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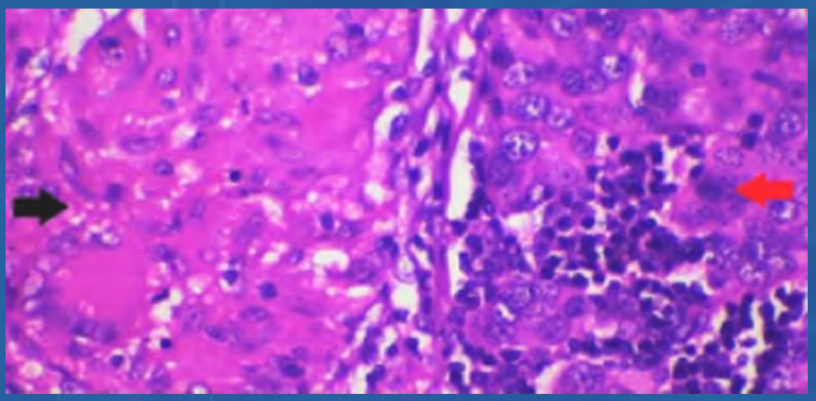
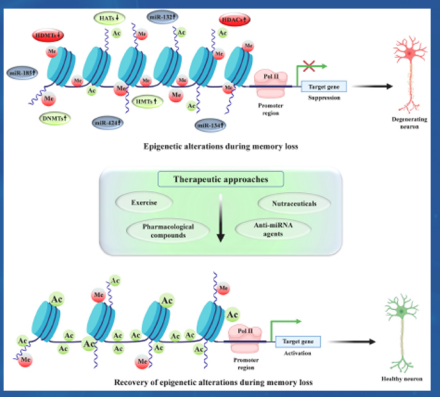


Epithelioid granuloma (black arrow) with invasive breast carcinoma (red arrow) (Haematoxylin & Eosin ×400).



Epigenetics in memory decline during aging

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Annals of the National Academy of Medical Sciences (India)

Review Article

Recovery of memory decline during aging - role of epigenetics

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ABSTRACT

Aging is a natural phenomenon associated with the accumulation of multiple alterations, including memory loss. Such deterioration of memory is based on the susceptibility of specific brain regions and the disorders that coincide with aging in those areas. Previous findings suggest that the optimal expression of synaptic plasticity-related genes is essential for memory formation and consolidation. Epigenetic modifications are one of the most crucial factors that cause memory deterioration by inducing the differential expression of synaptic plasticity-related genes. Understanding the fundamental cause of cognitive alterations that arise with aging is very essential for the development of therapeutic and/or preventive approaches. Several strategies have been employed to restore or reverse the memory decline caused by age-associated epigenetic alterations. The present article emphasizes the role of epigenetic alterations caused by histone modifications, DNA methylation, and non-coding ribonucleic acids (RNAs) on memory during aging. Also, we highlight the mechanistic switches of brain aging, including physical exercise, nutraceuticals, epigenetic modifiers, modulators of non-coding RNAs, and associated targets for therapeutic interventions. The emerging field of neuropharmacology and pharmacoepigenomics provides evidence that small drug molecules are currently employed to treat memory loss associated with aging, particularly by targeting epigenetic systems like DNA methylation, chromatin remodeling, histone modifications, and small non-coding RNAs. Therefore, targeting epigenetic modifications could be a potential therapeutic approach for the improvement of synaptic plasticity, neuronal activities, memory, and other brain functions during aging.

Keywords: Aging, Epigenetics, Memory, Pharmacoepigenomics, Therapeutic Recovery

INTRODUCTION

Memory-related issues are common complications in aged individuals and are considered as one of the prime concerns in even those who are healthy and do not have any neurological disorder.[1,2] The changes in molecular, chemical, and physical characteristics of neurons with advancing age result in cognitive deficits, memory loss, dementia, and behavioral alterations.[3] A key mechanism involved in the modification of neuronal networks and the formation of memory is attributed to the activation of neuronal plasticity-related genes. Age-associated cognitive decline is largely attributed to the progressive loss of synaptic plasticity caused by intracellular and intercellular alterations in the hippocampus.[4] Studies on the effect of aging on memory have attracted attention due to the growing aged population.[5] In several human subjects and animal models, the expression of synaptic plasticity-related genes is altered during the formation and/or loss of memory. The storage and duration of long-term memory occur at the cellular level as a result of gene transcription and translation. However, long-term memory declines with normal aging and in a variety of pathological conditions like dementia, neurodegenerative ailments, depression, and neuropsychiatric issues.[6] Previous studies have reported alterations in the expression of various synaptic plasticity-related genes, including cAMP response element binding (CREB) protein, early growth response 1 (EGR1), glutamate receptor (GluR), activity-regulated cytoskeletal-associated (ARC) protein, and brain-derived neurotrophic factor (BDNF) during normal aging as well as pathological conditions.[6–8] However, the molecular mechanism regulating the expression of synaptic plasticity-related genes is not elucidated. A study has demonstrated that these synaptic plasticity-related genes are significantly regulated at the transcriptional level by epigenetic mechanisms.[9] Therefore, stable behavioral consequences, including the formation of long-lasting memory, may depend on the healthy function of the neuronal epigenome.

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The epigenetic-driven changes in the transcriptome impair many of the brain processes with aging, including synaptic plasticity, learning, and memory.[9] These impairments are caused by epigenetic alterations such as histone acetylation and methylation and DNA methylation, which regulate the expression of memory and synaptic plasticity-related genes. Several epigenetic mechanisms, including histone modifications, nucleosome remodeling, DNA methylation as well as non-coding RNAs (ncRNAs)-mediated gene regulation are known to alter with age.[10] Considering the remarkable role of epigenetic modifications in the regulation of neuronal plasticity, several pharmacological antagonists and agonists are being used to improve cognitive functions by targeting these modifications that cause alterations in the transcription of memory-associated genes during aging.[9] Thus, epigenome-targeted interventions are one of the most promising fields of anti-aging research because of the outstanding adaptability and flexibility of the neuroepigenome. In addition, various therapeutic approaches such as calorie restriction, antioxidant dietary supplements, lifestyle adjustments, brain-training exercises, and molecular targeting with epigenetic modifiers such as synthetic pharmacological compounds and natural bioactive compounds have been employed.[11] The current review deals with memory and its regulation, the role of epigenetics in memory during aging and therapeutic approaches for memory recovery.

MEMORY AND ITS REGULATION DURING AGING

Memory is the ability to recite or recall information that has been learned and stored after any event.[12] A crucial aspect of memory formation is attributed to neuroplasticity, which brings about long-lasting changes in the brain and behavior from brief stimulations.[13] It has been widely accepted that the ability of an organism to adapt to a transient environmental stimulus over time is dependent on the capacity of the central nervous system for functional and structural plasticity.[14] This plasticity depends on a meticulously controlled series of post-synaptic receptor activation, neurotransmitter release, intracellular signaling cascades, transcription of synaptic plasticity-related genes, and consequent protein synthesis. The comprehensive cellular and molecular alterations in the brain regions are responsible for acquisition, consolidation, and maintenance of long-term memories.[9] The two key components, long-term depression (LTD) and long-term potentiation (LTP) are accountable for the neuroplasticity to induce prolonged changes in the brain and behavior.[15] The ability to encode, store, and retrieve environmental information by the brain deteriorates with age under the influence of several physiological and epigenetic factors.[16–19] Memory loss and the downregulation of synaptic plasticity-related genes are the consequences of several morphological, physiological, and molecular changes that take place in the brain with aging.[20,21]

Aging causes a delay in encoding, processing, and response time and has diverse effects on various types of memories.[22] For instance, recognition memory, working memory, episodic memory, and prospective memory all significantly deteriorate with normal aging, whereas procedural memory and some perceptual memory skills exhibit negligible age-related changes.[4] Although the capacity to recall extremely detailed information declines dramatically with age, semantic memory lasts well in an elderly person, if the information is used routinely.[23] Free recall task tests revealed that older adults perform worse than younger adults, implying that to reach high levels of performance, advanced encoding and retrieval techniques are employed, which deteriorate with advancing age.[24] During aging, deterioration in various cognitive functions including attention, episodic memory, and executive functions is dependent on the hippocampus and prefrontal cortex.[25] The course of cognitive aging varies according to genetic and epigenetic factors that influence cellular damage and vulnerability or resistance to age-related stress.[9] The limitation of the transient nature of memory-related biomarkers has been envisioned for the identification of more stable and adequate biomarkers with a longer duration even under the influence of several epigenetic factors. Therefore, an extensive investigation is required in the area of neuroepigenetics to understand the details of memory and its regulation during aging.

ROLE OF EPIGENETICS IN MEMORY

The covalent modifications of DNA and histone proteins can cause the conformational and structural alterations of chromatin, thereby upregulating or downregulating the transcription of associated genes and eventually leading to differential gene expression.[26,27] Some enzymes and processes, such as DNA methylation and post-translational changes in histone proteins, in particular methylation, phosphorylation, ubiquitination, and acetylation, result in these alterations. During aging, the epigenetic dysregulation caused by DNA methylation and histone acetylation is responsible for memory impairment.[13] Chromosomes are typically compressed, making it challenging for transcription factors to attach, and this leads to the suppression of memory-related genes. During chromatin relaxation, transcription factors bind easily to DNA and lead to the activation of genes related to memory. Therefore, chromatin expansion and contraction regulate the expression of various immediate early genes (IEGs) that are responsible for cognitive functions [Figure 1]. The epigenetic

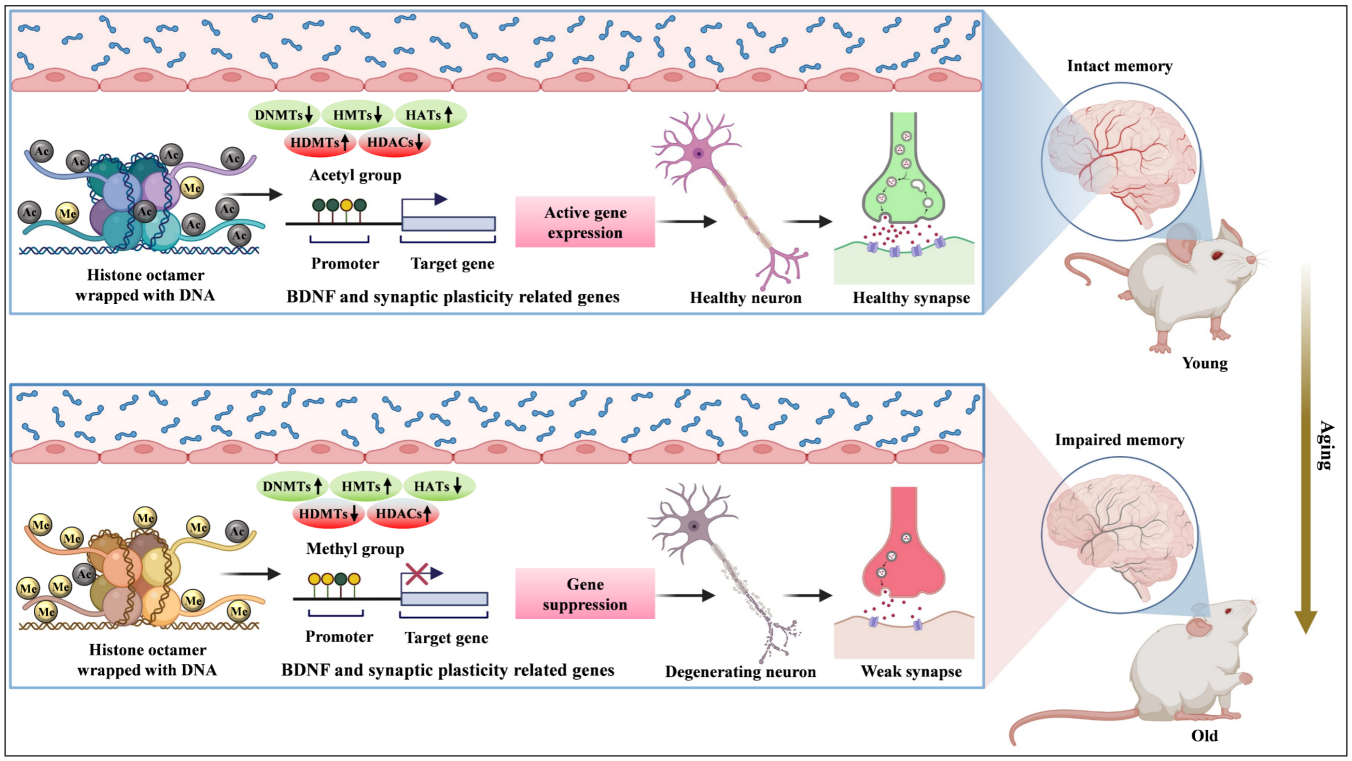

Figure 1: Summary of age-related alterations in neuroepigenome driven by epigenetic modification of memory-related genes and proteins. In the young brain, the gene promoter is epigenetically modified by differential expression of chromatin-modifying enzymes (DNMTs, HMTs, HATs, HDMs, and HDACs). Increased expression of HATs and HDMTs, and decreased expression of DNMTs, HMTs, and HDACs results in the activation of genes related to cognitive functions and synaptic plasticity leading to intact memory in young. In contrast, the increased expression of HMTs, DNMTs, and HDACs, along with decreased expression of HATs and HDMTs is observed in old. These alterations result in the suppression of cognitive functions and synaptic plasticity-related genes leading to impaired memory in the old.

