribution

N.A. did data collection and manuscript draft preparation. V.G. contributed in conceptualization and review of manuscript. S.N. and A.A. did the critical review of manuscript.

Conflict of Interest

None.

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Effectiveness of Video-Based Educational Intervention on Knowledge, Attitude, and Practice (KAP) of COVID-19 Health Care Workers: Lesson for Future Pandemic Preparedness

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Abstract

Introduction As we have seen from the previous health emergencies, most recently the COVID-19 pandemic, public health education is often the first line of defense in any disease prevention. Through a short educational video intervention, this study aims to improve the awareness regarding COVID-19 infection control practices to be followed in a clinical laboratory.

Methods An online pre- and posttest survey was conducted. After pretest, an educational video was shared among the study participants followed by post test survey. This questionnaire contains items on three domains: knowledge, safe laboratory practices, and attitude.

Results Significant improvement occurred in all three domains in study participants post educational intervention.

Conclusion Simple educational video intervention can substantially improve the readiness of frontline workers to deal with any kind of pandemic situation.

Keywords
- COVID-19
- health care personnel
- medical education
- preparedness

Introduction

Coronavirus disease 2019 (COVID-19) has exposed the lack of preparedness of the health care system in dealing effectively with a pandemic. Learnings and experience of previous waves of the current pandemic should be harnessed to enhance our preparedness for any subsequent insults. With its first case reported in Wuhan, China, in December 2019, India has witnessed three catastrophic waves of COVID-19 so far. This novel corona virus is known to keep mutating its own genetic material quite fast,[1] and we have seen the severity of alpha, delta, and omicron variants in the Indian population. India is brutally affected with 44.7 million confirmed positive cases and 531,000 recorded deaths (last updated on January 15, 2023).[2] Some countries are now regaining a degree of normality, although the threat of

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another variant-induced wave of disease remains. Omicron BF.7, the latest variant of concern, has been on the rise since the beginning of 2023.[3]

As the pandemic has entered its third year, it is speculated that virus may reach an endemic stage in India rather than completely disappear.[4] Although India is well prepared to deal with another COVID wave if it happens in terms of sufficient corona-bedded hospitals, oxygen supplies, personal protective equipment (PPE) kits, genomic surveillance, and vaccine drives, the at-risk population has not backed up sufficiently in terms of continued educational interventions regarding corona pandemic preparedness. An effective public health education program is essential to disease prevention. Data from the 2002 severe acute respiratory syndrome (SARS) and 2012 Middle East respiratory syndrome (MERS) outbreaks support that knowledge, attitude, and practice (KAP) toward disease outbreaks affects individual action.[5] Health care workers are the frontline heroes in our fight toward effective pandemic response. A routine practice of precautionary behaviors among them must become essential. The objective of this study is to assess the effectiveness of educational intervention regarding recent corona outbreak on KAP of health care workers. This study also aims to improve knowledge, implement infection control practices, and promote positive attitude of health care workers regarding COVID-19 to prepare themselves for the next wave if it were to occur or in case of similar future pandemics through a short video-based intervention.

Methodology

This study was conducted in two parts: pretest and posttest questionnaire. A convenient sampling method targeting the medical personnel, medical students, and laboratory staff of Lok Nayak Jai Prakash Hospital, Delhi, India, was chosen. The questionnaire was given in the form of an online Google Form in the English language. Hard copies of Google Forms in their native language (Hindi) were also given to those staff who were not well versed with Google Forms. Consent was taken from all the participants in their native language. No financial incentive was provided. The institutional ethics committee (IEC) reviewed the study-related documents: F.1/IEC/MAMC/(73/01/2020) No. 146. The questionnaire was prepared mainly from frequently asked questions on the World Health Organization (WHO) Web site. The questionnaire was first provided to 10 individuals for content validation. Their responses were not included in the final analysis but used to improve the quality of the questionnaire.