DNMTs: DNA methyltransferases; HMTs: Histone methyltransferases; HATs: Histone acetyltransferases; HDMTs: Histone demethylases; HDACs: Histone deacetylases; BDNF: Brain-derived neurotrophic factor.

modifiers are known to upregulate the IEGs by inducing some of the specific transcription factors such as CREB and Elk1 which lead to the conversion of short-term memory into long-term memory.[28,29] The IEGs such as Arc, BDNF, c-fos, CREB, and Zif268 are important factors responsible for LTD and LTP, and further, lead to the formation of long-term memory and its consolidation.[29] Previous study revealed that the dysregulation in epigenetic regulatory mechanisms associated with the aging epigenome includes abnormally produced transcription factors, epigenetic modulators, enzymatic dysfunctions, and ncRNAs, which together cause a gradual loss of sensitivity to environmental cues and accelerate the aging process.[27] Moreover, the impairments in the function and biogenesis of various ncRNAs cause initial and sustained synaptic loss and cognitive deficits in aging models.[30] Further investigation in the field of neuroepigenetics is required to comprehend the underlying mechanisms of memory persistence.[26] The subsequent sections deal with the involvement of various epigenetic factors that affect memory and its consolidation during aging.

DNA METHYLATION IN MEMORY DURING AGING

DNA methylation can be dynamically changed throughout life, altering synaptic connectivity and cognitive functions with aging.[10] During DNA methylation, a methyl group (CH_3) is covalently attached to the 5' site of cytosine residues in CpG dinucleotides. Penner *et al*. reported that aged rats show considerably higher levels of methylation at the Arc promoter than young rats.[31] It has also been shown that a wide range of agonists of DNA methyltransferase (DNMT) activity modify DNA methylation status by binding to the promoter region of several neuronal plasticity-related genes such as Reelin, PP1, and BDNF. The promoter of the PP1 gene undergoes hypermethylation as a result of fear conditioning, which leads to the suppression of PP1 synthesis.[32] On the other hand, Reelin supplementation in the CA1 region of the hippocampus enhances the memory formation, learning ability, and synaptic plasticity for an extended period. This suggests that reduction in Reelin signaling might cause diminished cognitive function in age-related neurodegenerative

disorders.[32] The hypomethylation at the promoter region of Reelin leads to the increased transcription of Reelin, which enhances the synaptic plasticity and cognitive functions.[33] Another study revealed that the DNMT-knockout *in vivo* model exhibits deficiency in the Morris maze, contextual fear training, and hippocampal LTP.[34,35] Furthermore, deficits in learning and LTP were demonstrated in an *in vivo* mice model having double knockout for DNMT3a and DNMT1 genes in neuronal cells of the forebrain but not in the single knockout of either DNMT3a or DNMT1 gene.[34] This study suggests that the regulation of synaptic function and maintenance of DNA methylation status can be sustained by the presence of either of these isoforms. Memory formation involves both increased and decreased methylation at the promoter region of memory suppressor genes and memory enhancer genes, respectively. Therefore, either hypermethylation or hypomethylation might be responsible for memory function. These findings collectively imply that the regulation of DNMT activity is essential for memory and DNA methylation may cooperate with histone modifications, which have previously been linked to memory development and storage.[35,34] Additionally, several studies have found abnormal DNA methylation patterns in many areas of the brain, notably at CpG and non-CpG sites, suggesting that aberrant DNA methylation potentially aids in neurodegeneration.[36]

HISTONE MODIFICATIONS IN MEMORY DURING AGING

Histone modifications alter the fundamental structure of chromatin by modifying histone tails post-translationally.[10] A change in histone variants is associated with both gene activation and gene suppression. Acetylation of histones is linked to increased expression of genes, whereas deacetylation is linked to suppressed gene expression. In a study, the expression of histone deacetylase 2 (HDAC2) was elevated but there was no change in the level of CREB-binding protein (CBP) with HAT activity in the hippocampus of old mice.[16] Furthermore, another study showed that the complex association of CREB with CBP leads to the inhibition of HDAC and thereby affects the long-term memory for novel object recognition in a mouse model.[37] In the brain of aging mice, an increase in HDAC2 expression was linked to a worsening in recognition memory consolidation.[38] Epigenetic instability has also been linked to the loss of memory in neurodegenerative diseases. For instance, after fear training, histone acetylation is decreased in the hippocampus of APP/PS1 mice, which may be attributed to an increased level of HDAC activity. This is supported by the finding that contextual fear conditioning and LTP deficiencies in aged APP/PS1 mouse models may be cured by inhibiting HDACs.[39]

The histone methyltransferases control both the epigenetic inheritance of genes and the integrity of the genome. Histone methylation is crucial to distinguish between the integrity of the genome and the genes which give identities to the cells. The addition of methyl groups to histones regulates transcription by blocking or enabling the transcription factors to bind to the promoter region of numerous IEGs and memory-associated genes. Moreover, transcriptional activation or repression of memory-associated genes depends on the position and the degree of histone methylation. It has been demonstrated that the pattern of histone methylation changes with aging, depending on the tissues and organisms. The expression level of H3K9me2 in the prefrontal cortex is higher in age-related neurodegenerative mice models, and the suppression of methyltransferases reversed deficiencies in spatial, working, and recognition memory.[40] Among the H3K9me3-specific histone methyltransferases, SUV39H1 expression was noticeably elevated in aged mice. The global H3K9me3 level was also high in the hippocampus of aged mice. Old mice exhibited higher amounts of H3K9me3 at the IEGs promoter than young mice. The binding of H3K9me3 at the IEGs promoter further leads to a decline in recognition and spatial memory.[41] The specific pharmacological antagonist is used to block SUV39H1, which result in a low level of hippocampal H3K9me3 and increases the level of BDNF protein and spine density in the aged mice model. These alterations lead to enhanced performance of behavioral tests, including fear conditioning, memory for object locations, and complicated spatial learning.[42] Altogether, memory loss and its pathophysiology are largely attributed to the alteration in epigenetic pathways.

NON-CODING RNAS (NCRNAS) IN MEMORY DURING AGING

Non-coding RNAs can regulate the activity of genes by interacting with mRNAs, transcriptional stimulators, and other small RNAs and thereby restrict them to code for essential proteins related to memory.[43] Aging and age-associated diseases are correlated with dysregulations in the biogenesis and functioning of ncRNAs. In functionally partitioned neurons, such regulation is crucial, which may explain the abundant biogenesis of potential ncRNAs in the brain.[44] For optimal synaptic function, the pathways followed for the transportation of mRNAs to the synapse are essential, and their synaptic translation is managed in an activity-dependent way. In synaptic structures, a subset of conserved neuronal miRNAs is more prominent and involved in the regulation of transported transcripts associated with synaptic plasticity and memory.[30] The upregulation of the evolutionarily conserved ncRNA, miR-132 is mediated by CREB and BDNF-dependent molecular pathways. The *in*

vivo study showed the involvement of a neuronal activity regulating miR-132 in memory consolidation, learning, and synaptic plasticity in mice models. Further, a study revealed that in the hippocampus of transgenic mice, a comparatively lower expression level of miR-132 results in the enhancement of learning, novel object recognition capacity, and the induction of synaptic plasticity.[45] These findings demonstrated that the function of neuronal cells, memory formation, learning, and synaptic plasticity are extremely sensitive to the concentration of miRNAs i.e., an increase in the expression level of miRNAs leads to neurodegeneration, which might be due to the inhibition of various essential mRNAs in an "off-target" way.[46,47] The expression of three miRNAs such as miR-182, miR128b, and miR-134 is regulated during the formation of fear memories. The brain-specific miRNA, miR-134 regulates synaptic plasticity, learning, and memory formation by binding to the promoter region of BDNF and CREB.[48] Furthermore, the deacetylase sirtuin 1 (SIRT1) regulates miR-134 in the brain, and its absence suppresses CREB and BDNF proteins, impairing learning and synaptic plasticity. Notably, the loss of miR-185 and miR-25 has shown age-dependent effects on synaptic plasticity in the hippocampus.[44] In the coming future, it will be interesting to investigate the role of ncRNAs in controlling and maintaining the epigenetic factors associated with synaptic plasticity and memory formation in the aging brain.

THERAPEUTIC APPROACHES FOR THE RECOVERY OF MEMORY

Memory restoration involves the formation of new neurons and neuronal connections by providing additional resources and further re-establishes the structure and function of the neurons.[48] Evidence from the emerging field of neuroepigenetics suggests that regulating the epigenetic mechanisms responsible for memory impairments could be a potential therapeutic strategy.[49] An increasing line of evidence suggests that in memory loss conditions, the neuroepigenome can be altered by employing various strategies such as regular aerobic exercise, nutraceuticals, and pharmacological compounds to improve cognitive function.[50] Memory is influenced by a variety of factors, some of which act as enhancers and others as inhibitors. In subsequent sections, we will be discussing some of the approaches that are involved in the recovery of memory.

PHYSICAL EXERCISE

Regular aerobic physical exercise helps in the maintenance of cognitive function during aging and prevents the onset of neurological diseases by altering the neuroepigenome.[51] Intense exercise can promote DNA hypomethylation as it changes the methylation state of genes that respond to it. The capacity of neuronal cells to modify their form or to become more plastic depends on the position of methylation at CpG sites. Recent research in postnatal brain tissues has pinpointed the CpGs locations where physical training acutely modifies methylation state.[10,52] The variations in the intensity of methylation at the CpG site caused by aerobic exercise affect the memory-associated molecular mechanisms. Changes in DNA methylation brought on by exercise may impact a variety of processes, including increased muscle contraction and other molecular alterations that are oxidative and non-oxidative, as well as the mitochondrial number. A previous study suggested that the downregulation of BDNF is observed in normal aging and age-related memory loss.[53] The synthesis of neurotrophic factors such as BDNF is reported to be enhanced by physical exercise and is known to play a potential role in preserving neuronal health and memory. Moreover, physical exercise increases the expression level of BDNF by lowering the expression of HDAC.[54] Running exercises have also been shown to upregulate the expression of hippocampal BDNF and synapsin-I, and thereby improving spatial memory. Exercise improves the ratio of acetylation to total protein for histone H3, but it does not affect histone H4. The HDAC5 enzyme, which is involved in regulating the expression of the BDNF gene, is likewise lowered by exercise at both protein and transcript levels.[54] Previous studies show that physical exercise improves memory by inducing dendritic morphological alterations, such as an increase in dendritic arborization and dendritic spine density.[51,55] Thus, regular exercise can help maintain intact memory by enhancing postsynaptic architecture and synaptic protein production, which can boost synaptic transmission and encourage synaptic plasticity.

NUTRACEUTICALS

The epigenome of an individual can be affected by the diet, which can alter gene expression and affect health.[56] Epigenetic control and associated processes, including DNA methylation and one-carbon metabolism, are influenced by nutrition and dietary components, such as vitamin B_{12}, folate, and methionine. To prevent or reverse the detrimental epigenetic alterations associated with aging, several dietary components have been discovered as epigenetic modifiers and used as an effective strategy to avoid memory loss.[57–59] Different fruits and vegetables, such as apples, parsley, berries, onions, broccoli, and celery, contain a varying degree of polyphenols, including phenolic acids, flavonoids like anthocyanins or flavonols, and tannins. Various studies on *in vivo* models suggested that the administration of flavonoid-rich diets, such as ginkgo biloba, blueberries, and green tea, improve memory.[60] The nutraceutical diets are believed to play a critical role in cognitive function due to their significant ability to

scavenge free radicals, as well as their anti-inflammatory and neuroprotective properties,[61] and their notable function as epigenetic modulators. Additionally, dietary substances can block the activity of DNMTs and thereby modify the methylation of related proteins.[62,63] As a result, nutriepigenetic and nutriepigenomic molecules, which affect fundamental human health, have emerged as a potential area of research. For instance, the unique interaction between the environment and the genome is highlighted by polyphenols, especially at physiological doses. The administration of a high dose of polyphenols exhibits a substantial effect under the condition of restricted levels of methyl donors. S-Adenosylmethionine (SAM), the universal methyl donor for DNA and protein methyltransferases is produced in the methionine cycle from a variety of dietary precursors such as betaine, choline, folate, methionine, and vitamins, including B_2, B_6, and B_{12}. Therefore, poor SAM production and widespread hypomethylation of DNA should occur when methyl donors are less readily available, and vice versa.[63] The nutraceuticals are known to influence cerebral blood flow and synaptic plasticity-related changes and enhance the neuronal connection in the hippocampus, that alters memory processing.[60] Fruits and vegetables with various nutraceutical properties and a healthy lifestyle offer an innovative therapeutic strategy for the prevention and treatment of memory-related problems. It was found that curcumin reduces the DNA hypermethylation at CpG sites present at the promoter region of peroxisome proliferator-activated receptor-alpha (PPAR-α). Additionally, ascorbic acid-induced differentiation and hypomethylation have been linked to a reduction in the expression of DNMT.[58] Overall, these findings suggest that ascorbic acid may have the potential to act as a (co-) therapeutic agent for age-associated malfunction in memory as well as for reducing the inflammatory diseases linked to aging through DNA and histone demethylation.[63] The benefits of dietary intervention in modulating the detrimental epigenetic modifications involved in memory loss during aging are now the subject of considerable investigation.

PHARMACOLOGICAL COMPOUNDS

The risk of acquiring a range of age-related complications may rise if the aging epigenome is changed or less sensitive to both preventive and curative epigenetic-modifying pharmacological compounds.[64] Epigenetic modifiers can partially reverse the abnormal aging brought on by epigenetic drift.[65] According to the genetic studies of children with neurodevelopmental problems, epigenetic modifiers that regulate DNA methylation and chromatin remodeling are necessary for brain development.[66] Additionally, the epigenome changes have been linked to various neurodegenerative diseases including Alzheimer's disease.[67] The application of epigenetic modifiers including pharmacological compounds, enzyme inhibitors, nutraceuticals, physical exercise, and modulators of ncRNA alters the epigenetic modifications and thereby enhances memory consolidation [Figure 2].

In light of recent advances in drug discovery and development, a variety of natural and synthetic bioactive compounds with pharmacological significance have been discovered to target epigenetic alterations, in particular, histone modifications and DNA methylation [Tables 1a and 1b]. Donepezil, Verapamil, Agmatine, Suvorexant, Memantine, Galantamine, and Rivastigmine have shown significant pharmacological activities in terms of cognitive improvement, responders' rate, and dropout cases during aging.[68] Donepezil promotes the activation of various genes by increasing the acetylation through decreased expression of HDAC2, and decreasing the histone methylation H3K9me2.[69] Furthermore, the hypermethylation in the CpG island of the promoter region of muscarinic receptor type 3 may reduce the recognition memory in both humans and animals. Consequently, several muscarinic receptor antagonists work by altering the histone modification and DNA methylation status, thereby improving synaptic plasticity and memory.[70,71]

Since DNMTs are primarily known as negative regulators of memory, their inactivation could be targeted to restore memory during aging.[71] The two most used commercial chemicals are 5-Azacytidine (5-Aza) and Zebularine available as DNMT inhibitors and function similarly to cytosine. These commercially available chemicals are DNA-binding agents that bind to DNA during replication and prevent the covalent binding of DNMTs, which results in demethylation and gene reactivation.[63] The administration of pharmacological inhibitors of DNMTs such as 5-Aza and Zebularine reduces the methylation state of reelin and shows the region-specific impact of DNMT inhibition on the promoter of BDNF, which improves the synaptic plasticity. The incubation of rat hippocampus slices with the methylation inhibitor 5-Aza exhibits decreased methylation of the BDNF gene than that of the untreated slices.[72] Administration of DNMT inhibitors 5-Aza, Zebularine, and RG-108 into the anterior cingulated cortical area reduces methylation of calcineurin gene and thereby impacts long-term memory.[72] Zhao *et al*. demonstrated that intrahippocampal infusion of 5-Aza followed by behavioral training enhances the consolidation of recognition memory.[73]

Trichostatin A (TSA) and sodium butyrate (NaB) inhibit different classes of HDACs.[73] In a mouse model of neurodegenerative disease, the non-specific HDAC inhibitor NaB dramatically improves the consolidation of spatial and associative memories.[74] In agreement with the previous findings, intrahippocampal injection of NaB improves

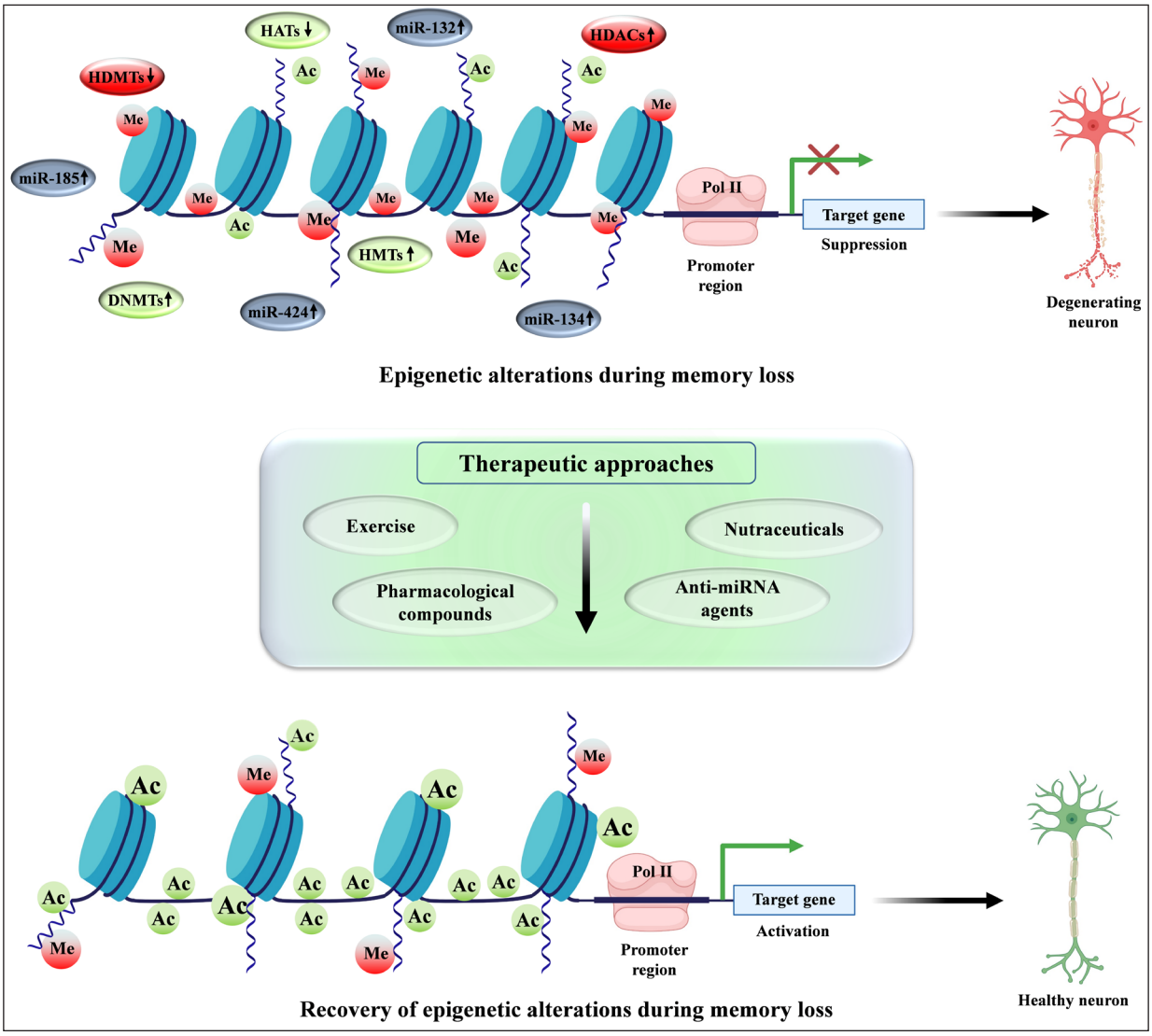

Figure 2: A schematic model illustrating the recovery of memory decline using various therapeutic interventions. Increased methylation at the promoter region causes the suppression of various synaptic plasticity and memory-associated genes which lead to neurodegeneration and impaired memory. However, various therapeutic interventions including physical exercise, nutraceuticals, and pharmacological compounds work as memory enhancers and modulators. These therapeutic interventions lead to neuroprotection and memory restoration by following several biochemical pathways. These epigenetic modifiers cause increased acetylation and demethylation which in turn activates the transcription of various memory-related genes which leads to neuroprotection and thereby enhances synaptic plasticity and memory. These memory enhancers, including epigenetic modifiers, could potentially serve as ideal therapeutic targets in neuroprotection and memory loss conditions. HDMTs: Histone demethylases; DNMTs: DNA methyltransferases; HMTs: Histone methyltransferases; HATs: Histone acetyltransferases; HDACs: Histone deacetylases; Pol II: Polymerase II; miRNA: MicroRNA; Ac: Acetylation; Me: Methylation.

the acetylation level of H4K12 in the promoter region of the synaptic plasticity-related gene and associated fear-conditioned memory. The administration of NaB in the hippocampus of CBP mutant mice exhibits the restoration of object location memory consolidation.[75] Also, post-training treatment of NaB in aging rats leads to increased levels of histone H4K8 acetylation in the promoter region of the synaptic plasticity gene BDNF than that of the control. Additionally, in scopolamine-induced amnesic mice, NaB therapy enhances memory consolidation by increasing the acetylation of total histone H3K9 and H3K14 as well as the promoter region of the synaptic plasticity genes such as BDNF and Arc.[75] To improve memory and learning, suberoylanilide hydroxamic acid (SAHA) is also extensively employed as an HDAC inhibitor. In CBP mutant mice, intraventricular injection of SAHA improves the consolidation of fear

Table 1: Pharmacological compounds responsible for alteration in the neuroepigenome.

(a) Natural pharmacological compounds, their targets and role

Natural pharmacological compounds	Target	Role of the compound
Spermidine	HATs	Significantly affects cognitive function
Garcinol	HATs	Anti-inflammatory and antioxidant properties
Spermine	HATs	Improves memory impairment via BDNF and TrkB activation
Curcumin	HATs	Antioxidant and cognitive enhancer
Epigallocatechin gallate	HDACs	Lowers inflammation, stimulates endogenous antioxidant defense, and has an anti-aging skin impact
Folate	HMTs	Deduces oxidative stress and keeps neurons intact during aging, all of which contribute to improved memory
Vitamin B_{12}	HMTs	Improves neurocognitive problems
Catechins	DNMTs	Inhibits age-related cognitive loss by increasing the expression of IEGs that are engaged in long-term changes in the plasticity of synapses and brain circuits
Caffeic acid	DNMTs	Enhances hippocampal neurogenesis and memory
Resveratrol	DNMTs, HATs, HDACs, SIRT1	Beneficial effect on memory and aging
S-Adenosylmethionine	HMTs	PSEN1 repressor and memory enhancer
Nicotinamide	SIRT	Inhibits gene silencing and promotes replicative aging

(b) Synthetic pharmacological compounds, their targets and role

Synthetic pharmacological compounds	Target	Role of the compound
5-Azacytidine	DNMTs	Restores cognition in sevoflurane-exposed rats with cognitive impairment, object recognition memory in scopolamine-induced amnesic mice, and recognition memory in ovariectomized mice.
ETP69	HMTs	Gives the aging brain procognitive benefits, and this effect is proof that H3K9me3 is involved in memory function. It boosts the quantity of both stubby and thin spines required for the formation of new synapses, which makes them excellent for fostering rapid cognitive advances.
BIX-01294	HMTs	Restores the consolidation of fear memory.
Tranylcypromine	HMTs	Prevents the death of dopaminergic neurons and restores the memory.
Entinostat (MS-275)	HDACs	It enhances social connection and serves as an antipsychotic in a mouse model of social defeat.
Suberoylanilide hydroxamic acid (SAHA)	HDACs	Restores spatial memory in mice with impaired memory caused by sevoflurane as well as fear memory in CBP mutant mice and APP/PS1 AD mice. Improves mice's recollection of their fears. Improvements in social interaction and antidepressant-like effects in a mouse model of social defeat.
Trichostatin-A (TSA)	HDACs	Restores memory for identification in ovariectomized mice, and rescues memory for fear in CBP mutant mice. enhances memory for object identification in a mouse model of neurodegeneration treated with kainic acid, and improves the HD mouse model's long-term memory impairment.
Sodium phenylbutyrate	HDACs	Increases neurotrophin levels in the CNS and thereby enhances synapse function. Memory improvement, Amyloid burden reduction.
Valproic acid	HDACs	Improves working memory difficulties in the hippocampal region and neurogenesis. Mood stabilizer, GABA modulator.
Sodium valproate	HDACs	Reduces the defects in synaptic plasticity, ameliorates hippocampal neurodegeneration, and improves spatial memory.
Vorinostat	HDACs	Memory improvement
Sodium butyrate	HDACs	Restores object localization, spatial, and contextual fear memories in CBP mutant mice, fear memory in APP/PS1 AD mice, and fear memory in scopolamine-induced amnesic mice. promotes long-term memory retention while maintaining short-term memory function, improves fear memory and object recognition memory in elderly mice, and enhances recognition memory in cognitively deficient, maternally deprived rats. enhances both short-term and long-term memory for objects in a mouse model of neurodegenerative disease caused by kainic acid. Improves the behavior of mice with depression.
Donepezil	HDACs, HMTs	Prevents neurodegeneration and memory improvement.
Zebularine	DNMTs	Regulates synaptic plasticity, LTP induction, and learning and memory functions in the hippocampus.