The questionnaire was broadly categorized into four domains: demographics, knowledge, laboratory practices, and attitude. Cronbach's alpha for questionnaire reliability was 0.731, with a value greater than 0.6 considered satisfactory. Demographic sections included name, age, gender, occupation, and education. Knowledge section had 12 questions based on the origin, symptoms, incubation period, mode of spread, high-risk population, mortality, and vaccines. Laboratory practice section had 18 questions regarding social distancing, facemask, cough hygiene, waste disposal, some blood sample processing procedures, disinfectant procedures, and PPE kit. The attitude section had five questions. Each question was scored "1" if the response was correct and "0" if the response was incorrect. The number of correct responses in each question was added to give a cumulative score in that domain.

The period of pretest survey was from May 5 to 9, 2020. On May 10, 2020, an 8-minute educational video with audio in Hindi was shared among all the participants of pretest questionnaire. It was a PowerPoint presentation created by the authors, used for regular departmental academic activities. It contained basic information regarding coronavirus, transmission, clinical symptoms, prevention, and myths. Good laboratory practices for health care workers in clinical laboratory and hospital to prevent the spread of virus were also described. It was circulated through social media apps and was shown personally to all housekeeping staff. Then all the participants were given a post video questionnaire again through an online Google Form in the English language and also through hard copies in the native language. It contained the same questions as in the pretest questionnaire. The period of posttest questionnaire was from May 10 to 14, 2020. Data were tabulated in Microsoft Excel spreadsheet. The distribution of responses was presented as frequency and percentages. The descriptive statistics were applied. Individual pairwise comparison of all subgroups for pretest questionnaire and post video questionnaire were done using the paired Student's *t*-test. A value less than 0.05 was considered significant.

Results

Of 125 likely participants, only 100 responded for the pretest study. Eighty responded for both pre- and posttest studies. In total, only 80 participants completed the study. All the participants were health care workers. Of all the participants, 49 (61.25%) were females and 31 (38.75%) were males. The average age was 33.07 ± 8.6 years. This study population includes 43.75% doctors, 31.25% technicians, 16.25% MBBS students, and 8.76% housekeeping staff.

A comparison of the average percent of correct response in pre- and posttest in each of the three domains (knowledge, practice, and attitude) is given in ►**Table 1**. The average percentages of correct response pretest in the knowledge, practice, and attitude domains were 63.75 ± 11.3, 71.80 ± 10.14, and 79.25 ± 18.4%, respectively. A significant improvement was seen among participants in all three domains posttest. The average percentages of correct response posttest in the knowledge, practice, and attitude domains were 86.25 ± 13.72, 91.38 ± 10.6, and 86 ± 17.38%, respectively. Improvement in the average percent of correct response from pretest to posttest was 22.5% in the knowledge domain ($p < 0.001$), 19.58% in good lab practice ($p < 0.001$), and 6.75% in positive attitude among the study participants ($p < 0.017$). Of all the study groups, doctors and MBBS students showed maximum improvement in all three domains post video exposure. But technicians and housekeeping staff did not show any significant improvement in

Table 1 Comparison of average percent correct response in pre- and posttest in each of the three domains

	Pretest	Posttest	Improvement (posttest–pretest)	*p* value
Knowledge	63.75 ± 11.33%	86.25 ± 13.72%	22.5%	<0.001
Practice	71.8 ± 10.14%	91.38 ± 10.6%	19.58%	<0.001
Attitude	79.25 ± 18.4%	86 ± 17.83%	6.75%	0.017

Table 2 Comparison of the number of participants pre- and posttest in three domains with respect to the pretest performance