HATs: Histone acetyltransferases; HDACs: Histone deacetylases; DNMTs: DNA methyltransferases; SIRT: Silent Mating-Type Information Regulation; HMTs: Histone methyltransferases; BDNF: Brain-Derived Neurotrophic Factor; TrkB: Tropomyosin receptor kinase B; IEGs: Immediate Early Genes; PSEN1: Presenilin 1; CBP: CREB-binding protein; APP/PS1: Amyloid precursor protein/presenilin-1; HD: Huntington's disease; CNS: Central nervous system; LTP: Long-term potentiation; GABA: Gamma-aminobutyric acid.

memories and raises histone H2B acetylation levels and late-phase LTP. The proteome-based study revealed that HDAC1 and HDAC2 were identified as major targets of the SAHA-based affinity probe, suggesting that they may be pertinent targets for HDAC inhibition-induced memory improvement.[76] Further studies suggested that intraperitoneal administration of SAHA improved the consolidation of fear memories in HDAC2 overexpressing mice.[77] Besides the pharmacological compounds, several ncRNAs are also targeted for the restoration of memory.[67] MicroRNAs have the potential to play a significant role in the processes that underlie memory loss brought on by aging and neurodegeneration. In the *in vivo* epilepsy model, the administration of miR-134 antagonists or miR-22 mimic-miRs was able to diminish neuronal loss and seizure intensity. The efficacy of administering anti-miRNA agents demonstrates an acceptable level of treatment tolerability, suggesting their potential utility for therapeutic interventions, particularly in the context of neurological disorders.[46,78] These findings collectively imply that modifications in the gene-specific targeting of histone modifications may affect memory across the lifetime.

CONCLUSION

Memory acquisition and preservation are intricate processes that must be deliberately regulated for normal day-to-day activity. The ever-improving understanding of the physiology of memory is crucial for the development of novel therapeutic approaches to mitigate memory-related problems. Various epigenetic modifiers and their upstream molecules are responsible for the differential expression of genes and proteins related to synaptic plasticity and memory impairment or consolidation during aging. Moreover, memory impairment is potentially driven by the disruption of chromatin markers that lead to significant drift in neuroepigenome over time during aging. However, the detailed molecular mechanism causing alteration in these epigenetic markers during aging needs to be elucidated. Despite considerable research, efforts aimed at targeting the epigenetic markers for memory restoration during aging and various neurological disorders, several unresolved queries still need to be addressed. (A) How is aging correlated with the accumulation of epigenetic alterations in diverse types of epigenomic signaling involved in memory, and how do these modifications crosstalk? (B) What are the underlying mechanisms involved in the neuroepigenome of a stable or transient model of memory impairment following exercise and administration of the pharmacologic agents? (C) What is the significance of investigating neuroepigenetics to understand memory and its regulation during aging, considering the limitation of transient memory-related biomarkers and the need for more stable and long-lasting biomarkers that can withstand the influence of various epigenetic factors? (D) How do nutriepigenetic and nutriepigenomic molecules modulate epigenetic alterations and improve learning and memory? (E) What is the mechanism by which dietary intervention mitigates the adverse impact of epigenetic modifications linked to memory impairment in the aging process?

Several recovery approaches have been utilized to mitigate the problems associated with memory impairment caused by epigenetic alterations. Moreover, in-depth research is needed for the development of improved detection methods and the discovery of pharmacological drugs, including epigenetic modifiers to gain a complete understanding of the molecular pathways involved in memory. The understanding of epigenetic modifiers that control cognition and cause the reversal of age-related cognitive deficits will be a better therapeutic approach in the future. The implementation of innovative epigenetic-based prevention and therapeutic techniques will be helpful in diminishing memory-related problems. In addition to pharmacological compounds, physical exercise and numerous dietary components that alter neuroepigenome have also been found to delay aging and ward off associated problems. Further research is required to ascertain the ideal dose and time window for a range of dietary components and synthetic compounds, to effectively cure detrimental epigenetic effects against aging to improve the overall quality of life.

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Authors' contributions

EM: Conceptualization, Software, Visualization, Writing–original draft, Writing – review & editing; **MKT:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – final review & editing.

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The authors declare that they have no real or potential conflicts of interest in terms of their personal, intellectual or financial interests.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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Review Article

A narrative review on rebound acid hypersecretion due to long-term use of proton pump inhibitors

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ABSTRACT

Objectives: Proton pump inhibitors (PPIs) are the most commonly used drugs to reduce hyperacidity. The usage of PPIs reduces the secretion of gastric juice; their prolonged usage results in gastric acid suppression with hypergastrinemia while their stoppage results in hypersecretion of gastric juice. This kind of paradoxical reaction is seen in the rebound effect of drugs. Dr. Samuel Hahnemann gave us the vital principles of homeopathy, the law of similitude, i.e., "similia similibus curentur" derived from the "Nature's Law of Cure". This also tells us that the primary action of medicine stimulates the dynamic expression of an organism (vital force), which results in the counteraction called secondary action by the organism.

Material and Methods: Review of literature on the effects of long-term use of PPIs and rebound hypersecretion of gastric juice due to PPIs.

Results: For this review article, 16 most relevant articles are selected from the search results. Thirteen systematic reviews, two randomized control trials, and one pilot study are included. Rebound acid hypersecretion (RAHS) occurs after prolonged treatment with histamine-2 blockers and PPIs, causing gastric hypoacidity and hypergastrinemia. Longer PPI durations can result in prolonged hypersecretion, with moderate hypergastrinemia and increased enterochromaffin-like (ECL) cell hyperplasia. Deprescribing PPIs is crucial to reduce RAHS and safety concerns. Long-term usage can lead to nutritional deficiencies, respiratory infections, and bone fractures.

Conclusion: Homeopathic remedies have shown significant results in treating symptoms caused due to gastritis, ulcers, gastroesophageal reflux disease, etc., and further research is needed to reduce RAHS caused due to the long-term use of PPIs.

INTRODUCTION

"Proton pump inhibitors (PPIs)" are a class of medicines that are most prominently known for their use in acid-related disorders. They include Omeprazole, Esomeprazole, Lansoprazole, Pantoprazole, Rabeprazole, and Dexrabeprazole.[1] H+-K+-ATPase plays a major role in the final step of acid secretion and this led to the development of PPIs. These drugs specifically target this H+/K+ ATPase enzyme in the proton pump. In the management of acid-peptic diseases, PPIs have displaced H2 blockers, as their fundamental pharmacological effect is the dose-dependent inhibition of gastric acid secretion without having any anticholinergic or H2 blocking effects. PPIs completely suppress Hydrochloric acid (HCI) secretion, both at rest and when stimulated by food or any of the secretagogues, in addition to inhibiting the secretion of gastric acid, without much effect on the gastric motility volume of gastric juice and other hormonal factors that influence the gastric acid secretion.[1]

Due to recent lifestyle modifications, most individuals are facing challenges regarding gastric complaints. To combat those symptoms, PPIs are used. Easy availability of this medication over the counter without any prescription and immediate relief of symptoms regardless of the recurrence led to the relentless usage of PPIs. This long-term use and abrupt stoppage of PPIs ultimately results in various side effects and complications in many individuals. The withdrawal of these medications must be attempted for patients who are receiving PPI therapy for uncertain symptoms or in situations where there is a minimal indication for PPI use. Rebound acid hyper-secretion (RAHS) is a condition where gastric acid production abruptly increases after a sudden discontinuation of PPIs. As a result, this could cause or exacerbate upper gastrointestinal (GI) symptoms. It's vital to avoid abrupt PPI withdrawal, especially in chronic users, and to implement a plan to decrease the RAHS phenomenon. Homoeopathic medicines are useful in the prevention and treatment of

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numerous diseases and can help us resolve the withdrawal symptoms of stopping PPIs.

Homoeopathy is a holistic system of medicine with the principle of similitude, i.e., "similia similibus curentur" (Like cures like), which is derived from the "Nature's Law of Cure". A homoeopathic remedy has mainly two effects, i.e., primary action and secondary curative action. The primary action is the initial response produced by the remedy when it is administered. This response is often an aggravation or intensification of the existing symptoms. This aggravation is considered a positive sign in homeopathy, indicating that the remedy has been properly matched to the individual's symptoms. Secondary action is the curative action or the desired therapeutic effect that occurs after the primary action subsides. Following the initial aggravation, the body's self-healing mechanisms are stimulated, and the symptoms gradually improve. The secondary action involves a restoration of the body's balance and resolution of the underlying cause. It's important to understand that the primary action is a transient and temporary exacerbation of symptoms, while the secondary action leads to long-lasting improvement and healing. The primary action is considered a necessary step in the curative process of homeopathy, as it indicates that the remedy is actively interacting with the body's vital force and stimulating the healing response. It's worth noting that not all individuals experience a noticeable primary action with each homeopathic remedy. The intensity and duration of the primary action can vary depending on the susceptibility of the individual and the nature of the complaint being treated. Homeopathy aims to stimulate the body's innate healing abilities, and the primary and secondary actions are integral parts of the healing process within this holistic approach.

Dr. Samuel Hahnemann presents several instances of primary action and the vital force's secondary curative action. The vital force acts instinctively to maintain homeostasis while simultaneously causing potent and antagonistic symptoms to the initial alteration of vitality. Hahnemann's Organon of Medicine, paragraph 65 states that "*Excessive vivacity follows the use of strong coffee (primary action), but sluggishness and drowsiness remain for a long time afterward (reaction, secondary action) if this be not always again removed for a short time by imbibing fresh supplies of coffee (palliative). After the profound stupefied sleep caused by opium (primary action), the following night will be all the more sleepless (reaction, secondary action). After constipation produced by opium (primary action), diarrhea ensues (secondary action), and after purgation with medicines that irritate the bowels, constipation of several days" duration ensues (secondary action). And in like manner, it always happens, after the primary action of a medicine that produces in large doses a great change in the health of a healthy person, that it is the exact opposite when, as has been observed, there is actually such a thing, is produced in the secondary action by our vital force*".[2] In a similar manner, initially, when patients use PPIs to reduce gastric acidity, primary action acidity decreases, but whenever the medicine is stopped in secondary action, it results in an increase in the secretion of acid by the parietal cells. In a similar way, RAHS is the organism's secondary action to the prolonged use of PPIs.

MATERIAL AND METHODS

The literature in books is referred to, and search engines like Google Scholar, ResearchGate, and PubMed databases are used along with the keywords proton pump inhibitors, rebound hypersecretion, randomized control trials, and literature in homoeopathy to select the scientific evidence in the most relevant articles.

RESULTS

Physiology of gastric acid secretion

The stomach is lined with mucous cells, parietal cells, chief cells, and neuro-endocrine cells (G-cells, ECL-like cells, and D-cells). Each of the cells has its specialized function. The mucus cells produce mucus, which functions as a protective barrier from the acidic pH of the gastric juice. The parietal cells secrete the intrinsic factor and HCl. Vitamin B12 absorption in the small intestine depends on the intrinsic factor. HCl helps with the digestion of proteins and kills the bacteria present in the food. Chief cells secrete the pro-enzyme pepsinogen, which is essential for the digestion of proteins after its conversion to pepsin by HCl. G-cells produce a neuroendocrine hormone called gastrin, which increases HCl production both directly and indirectly. The ECL-like cells produce histamine, which indirectly increases HCl production. D-cells secrete somatostatin (SST), an inhibitory hormone that decreases the production of gastric acid by inhibiting the secretion of gastrin.

Gastric acid secretion is a multi-step, complicated process. Acetylcholine (ACh), histamine, and gastrin synergistically stimulate acid release. Vagal stimulation caused due to sight, smell, or the presence of food causes the release of ACh. It stimulates the nicotine receptor (N) and muscarinic receptor (M1) in the enteric nervous system (ENS ganglion cell). The postganglionic ENS neurons release gastrin-releasing peptide (GRP) and ACh. Stretch receptors are stimulated with distension of the stomach due to the presence of food; raising pH activates the chemoreceptors; along with the stimulation by stretch receptors and chemoreceptors, GRP and ACh also act on G-cells, facilitating the release of the hormone "gastrin". ACh also stimulates the muscarinic receptors (M, M3), and gastrin stimulates the cholecystokinin (CCK2) receptors on

the ECL cells and parietal cells. Gastrin and ACh aid in the production of HCl's direct and indirect mechanisms. ACh indirectly inhibits SST secretion, thus promoting gastric acid secretion. The hormone "histamine" is secreted by ECL cells with the stimulation of M and CCK2 receptors which stimulates H2 receptors on parietal cells, thus stimulating the adenylate cyclase (AC), generating cAMP in the parietal cell lumen, aiding indirectly in the secretion of HCl. The direct mechanism involves the direct stimulation of parietal cells. The stimulated M3 increases the intracellular calcium, and the phospholipase C is activated by CCK2 receptors to increase the cytosolic calcium release. Both mechanisms increase H+K+ ATPase activity. The proton pump, H+K+ ATPase, is activated in the lumen of the parietal cell by downstream protein kinases that are triggered by intracellular calcium and cAMP-dependent signaling pathways. H+K+ ATPase catalyzes the electro-neutral exchange of luminal K+ for the cytoplasmic H+. This reaction is coupled with the extrusion of Cl- and K+ ions through an apical chloride channel and apical potassium channel, thus producing HCl. ACh indirectly inhibits the secretion of SST, which is mediated by the inhibition of atrial natriuretic peptide (ANP) secretion from ECL cells. Vasoactive intestinal peptide (VIP)-expressing neurons are activated by distension of the stomach and stimulate SST and Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) neurons, and the release of ANP also stimulates the release of SST and, thus inhibits gastric secretion.[3–8]

Pathophysiology

Peptic ulcer: Ulceration is caused due to any kind of imbalance between the gastric mucosal defending factors and aggressive factors.[1] Pepsin and HCl are the aggressive factors, whereas neuropeptide Y, calcitonin, neurotensin, prostaglandins, interleukin 1, corticotropin-releasing factor, bicarbonates, mucin, and neurotensin are the defending factors.[9] Treatment includes restoring the balance between these aggressive and defending factors. Duodenal ulcers (DU) are caused due to secretary abnormalities. Average basal and nocturnal gastric acid secretion appears to be increased in DU10. Gastric ulcers (GU) are caused due to weakening of the mucosal defense mechanisms. The majority of GUs are caused by either H. pylori or Nonsteroidal antiinflammatory drug (NSAID)-induced mucosal damage.[10] Ulcer formation, GI bleeding, or related perforation is at a 2–4% risk in chronic NSAID users. NSAIDs quadruple the risk of potential complications in ulcer patients.[9] PPIs and H2-receptor antagonists aid in preventing and managing the relapse of peptic ulcers.

Gastroesophageal reflux disease (GERD): It results from the normal anti-reflux barrier failing to protect against persistent and excessive gastroesophageal reflux. It is caused due to esophageal injuries, adenocarcinoma, stricture, and Barrett's esophagus.[11] In healthy people, gastroesophageal reflux episodes happen occasionally. The esophageal peristaltic waves that often follow reflux effectively evacuate the gullet, alkaline saliva neutralizes any remaining acid, and symptoms are avoided. When the esophagus mucosa is exposed to gastroduodenal contents for extended periods of time, then GERD occurs and, in some instances, esophagitis. It is recognized that a number of factors contribute to the onset of GERD.[12] The main causes of GERD are defective anti-reflux barriers like hiatus hernia (sliding type), abnormalities of the lower esophageal sphincter which causes relaxation and reduction of the lower esophageal sphincter's tone, and cardiomyotomy and vagotomy also reduce the efficiency of the lower esophageal sphincter. Drugs like aminophylline, beta-agonists, nitrates, and calcium channel blockers also reduce lower esophageal sphincter (LES)'s tone. Crural diaphragm, increased intra-abdominal pressure, and prolonged/delayed esophageal clearance of refluxed acid in which there is impaired peristalsis, reduced salivation, and body position cause GERD. Insufficient esophageal clearance causes a longer period of acid exposure. Defective gastric emptying increases the amount of stomach content available for reflux. The potential causes are anticholinergic medicines, fatty meals, and obstruction of the gastric sphincter.[11]

Esophagitis: Despite having completely normal appearances, one can recognize a wide range of endoscopic findings. These findings can be limited to mild redness or severe bleeding ulceration with stricture formation. The symptoms and histological and endoscopic findings have a proper correlation between them.

Barrett's esophagus: Barrett's esophagus is a premalignant condition. In this condition, a columnar mucosa replaces the normal squamous lining of the lower esophagus. It may have certain areas of intestinal metaplasia. Among all the patients undergoing gastroscopy for reflux symptoms, 10% of them may have it as an adaptive response to chronic gastroesophageal reflux. It can be associated weakly to smoking but not with alcohol intake. The danger of cancer appears to relate to the brutality and duration of reflux rather than the presence of Barrett's esophagus, and it has been advised that duodenogastro-esophageal reflux of bile, pancreatic enzymes, and pepsin, as well as gastric acid, will be significant in the pathogenesis.

Zollinger–Ellison syndrome (Z-E syndrome): Gastrin may be produced by non-b-cell tumors of the pancreatic islets in quantities high enough to promote the release of stomach acid. Uncontrolled hyperchlorhydria results in serious gastroduodenal ulcerations and other problems. The treatment goal is to reduce the

hypersecretion of gastric acid. PPIs are unquestionably the drug of choice. The controlling of hyperacidity in Z–E syndrome is done more effectively by Omeprazole than by histamine H2-receptor antagonists (H-2 blockers).

DISCUSSION

Generally, the RAHS can be simply represented as the increased acid secretion seen after a definite period of acid suppression. This RAHS has been recorded after treatment with H-2 blockers as well as PPIs. PPI causes gastric hypoacidity associated with hypergastrinemia. Unremitting stimulation of the ECL cells causes hypergastrinemia and hyperhistaminemia without increasing gastric acidity because the proton pump is effectively blocked. Continuous stimulation of the ECL cells causes the proliferation of the ECL cell mass. When the drug is discontinued, the mass effect is noticed to persist longer than the effect of actual PPI. Rebound hypersecretion occurs after 2 weeks from the sudden stoppage of prolonged PPI treatment until the ECL cell mass is restored to normal. Due to a potential impact on the parietal cell mass, lengthier PPI treatment durations are anticipated to result in longer RAHS.[13] These findings are also seen in *Helicobacter pylori*-positive patients with noticeably amplified gastrin levels. If the acid release is intact, *H. pylori* colonizes predominantly in the gastric antrum, causing antral-predominant gastritis, and if acid release is suppressed (by using PPIs), it colonizes in the body of the stomach, causing corpus-predominant gastritis. Inflammation of the antral mucosa causes increased production of gastrin, maintaining the acid production. However, inflammation in the antrum further impairs acid secretion and causes hypergastrinemia.[14] A systematic review done by L. Lundell *et al.* showed that long-term PPI therapy brought about reasonable hypergastrinemia in most patients and also an increased prevalence of ECL cell hyperplasia. It also shows that *H. pylori*-positive patients receiving long-term PPI therapy have a higher risk of corpus atrophy than *H. pylori*-negative patients.[15]

A randomized, double-blind, placebo-controlled trial done by Christina Reimer *et al.* with 120 healthy volunteers was conducted. The first group is given eight weeks of Esomeprazole 40 mg/day followed by four weeks of placebo, and the second group is given 12 weeks of placebo. Weekly symptom evaluations were done using the Gastrointestinal Symptom Rating Scale (GSRS). A score > 2 (equivalent to symptoms causing mild to severe discomfort) for heartburn, regurgitation, and dyspepsia indicates a clinically relevant acid-related symptom. Results showed that there were significantly higher GSRS scores for acid-related symptoms in the PPI group at week 10 (1.4 1.4 vs. 1.2 0.9; P = .023), week 11 (1.4 1.4 vs. 1.2 0.9; P = .009), and week 12 (1.3 +/- 1.2 vs 1.0 +/- 0.3; P = .001). In weeks 9–12, 44% of those randomly assigned to PPI reported one acid-related symptom versus 15% in the placebo group. In the PPI group, 13 of 59 patients (22%) at week 10, 13 of 59 patients (22%) at week 11, and 12 of 58 patients (21%) at week 12 reported having dyspepsia, heartburn, or acid regurgitation. Accordingly, the placebo group's numbers at weeks 10 (P = .034), 11 (P = .013), and 12 (P = .001) were 7%, 5%, and 2%, respectively. This study highlights overlooked PPI withdrawal symptoms and adds credibility to the hypothesis that RAHS has clinical consequences.[16] This study shows that the usage of PPI without a clear indication can cause RAHS symptoms even in healthy human beings. PPI consumption, which has spread like a disease around the world, remains to be exacerbated by excessive and improper prescribing of PPIs in daily practice. Physicians should take great care while prescribing PPIs in their everyday practice. These include a written indication, a treatment duration plan, and a defined review date to reassess the necessity for continuous treatment.[17]

The purpose of the randomized control trial by Andres Vales *et al.* was to evaluate how structured alginate use affected the severity of symptom burden in GERD. Forty-eight participants, who were receiving PPI therapy for about four weeks, were referred for manometry and 24-hour pH/impedance testing. PPIs and H2 receptor antagonists had to be stopped for a week, and antacids and alginates were permitted to be used up until the night before the course of the probe. The treatment group received the same instructions while taking Gaviscon Advance four times per day, while the control group was randomly assigned to follow the standard instructions. Gastro-Esophageal Reflux Disease Health-Related Quality of Life Score change was the main outcome assessed. The findings demonstrated that structured alginate use prevents symptom exacerbation during pre-investigation PPI washout, which is advantageous for thousands of patients each year who are being investigated for gastroesophageal reflux. To evaluate this impact in more detail, additional research is sustained on PPI deprescription.[18]

Deprescribing PPIs is also important to reduce RAHS due to extensive usage of PPIs and their associated safety considerations. As mentioned by Helgadottir H *et al.* in deprescribing studies, various interventions can be used to reduce a patient's PPI dosage by up to 80%, and about 30% of patients on long-term PPI therapy can discontinue long-term PPI therapy.[17] When there is no indication for long-term therapy, PPI deprescribing should be taken into consideration. Evidence points to a patient-centered strategy that calls for decreasing the dose before stopping or switching to PRN (as required) use. A gradual dose tapering before discontinuation can minimize the risk of short-term RAHS, which can be treated on-demand (PRN). Patients should be

involved in the development of the deprescribing plan as well as the discussion of the reasons for deprescribing.[19]

A study done by Fossmark R *et al.* showed that patients with anti-reflux surgery who used PPIs for over a year discontinued acid-suppressing drugs after the operation. Postoperatively, basal and pentagastrin stimulated acid output was measured, and oxyntic mucosal biopsies were collected for histidine decarboxylase (HDC) immunoreactive cells. Results showed higher pentagastrin-stimulated acid secretion at 4 and 8 weeks, reduced gastrin and CgA at 4 and 8 weeks, and a 60% reduction in HDC immunoreactive cells at 26 weeks postoperative.[20]

The systematic review done by Teixeira MZ *et al.* showed evidence that the acid rebound happens 1 hour after using antacids, 2 days after taking H2-receptor antagonists for weeks, and 1–2 weeks after taking PPIs for 4–8 weeks. After 4 weeks of H2-receptor antagonists, the rebound phenomenon lasts for 10 days; after 4 or 8 weeks of PPIs, it lasts for 2 or 4 weeks. RAHS was reported by almost 40% of PPI users. The American Hospital Formulary Service reported that 41% of patients experienced a recurrence of peptic ulcers after ceasing their long-term cimetidine medication after 1–4 weeks.[21, 22] One study published in the journal *Gut* in 2009 found that patients who stopped taking PPIs after 8 weeks of treatment experienced a significant increase in acid production, with symptoms of heartburn and acid regurgitation returning in many patients within 2weeks. However, the study also found that the rebound effect was generally mild and transient, lasting for only a few weeks. Another study published in the *American Journal of Gastroenterology* in 2013 looked at the rebound effect in patients who had been taking PPIs for 6 months or longer. The study found that patients who stopped taking PPIs experienced a significant increase in acid production, with symptoms returning in many patients within a week. However, the study also found that the rebound effect was generally mild and that most patients were able to successfully manage their symptoms with lifestyle modifications.