	Knowledge		Practice		Attitude	
Pretest level	Pretest, *N*	Posttest, *N*	Pretest, *N*	Posttest, *N*	Pretest, *N*	Posttest, *N*
0–50%	4 (5%)	1 (1.25%)	0%	1 (1.25%)	5 (6.25%)	5 (6.25%)
50–75%	55 (68.75%)	11 (13.75%)	48 (60%)	4 (5%)	20 (25%)	5 (6.25%)
75–100%	21 (26.25%)	68 (85%)	32 (40%)	75 (93.75%)	55 (68.75%)	70 (87.5%)
Total (*N*)	80	80	80	80	80	80

their attitude. We have stratified the population based on the pretest level of KAP average percent of correct response in the three groups from 0 to 50, 50 to 75, and 75 to 100%, respectively, in ►**Table 2**. The pretest results showed that in the knowledge domain only 21 of 80 participants scored more than 75%. The results improved after video intervention, with 68 of 80 participants scoring more than 75%. Similarly, 32/80 participants and 55/80 participants scored more than 75% pretest in the practice and attitude domains, respectively. However, there was marked improvement after the intervention of a short video, with 75/80 and 70/80 participants scoring more than 75% posttest in the practice and attitude domains, respectively. As can be seen in ►**Table 2**, there was substantial improvement to more than 75% correct response in each of the three domains among participants who had at least a 50% pretest level of KAP following the intervention of our educational video. No further significant improvement posttest was seen in the participants who already had less than 50% correct response, indicating that such minor educational interventions will further be useful to only those who were already aware of pandemic preparedness.

Discussion

What we are learning from the COVID-19 pandemic is similar to what we learnt in previous pandemics, that is, preparedness is crucial. Since COVID-19 has been here for more than 2 years, health care workers have now become so used to it that they frequently lack accurate knowledge and harbor negative attitude toward health guidelines, resulting in failure to practice essential hygiene and other forms of misconduct. Misconduct is often more prevalent among young people who myopically believe the risk of noncompliance is low in them. We all know that this is not the last pandemic and many first-world countries had failed to detect or respond decisively to the early signs of the outbreak. Regular consolidated and targeted instructional educational intervention might be more effective in improving individual KAP.[6]

To the best of our knowledge, this is among the first few studies to show the preawareness as well as improved postawareness responses regarding COVID-19 after an educational intervention among health care workers. This study has helped us to identify and fill the existing gaps in knowledge, safe laboratory practice, and psychology. Substantial improvement has been noted in study participants with a short educational video. From various responses we received from the participants, we observed that health care workers were already aware of the symptoms of COVID-19, mode of transmission, and COVID waste disposal. However, the awareness was not so common when it comes to practices like de-capping of vials, disinfection with hypochlorite solution, and correct sequence of donning/doffing of PPE. After the intervention, health care workers who were not in direct contact with coronavirus-infected patients could recognize and appreciate that even cloth masks along with social distancing and good personal hygiene were effective for prevention of coronavirus. This resulted in a decrement of hue and cry for N95 masks among them. They were now more satisfied with hospital administration after intervention. This faith in hospital administration is important to maximize the efficiency of health care workers, especially in a government hospital. More than 99% of the participants believed that this study not only improved their knowledge and awareness but also helped in reducing panic among health care workers. However, the improvement in attitude (6.75%) was not as much compared to the improvement in practice (19.58%) and knowledge (22.5%). Such short videos might be not able to bring a change in the attitude of participants so quickly, but it improved their knowledge and good lab practices, which are more the essential and practical needs to deal with any such future crisis. Probably more such repeated or long-term educational interventions will be required to improve their attitude as well.

Limitations of this study is that we were not able to determine the level of engagement with the video for

electronic or face-to-face participants. However, the increase in knowledge posttest helps validate participant engagement. Questionnaire responses were not in the form of Likert's scale, which would have made their responses even more objective. We did not include any control group without intervention, which could have added value to the study.

To conclude, lesson learnt from the COVID-19 pandemic is that we need to invest in preparedness in the form of medical education for health care workers, since we do not know when the next mutated virus will hit us hard.

Authors' Contribution

S.G. prepared the questionnaire, collected the response from participants, analyzed the results, and drafted the manuscript. B.G. developed the original idea of research and guidance. S.V.M. contributed to proofreading of the manuscript and guidance.

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Conflict of Interest

None declared.

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