Due to the prevalent usage of these medications and their large margin of safety, prolonged PPI use has been a major cause for concern. Long-term PPI usage, however, raises questions about potential negative consequences, including an elevated risk of respiratory infections, clostridium difficile infection, and bone fractures. PPIs have negligible renal clearance and a rapid first-pass and systemic hepatic metabolism by hepatic cytochrome P, notably cytochrome P2C19 and cytochrome P3A4. PPIs typically have minor side effects, with headaches, nausea, abdominal pain, constipation, flatulence, and diarrhea being the most frequent ones. However, PPI side effects over the long run have recently received more attention, and numerous studies have examined a range of side effects that could be related to long-term PPI use. Long-term effects include nutritional deficiencies like vitamin B12 deficiency, iron deficiency, and calcium deficiency with a risk of osteoporosis and hypomagnesemia, risk of infections like clostridium difficile infections and other enteric infections, risk of respiratory infections like community-acquired pneumonia, kidney disease, and acute kidney injury. It can also cause hypergastrinemia and malignancy like gastric polyps, gastric cancer, colon cancer, etc.[15,23,24]

HOMOEOPATHY

A review done by Debjit Bhowmik *et al.* on peptic ulcers showed that homeopathic medicines like argentum nitricum, arsenicum album, kali bichromicum, lycopodium, nitric acid nux vomica, phosphorus, pulsatilla, etc., help to eliminate the symptoms caused by *H. pylori* by healing the ulcers.[25] Another review article done on the management of peptic ulcer through homoeopathy by Partha Ratnaparikh and Sonika S. Adkine in Guru Mishri Homoeopathic Medical College PG Institute also showed that homoeopathic medicines are very helpful in treating ulcers.[26] A pilot study done by Mittal R *et al.* on nonerosive gastroesophageal reflux disease (NERD) characterized by reflux-related symptoms without esophageal erosions showed that homoeopathic medicines were used to treat symptoms like heartburn and regurgitation and found that homoeopathic medicines were effective in treating NERD. This study involved 78 patients with heartburn and/or regurgitation symptoms and a GERD symptom score of more than 4. Results showed significant differences in GERD symptom scores and VAS for heartburn, as well as improvements in psychological health, social relationships, and environmental domains. These findings suggest that further studies on reflux disease may be beneficial.[27] A review done on the scope of homeopathic management in gastritis by Akhila Doppalapudi and Shankar Hulekar showed that homoeopathic medicines like abies Canadensis, arsenic album, argentum nitricum, bismuthum, bryonia alba, chamomilla, cantharis ves, carbo veg, ipecac, nux vomica, phosphorus, lycopodium clav, etc., showed significant results in the management of gastritis.[28]

CONCLUSION

RAHS occurs after a period of acid suppression, often seen after treatment with histamine-2 blockers and PPIs. PPIs cause gastric hypoacidity and hypergastrinemia, while continuous stimulation of ECL cells causes hypergastrinemia and hyperhistaminemia without increasing gastric acidity. Rebound hypersecretion occurs after two weeks of prolonged PPI treatment until the ECL cell mass restores to normal. Longer PPI treatment durations are expected to result in

longer RAHS. Long-term PPI therapy can cause moderate hypergastrinemia and increased prevalence of ECL cell hyperplasia, with *H. pylori*-positive patients having a higher risk of corpus atrophy. Deprescribing PPIs is crucial to reduce RAHS and safety concerns. Gradual dose tapering can minimize short-term RAHS risk. Patients should be involved in the deprescribing plan and discuss reasons for deprescribing. RAHS occurs 1–2 weeks after taking PPIs for 4–8 weeks. Long-term usage of PPIs has potentially negative consequences like nutritional deficiencies, elevated risk of respiratory infections, clostridium difficile infection, and bone fractures. Homoeopathic remedies showed significant results in treating the symptoms caused due to hyperacidity, ulcers, GERD, etc. Further research should be done on the homoeopathic treatment of gastric symptoms to reduce the long-term usage of PPIs.

Ethical approval

Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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Review Article

Risk stratification in multiple myeloma – A review and update

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ABSTRACT

Multiple myeloma (MM) is a hematological malignancy of plasma cell origin with a prevalence rate of 1% and 10% of all cancers and hematopoietic malignancies, respectively. Though the median survival time has improved dramatically in the patients diagnosed with MM with the administration of novel therapeutic agents, the disease, by and large, remains incurable with frequent progression and relapses. In the recent past, an increased understanding of MM pathogenesis has opened facets for improved diagnosis, prognosis, and response assessment in patients diagnosed with MM. This review focuses on the various laboratory and clinical features used to stratify the MM patients into high vs. low-risk groups. Furthermore, it also highlights the role of artificial intelligence-based innovative research tools for risk stratification and prognostication in MM patients.

Keywords: Ethnicity-specific staging, Multiple myeloma, Staging, Survival

INTRODUCTION

Multiple myeloma (MM) lies at the malignant end of the spectrum of plasma cell proliferative disorder with variable clinical outcomes. The long-term survival of the patients with MM is variable and improvements have been observed over time with the use of novel therapeutic agents and stem cell transplantation.[1,2] The marked heterogeneity in survival outcomes is attributed to clinical and laboratory parameters, which are used to categorize MM patients into different risk categories. Thus, the evaluation of various prognostic factors is essential to define as well as refine the therapeutic strategies to improve treatment outcomes. In the era of risk-adapted therapy, it is critical to identify high-risk patients to render effective treatment to achieve optimal response and good clinical outcomes. The current review deals with an appraisal of various aspects of risk stratification, including the historical staging systems, i.e. the Durie and Salmon Staging (DSS) and International Staging System (ISS), the current standard of care, i.e. the Revised International Staging System (R-ISS) and the latest artificial intelligence based staging systems like Modified Risk Staging System (MRSS), and Consensus-based Risk Stratification System (CRSS).[3–7] This review also focuses on the new prognostic markers that are gaining relevance in patients treated with novel agents. Besides, the concept of dynamic monitoring and its significance in MM has also been addressed.

INCIDENCE

Multiple myeloma constitutes 1% of all cancers, 10%–15% of all hematological malignancies, and accounts for about 20% of all blood cancer-related deaths.[8–10] Prevalence of MM is less in Asia and is about 1.1 per 1,00,000 individuals in contrast to the West where it accounts for 4.1 per 1,00,000 individuals.[11] Similarly, the prevalence of MM in India is low as compared to Western countries, although it has been documented to be slowly increasing in urban India.[11,12] The reported incidence of multiple myeloma in India is 1 per 1,00,000 individuals.[13] The distribution of various plasma cell dyscrasias reported at our center from 2011-2018 is shown in Table 1. The age-standardized disability-adjusted life years (DALY) rate for multiple myeloma has markedly increased from 5.6 to 63.1 (28.2%) in India from 1990 to 2016.[11]

Multiple myeloma is slightly more prevalent in males as compared to females and the data from 27 population-based cancer registries in India during 2012–2014 showed that MM constitutes 1.19% of all cancers (95% CI: 1.14%–1.24%) with crude rates of 1.27 (95% CI: 1.20–1.35)/1,00,000 in men and 0.95 (95% CI: 0.89–1.02)/1,00,000 in women).[14] The projected

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Table 1: Distribution of various plasma cell dyscrasias in patients evaluated from 2011 to 2018 (n = 1644).

Disease	Number of patients	%
Multiple myeloma	1371	82.7
Amyloidosis	89	5.4
Plasmacytoma	74	4.5
POEMS	56	3.4
MGUS	30	1.8
Smoldering myeloma	13	0.8
PPCL	11	0.7

POEMS, polyneuropathy, organomegaly, endocrinopathy, myeloma protein, and skin changes; MGUS, monoclonal gammopathy of undetermined significance; PPCL, primary plasma cell leukemia.

incidence of patients with MM in India for the year 2020 among males was 10,725 (cumulative risk = 1 in 465), among females was 7,756 (cumulative risk = 1 in 646), and for both sexes combined was 18,481 (cumulative risk = 1 in 541).[15]

PROGNOSTIC FACTORS FOR RISK STRATIFICATION IN MM

The identification of high-risk groups is relevant from a therapeutic and disease monitoring point of view. The risk stratification in MM has evolved over time to incorporate both clinical and laboratory parameters but the heterogeneity in presence and interpretation of the biomarkers is responsible for variable definitions of high-risk disease. The risk factors in MM can be broadly categorized into two , i.e. (1) patient-related factors and (2) disease-related factors.

1. ***Patient-related factors:*** Factors that are considered significant as individual patient-related factors are age, performance status, Frailty score, comorbidities, extramedullary disease, and renal failure.
   a. ***Age:*** MM is rare in young individuals, with a reported frequency of 0.02%–0.3% in patients below 30 years of age.[16,17] The median age of patients at diagnosis is approximately 65 years, with a third of patients over 75 years of age. However, in India, myeloma tends to occur in younger age groups with a median age of 56 years, signifying that MM occurs almost a decade earlier in India compared to the West.[11] The recent AI-based modeling on Indian datasets has shown that MM patients aged 67 or higher have inferior survival outcomes.[6,7]
   b. ***Performance status:*** Performance status is used to assess the daily living abilities of the patient to determine appropriate treatment, disease progression, and overall disease outcome. The various performance status tools considered in determining therapeutic options and clinical outcomes in myeloma include the Eastern Cooperative Oncology Group (ECOG) performance status and the Karnofsky Performance Status (KPS).[18]
   c. ***Frailty score:*** Around a third of MM patients are above 75 years of age but account for 3/4th of myeloma mortality. Elderly patients benefit less from therapy due to drug-related toxicities and poor body physiology. A comprehensive additive scoring system (range 0–5), called Frailty score, combines age, comorbidities, cognitive and physical conditions and, identifies three groups, namely, fit (score = 0, 39%), intermediate fitness (score = 1, 31%), and frail (score ≥2, 30%). The other frailty scoring system included the Katz Activity of Daily Living (ADL) and the Lawton Instrumental Activity of Daily Living (IADL), estimating the physical and cognitive conditions, respectively.[19] The International Myeloma Working Group (IMWG) frailty scale can predict survival and risk of toxicity from treatment in elderly and frail patients with MM.
   d. ***Comorbidities:*** Various comorbid conditions influence the overall outcome of MM patients, and renal disease is one of the most important comorbid conditions.[20] As MM is a disease of the elderly who tend to have higher comorbid conditions, several scoring systems were designed to assess the impact of comorbidities in this disease. The Charlson Comorbidity Index (CCI) and revised Myeloma Comorbidity Index (R-MCI) are the two most widely used scoring systems in MM.[21] The CCI assesses a combination of 19 comorbid conditions and R-MCI considers only five parameters such as renal disease, lung disease, age, frailty score, and KPS. The other myeloma-specific comorbidity indices include ADL, the Mini-Mental State Examination (MMSE), and the quality-of-life 12-Item Short Form Health Survey Physical Composite Scale (SF-12 PCS).[21] The various comorbidity parameters are a more objective and accurate way to assess the functional health status of myeloma patients and thus individualize treatment decisions. Engelhardt *et al.* in their study demonstrated that R-MCI is a reliable tool for risk prediction of overall survival (OS) and progression-free survival differences in a large patient cohort (n = 801).[20] It has been shown that various co-morbid states such as renal, lung and KPS impairment, frailty, and age are significant risks for OS with median OS rates of 10.1, 4.4, and 1.2 years, respectively in fit, intermediate fit, and frail patients as per R-MCI index.[20] In terms of risk prediction, the R-MCI and IMWG frailty scores generated comparable results. The 3-year OS

rates were 90%, 74%, and 43% via the R-MCI for fit, intermediate-fit, and frail patients, respectively (P = 0.0006).[20] Among other groups, the 3-year OS rates for fit and frail patients were 80% and 66% via the ADL (P = 0.0159), 78% and 48% via the MMSE (P = 0.0001), and 86% and 66% via the SF-12 PCS (P = 0.0091), respectively.[21] Furthermore, it was found that R-MCI was proven to be the best predictor of survival in comparison to other comorbidity indices.[20] Thus, the functional assessment of MM-specific comorbidity indices offers a precise evaluation of the prognosis and risk status in MM patients.

e. ***Extramedullary disease:*** Growth of malignant plasma cells beyond the bone marrow compartment is termed extramedullary (EM) myeloma. EM involvement in MM is an aggressive form of disease associated with poor survival outcomes.[22,23] The reported incidence of EM disease was remarkably low before the positron emission tomography (PET) with computed tomography (CT) era, but with the advancement of imaging modalities, it is currently reported in up to 10% of newly diagnosed MM (NDMM) and 20% of patients with relapsed and refractory disease.[23-25] Available data on EM involvement also highlights the site dependency on outcome, with Central Nervous System (CNS) involvement having the worst outcome, followed by soft-tissue plasmacytomas and para-skeletal involvement.[26]

2. ***Disease-related factors:*** These factors can be broadly divided into (1) factors related to the disease load and (2) factors related to disease biology.

   1) Factors related to disease load -- These include

   a. ***Bone marrow plasma cells*** – Quantification of residual malignant plasma cells at the follow-up time point, commonly known as measurable residual disease (MRD) is a well-established post-treatment risk stratification tool in MM and recently incorporated in IMWG response criteria.[27] MRD negative status is associated with survival benefits in all subsets of myeloma patients irrespective of demographic factors or treatment taken, as shown in a meta-analysis.[28] In transplant recipients too, gradual reduction in malignant plasma cells at day 100% post-Autologous stem cell transplant (ASCT) is associated with improved PFS and OS.[29] From a diagnostic time point perspective, it has been demonstrated that elevated malignant plasma cell % at diagnosis is associated with adverse clinical-pathological features, including anemia, elevated LDH levels, ISS3, RISS3, double hit and triple hit myeloma.[30]

   b. ***Serum beta 2 microglobulin*** – Beta 2 microglobulin is ubiquitously present on the surface of all nucleated cells and is elevated in pathological conditions with high cell turnover. Serum beta 2M is a crucial parameter of the international staging system of multiple myeloma and is the global determinant of clinical outcome, predicting not only the prognosis but also the risk of progression from asymptomatic disease.[4,31]

   c. ***Lactate dehydrogenase*** – Lactate dehydrogenase (LDH) is a biomarker of anaerobic glycolysis and thus invariably increases in various neoplasms and serves as an index of malignant transformation. Elevated LDH is associated with aggressive disease and high tumor burden, resulting in inferior progression-free survival (PFS) and overall survival (OS).[32] Due to its crucial role in disease prognostication, LDH has been incorporated into the Revised International Scoring System (R-ISS) for myeloma.[5]

   d. ***Monoclonal-protein (M-protein)*** -- A monoclonal spike on serum protein electrophoresis (SPE) is pathognomonic of MM except for true non-secretory variants. It is also elevated in other plasma cell dyscrasias (monoclonal gammopathy of undetermined significance – MGUS, Smoldering multiple myeloma (SMM)) and higher levels are associated with the risk of transformation to myeloma in the case of MGUS/SMM.[33] The deleterious effect of the M-component is due to the deposition of immunoglobulins in renal tubules as well as the infiltration of various organs. It may cause hyperviscosity syndrome leading to cerebrovascular accidents. Due to consistently elevated levels of M-protein in MM and gradual fall with appropriate therapeutic intervention, M-protein plays a critical role in response evaluation and treatment monitoring in MM.

   e. ***Serum-free light chains*** – Serum-free light chain (SFLC) assay has emerged as an important parameter for the diagnosis, monitoring, and prognosis of MM. MM cases are invariably associated with altered SFLC ratio (SFLCLR) with either kappa or lambda light chain excess.[34] Several studies have demonstrated that SFLC measurement has a greater correspondence to tumor load especially at follow-up time points with small amounts of residual paraprotein.[35] Furthermore, altered SFLCLR is an important risk factor with a higher probability of transformation to MM in patients with MGUS and SMM.[36,37] As per the latest IMWG guidelines, the SFLC assay is particularly useful in oligosecretory/non-secretory variants, provided the SFLCR is

abnormal, and the involved FLC level is ≥100 mg/L.

2) ***Factors related to disease biology*** – The disease biology of MM is influenced by multiple genetic alterations regulating the intrinsic biology of the plasma cells.[38] With sequential acquisitions of genetic aberrations and the process of clonal evolution, patients develop a progression of disease and resistance to therapy.[39,40]. The primary genetic events in MM include IgH translocations and hyperdiploidy, while the secondary genetic events include copy number abnormalities, DNA hypomethylation, and acquired several somatic mutations.[41]

   a. ***Primary genetic events: (i) IgH translocations*** – IgH translocations are considered as myeloma initiating events and are present in nearly half of the patients. They involve five chromosomal loci, 11q13, 6p21, 4p16, 16q23, and 20q11 which contain the *CCND1*, *CCND3*, *FGFR3/NSD2*, *MAF*, and *MAFB* oncogenes, respectively. Translocations t(4::14), t(14::16), and t(14::20) are associated with poor prognosis and are indicative of high-risk (HR) disease. On the other hand, t(11::14), t(6::14), and trisomies are considered to have been associated with standard-risk (SR) disease. ***(ii) Ploidy status*** – Hypodiploidy encompassing pseudodiploid, hypodiploid, and/or near-tetraploid variants carries poor prognosis.[42,43] Hyperdiploidy in MM is seen in 50–60% of patients with frequent trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 2 and is associated with better survival outcomes.[44]

   b. ***Secondary genetic events:*** Secondary cytogenetic abnormalities in MM are acquired over time and include gain(1q), del(1p), del(17p), del (13), *RAS* mutations, and translocations involving *MYC*. The deletion of tumor suppressor gene TP53 at 17p13 is observed in approximately 8–10% of NDMM and is associated with poor prognosis and poor survival.[45] Both deletion at 1p and gain at 1q are associated with poor prognosis in MM, irrespective of the type of therapy given.[46]

   c. ***Gene expression profiling*** - Evaluation of myeloma transcriptome with analysis of messenger ribonucleic acid (mRNA) and small non-coding RNAs has been used to assess its impact on outcome in MM.[47] Gene expression profiling (GEP) is a tool to assess molecular heterogeneity using a much larger series of genes.[48] In this context, the first whole genome sequencing by Chapman *et al.* revealed the presence of non-recurrent somatic alterations in MM.[49] Following this, several NGS studies of the MM genome and exome demonstrated the spectrum of gene mutations associated with tumor progression [50] and have been reported most in *KRAS* (23%–26%), *NRAS* (20%–24%), and *BRAF* (4%–6%) genes that play a key role in MAPK pathway, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB) signaling and *FGFR3, TRAF3*, and *TP53*.[51] Thus, cytogenetic profiling in MM, which reflects the disease biology predicting survival, is of critical importance in risk stratification and overall prognosis. Figure 1 shows the cytogenetic risk stratification of MM.

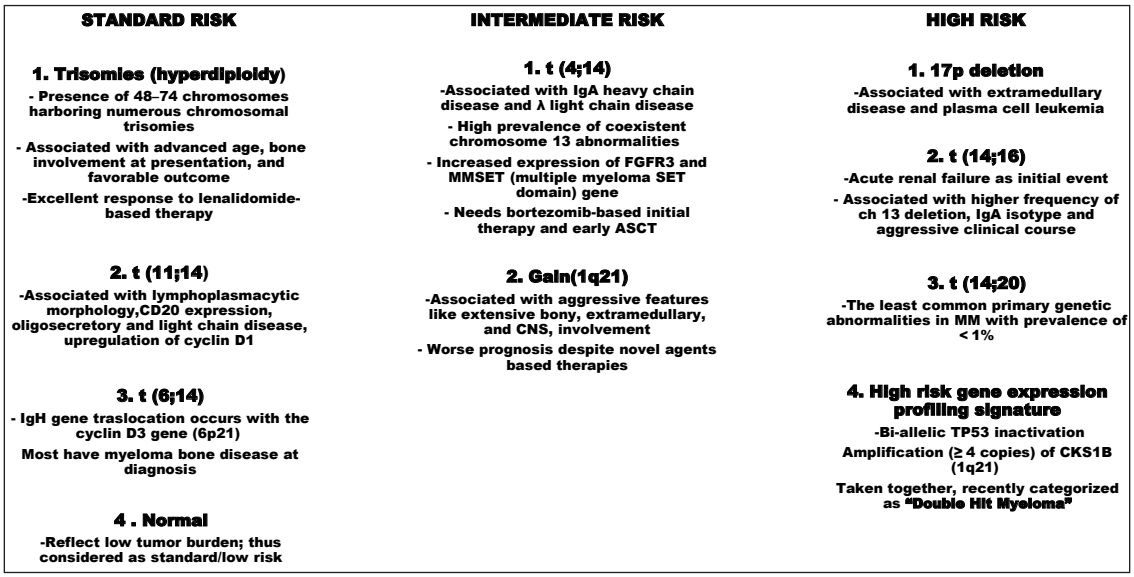

Figure 1: Cytogenetics-based risk stratification in multiple myeloma. ASCT - Autologous stem cell transplant; CNS - Central nervous system; MM - Multiple myeloma.

Over the last decade, novel tools have been used to assess molecular heterogeneity of the genes involved in MM, which include techniques like real-time reverse transcriptase polymerase chain reaction (RT-PCR), array comparative genomic hybridization, single nucleotide polymorphisms, and next-generation sequencing (NGS). Shaughnessy *et al.* investigated the gene expression profile (GEP) of myeloma cells in NDMM and found a 70-gene signature linked to shorter durations of complete remission, event-free survival, and overall survival.[52] Furthermore, in multivariate discriminant analysis, a 17-gene subset was identified that performed as well as the 70-gene model. Another large study published by Decaux *et al.* from the IFM group revealed 15-gene subsets involved in cell cycle progression and its surveillance and were identified to calculate a risk score that correlated with survival based on high-risk and low-risk groups.[53] Several large clinical trials have shown that a high-risk GEP signature is associated with inferior treatment outcomes.[54] Recently, a high-risk, "Double-Hit" group of NDMM was identified by genomic analysis using either (a) bi-allelic TP53 inactivation or (b) amplification (≥4 copies) of CKS1B (1q21) on the background of International Staging System III.[55] Double-Hit MM was associated with dismal outcome with median PFS = 15.4 months; OS = 20.7 months. Though GEP is acclaiming an era of personalized medicine including risk-adapted therapy in MM, a great amount of prospective work is needed in this field.

STAGING SYSTEMS

Based on various clinical, cytogenetics, and molecular markers defined above, several staging systems have been attempted since the mid-1970s to risk-stratify the patients diagnosed with MM.

1. ***The DS staging system:*** The first clinically recognized staging system for MM was introduced by Durie and Salmon in 1975, known as the Durie/Salmon (DS) system.[3] The DS system was based on various clinical parameters that predicted myeloma cell tumor burden and included hemoglobin, serum calcium level, the type and quantity of monoclonal protein, and bone lesions. Creatinine level was further used to stratify the patients into good vs. poor risk groups [Table 2]. The major limitation of the DS staging system was the number of lytic lesions on routine radiographs, which were found to be observer-dependent and subject to variations. Another limitation of the DS system was that it was based on the utility of clinical parameters, which was considered a means to measure tumor mass rather than tumor biology-related factors like adverse cytogenetics. As a result, the DS staging system was found to be supplementing myeloma-associated diagnostic Calcium elevation; Renal insufficiency; Anemia; Bone abnormalities (CRAB) criteria viz hypercalcemia, renal dysfunction, anemia, and bone disease rather than truly predicting outcomes. Additionally, in the setting of novel therapeutic agents, the DS system was less predictive of treatment outcomes as novel agents more effectively reduced the myelomatous tumor burden.[56] Thus, to ensure a precise and reproducible staging of newly diagnosed MM patients, several staging systems were proposed in the subsequent years;[57,58] however, the DS staging system remained the most widely used staging system for NDMM for almost 25 years.
2. ***The ISS risk stratification:*** In 2005 Greipp *et al.* proposed an international staging system (ISS) based on two simple laboratory parameters of serum albumin and beta2-microglobulin.[4] After the inception of the DS staging system, serum beta 2-microglobulin (Sβ2M) emerged as the single most reliable and powerful predictor of survival across various studies.[59,60] In addition to β2M, several clinical biomarkers have been introduced as predictors of survival, Greipp *et al.*[61-63] analyzed Sβ2M, serum albumin, platelet count, serum creatinine, and age by univariate and multivariate techniques, and found a combination of Sβ2M and serum albumin as the simplest and reproducible three-stage classification parameters [Table 2]. However, with the advent of cytogenetic abnormalities detected by iFISH as described above, the prognostication of MM patients was further refined and was later incorporated into the Revised International Staging System (RISS) as described below.
3. ***The RISS risk stratification:*** The fluorescent in-situ hybridization (FISH) cytogenetic revealed t (14; 16), t (14; 20), loss of p53 gene locus (del(17p), and monosomy 17 as a poor prognostic group across various studies[64,65]. Additionally, serum lactate dehydrogenase (LDH) level is found to be another key determinant of outcome in MM independent of conventional vs. novel therapeutic agents.[66] LDH level above the normal reference range reflects a higher proliferation rate of the tumor mass associated with an aggressive course of the disease including extramedullary and extraosseous involvement.[67] Thus, in 2015, Palumbo *et al.* combined ISS with cytogenetic features and serum LDH level and evaluated their prognostic relevance in NDMM, which they designated as the Revised International Staging System [Table 2].[5] In this context, Gupta *et al.* also demonstrated nucleic acid-based tests (multiplex ligation-based probe amplification – MLPA and quantitative real-time polymerase chain reaction – qRT-PCR) as a resource as well as a cost-effective method to determine cytogenetic abnormalities as per R-ISS.[68] Though the R-ISS staging system is a new risk stratification algorithm with an improved prognostic power compared with the ISS, the major limitation of RISS is the exclusion of host-related factors such as age, performance status,

Table 2: Staging systems for multiple myeloma.

Staging system	**DS-STAGING[3]**			**ISS[4]**			**RISS[5]**			**MRSS[6]**			**CRSS[7]**		
Parameters	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**1**	**2**	**3**	**1**	**2**	**3**
Age (in Years)										<67	***Fitting neither 1 nor 3***	>67	<67	***Fitting neither 1 nor 3***	>67
Hb (g/dL)	> 10	***Fitting neither I nor III***	< 8							>12.3		≤12.3	>12.3		≤12.3
Calcium (mg/dL)	<10.5		> 12							≤11.3		>11.3	<11		≥11
Albumin (g/dL)				≥3.5	< 3.5	-	≥3.5	***Fitting Neither I nor III***	-	>3.6		<3.6	>3.5		≤3.5
Beta 2 microglobulin (mg/L)				<3.5	3.5–5.5	>5.5	<3.5		≥5.5	≤4.85		>4.85	<4.78		≥4.78
LDH							Normal		High				Normal		High
M Protein (g/dL)	• IgG < 5; IgA < 3	***Fitting neither I nor III***	• IgG > 7; IgA > 5												
24-h Urine Protein (g/24h)	M-protein < 4		M-protein > 12												
Imaging	Normal bone structure on X-ray (scale 0), or solitary bone plasmactyoma		Advanced lytic bone lesions (scale 3)												
Cytogenetic and molecular abnormalities							*Standard risk	*Fitting neither 1 nor 3*	*High risk				*Standard risk	*Fitting neither 1 nor 3*	*High risk
Serum Creatinine (g/dL)/eGFR	**A:** serum creatinine < 2.0 **B:** serum creatinine ≥ 2.0									>48.1	*Fitting neither 1 nor 3*	≤48.1	>48.2	*Fitting neither 1 nor 3*	≤48.2

DS, Durie Salmon; ISS, International staging system; RISS, Revised International Staging System; MRSS, Modified Risk Staging System; CRSS, Consensus-based Risk Staging System; LDH, Lactate dehydrogenase; eGFR, estimated glomerular filteration rate (*For standard and high-risk cytogenetic abnormalities, refer to Figure 1).

and comorbidities, which play an important role in defining patient prognosis. Another pitfall in RISS is the lack of interlaboratory standardization of FISH analysis and heterogeneous cutoff levels for LDH across the laboratories. Thus, in clinical practice, a better definition of MM risk groups is essential to provide more effective personalized therapies.

4. ***Modified risk staging system (MRSS):*** The novel therapeutic agents in survival data of RISS comprised predominantly of immunomodulatory drugs, however, with the advent of proteasome inhibitors, it is observed that few adverse cytogenetics like t (4;14) lost their poor prognostic relevance.[69] Thus, from both clinical and research points of view, it is essential to characterize patients with poor clinical outcomes to develop effective therapies. In this context, Farswan *et al.* recently developed a machine learning-derived MRSS utilizing six clinical parameters, i.e., age, albumin, β2-microglobulin (β2M), calcium, estimated glomerular filtration rate (eGFR), and hemoglobin [Table 2].[6] This artificial intelligence (AI)-based staging system performed better than ISS and RISS as a predictor of long-term survival on the MM Indian (MMIn) data set. Additionally, for the Multiple Myeloma Research Foundation (MMRF) repository, MRSS showed a superior survival outcome than RISS but a comparable survival rate with ISS.[6] Thus, it is interesting to see the utility of this recently devised prognostication tool as a long-term predictor of survival across multiple prospective studies.
5. ***Consensus-based Risk Stratification System (CRSS):*** Another artificial intelligence (AI)-based staging system was also developed by the same group last year taking into consideration the ethnicity, designated as the Consensus-based Risk Stratification System (CRSS).[7] Recently the role of ethnicity in the differential laboratory parameters was identified in the two independent cohorts of MMIn and MMRF patients with NDMM belonging to two separate ethnic groups. Furthermore, an AI-enabled risk stratification system was proposed incorporating the ethnicity-specific cut-offs of the laboratory parameters viz. albumin, Sβ2M, calcium, estimated glomerular filtration rate (eGFR), hemoglobin, and age along with high-risk cytogenetics. The rationale behind the development of such a staging system was to find an ideal risk staging system based on all the known adverse prognostic factors, including clinical, ethnic, and molecular aberrations. High concordance-index and hazard ratios revealed the superior performance of CRSS as compared to R-ISS. Thus, it was demonstrated that risk-stratification achieved by AI-assisted CRSS can better separate the patients into different risk groups as compared to R-ISS [Table 2].

DYNAMIC RISK STRATIFICATION

All staging systems described above are directed at risk stratification at the time of diagnosis, however, MM is a continuously evolving disease with changes in disease biology during the course of disease, responsible for possible relapse. There has been documentation of changes in risk factors at relapse.[70] The evolving consensus is that, if a patient acquires high-risk features at relapse or progression, then that patient should be reclassified as having a high-risk disease. Therapy-related poor-risk features include progression while on therapy and short duration of response. Thus, in patients with relapsed disease, additional risk stratification criteria include the type of response and length of response to prior therapy. In this regard, Mayo Stratification for Myeloma and Risk-Adapted Therapy (mSMART) is a consensus opinion that considers genetically determined risk status and the various treatment strategies currently available.[71] In 2007, Mayo Clinic introduced myeloma risk stratification or risk-adapted therapy as an evidence-based system that is constantly evolving and updated.

Response to therapy is one of the significant measures of dynamic risk assessment. Response to the first therapy has been shown to be associated with PFS and OS. We have already demonstrated that response after the first induction therapy was a predictor of PFS as well as OS.[68] In post-transplant patients too, after ASCT, depth of response achieved is the most significant parameter of survival outcomes.[29]

The clinical parameter that is overlooked in most of the staging systems except DS staging is the utility of imaging as a prognostic tool. The number of bony lesions present in the DS system has already been considered prognostically insignificant due to low sensitivity as at least 30% destruction is required to visualize an intramedullary bone disease and its inability to detect extra-osseous lesions. There is a paucity of literature recommending the role of imaging studies in MM risk stratification. A study by Walker *et al.* showed that achieving magnetic resonance imaging (MRI) directed complete remission has a better prognosis than those with the presence of lesions.[72] In another study, the prognostic value of Fluoro-deoxyglucose positron emission tomography (FDG-PET) and MRI was compared and FDG-PET at diagnosis was the only imaging modality significantly associated with inferior survival outcome.[73] However, such studies depicting the role of imaging are limited, and thus a larger number of prospective studies are needed to demonstrate the utility of imaging as a prognostic parameter.

Measurable residual disease (MRD) assessment by flow cytometry or molecular techniques and its correlation with clinical outcomes in MM has been explored a lot in the last two decades.[74] Another technique (complementary to molecular or flow cytometric assessment) to evaluate MRD

is the use of imaging which can detect residual metabolically active focal lesions. In a recent study, MRD negativity is found to be associated with longer progression-free survival and overall survival and thus emerged as one of the strongest predictors of survival.[30] The recent myeloma MRD data are supportive of MRD becoming a regulatory endpoint for drug approval in NDMM, based on which the IMWG recently revised the response criteria for myeloma and included MRD negativity as the highest degree of response to treatment.[75] This emphasizes the fact that MRD is one of the single most important predictors of treatment outcome and thus should be incorporated in the MM risk stratification. Furthermore, MRD assessment also offers the advantage of dynamic risk stratification as it can be evaluated at any time during the disease, and based on MRD status, we can re-stratify the MM patients.

Risk stratification in resource constraint setting: Though a robust risk stratification system is mandatory for all myeloma patients for a timely institution of appropriate and adequate therapy, molecular studies and genomic profiling are available for a limited number of patients in a resource constraint setting. In this scenario, the use of various clinical and patient-specific factors becomes critical to annotate the patient as a good risk or vice versa. The MRSS system developed by Farswan *et al.* utilized six routinely evaluated parameters in MM, i.e., age, albumin, β2-microglobulin (β2M), calcium, estimated glomerular filtration rate (eGFR), and hemoglobin, to build a robust AI-based algorithm for survival predictions in MM.[6] The MRSS which does not require genomic features for risk stratification was demonstrated to perform equivalent to R-ISS. Besides, determinants of MM disease burden viz LDH, high proliferation rates, extramedullary disease, hypercalcemia, high serum-free light chain ratio, plasmablastic disease, and plasma cell leukemia may also be used as predictors of outcome in a resource constraint setting. Thus, at the global level, it is imperative to strategize staging and risk prediction models for resource-constraint as well as resource-rich settings to ensure their implementation in real-life settings.

CONCLUSION

Though a number of individual risk factors have been identified so far, the general agreement is to use a system that incorporates multiple factors. However, none of the staging systems described so far is adequate to risk-stratify the MM patients in all scenarios. Thus, a comprehensive and robust risk stratification is needed in patients diagnosed with MM which takes into consideration not only laboratory-based parameters and cytogenetic abnormalities but also host-related factors like age, ethnicity, and co-morbidities. In this regard, AI-based staging systems like MRSS and CRSS are simple, reliable, and reproducible methods highlighting the role of machine learning models as an efficient prognostication tool that may be useful in planning better therapeutic strategies in MM patients. Furthermore, genomic features as well as measurable residual disease (MRD) though, emerged as the strong independent predictor of survival, have not been included in any of the risk stratification systems so far. Thus, the additional risk factors that are worthwhile to explore include the role of imaging, novel genomic signatures, including subclonal evolution during the course of therapy, and MRD evaluation for further refining of MM risk groups.

Ethical approval

Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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Review Article

A review of the adverse impacts of allergic rhinitis on health-related quality of life and its evaluation

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ABSTRACT

It has been proven that successfully treating allergic rhinitis (AR) with non-sedating antihistamines, intranasal corticosteroids, and other widely used, approved treatments results in improvements in health-related quality of-life questionnaires by incorporating health-related quality of life measures in clinical trials. Analyze therapeutic modalities that provide reliable and practical solutions to minimize the effects of this illness. The effects of AR on people's health and quality of life should be examined by doctors. Better patient-provider communication and a deeper comprehension of AR may help with the treatment of this illness.

Keywords: Allergic rhinitis, Burden of illness, Impact on life, Quality of life

INTRODUCTION

Respiratory allergies are a common chronic issue that affects children's breathing in the United States. Today, respiratory allergies are fairly frequent, yet they were not even recognized a century ago. At that time, the term "allergy" did not exist. Only one in seven Americans in 1950 were allergic to their respiratory system.[1] In 1871, a Scottish physician by the name of C.H. Blackley made the initial discovery that plant pollen-induced hay fever; nevertheless, he is not well known in the medical field.[2] Grant L. Selfridge, another physician, practiced homeopathy, or the use of natural treatments for illness. He was one of the American Academy of Allergy's founders. In the west of the United States, he was the first to examine flora and pollen.[3] According to homeopaths, using a substance that produces the same symptoms as the sickness is the best approach to treat it. Although some modern allergy medications function in a similar way, mainstream medicine does not give homeopathy enough credit.[4] Allergic rhinitis (AR) has become more common in the United States and across the world.[5] The Allergies in America, Allergies in Latin America, and Allergies in Asia-Pacific surveys, as well as the Pediatric Allergies in American questionnaires, were developed by physician experts in the field of AR in collaboration with Abt Schulman, Ronca, and Bucuvalas, a national public opinion research organization. The survey in the United States was performed entirely by random digit dialing, whereas the survey outside the United States was conducted through either a telephone interview or an in-person interview.[6] Over the last three to four decades, high-income nations have experienced an allergy epidemic, with roughly one in every four children diagnosed with AR. The Indian subcontinent is additionally experiencing a rise in AR and asthma in the last 2 decades. India has one of the greatest concentrations of air pollution in the world due to biomass, fossil fuels, and automotive exhausts, and the usage of mosquito coils, incense, and dhoop sticks is a major source of interior pollution.[7]

Allergic rhinitis

Allergic rhinitis is a problem that makes the nose react to things in the air that some people are allergic to. It can affect many people around the world. It happens when some cells in the nose get inflamed because of the immune system. AR is caused by a mix of genes and environment.[8,9] Some of the signs of AR are runny nose, stuffy nose, sneezing, and itching. AR can also cause other issues, such as asthma, sinus infections,

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and eye allergies. It can make people's lives more difficult by interfering with their social lives, learning, employment, and sleep.[9,10] AR is classified into two types: seasonal and permanent. Plants emit pollen at specific periods of the year, resulting in seasonal AR. Persistent AR occurs when people are allergic to objects in the environment, such as animals, dust, mold, or chemicals.[11]

Epidemiology

Prevalence

To accurately quantify the prevalence of AR, it is necessary to differentiate it from other types of rhinitis caused by different factors, such as infections or non-allergic triggers. Between 1990 and 2010, the European Community Health Survey (ECRHS) and the International Study of Asthma and Allergy in Childhood (ISAAC) conducted two large-scale international surveys that investigated the prevalence of allergic diseases among adults and children in various parts of the world. According to the Phase III International Study of Asthma and Allergies in Childhood (ISAAC), the prevalence of AR ranged from 0.8% to 14.9% in children aged 6–7 years and from 1.4% to 39.7% in children aged 13–14 years.[12] According to one Indian study, the incidence of AR was 11.3% in children aged 6–7 years and 24.4% in children aged 13–14 years. In contrast to the high incidence of rhinitis, asthma, and eczema found in the ISAAC research, food allergy was shown to be low at 0.14% among Indian children aged 6–11 years.[13] In India, common aeroallergens associated with AR and asthma include home dust mites, cockroaches, pollen, and mold spores. In India, there are two pollen seasons: tree pollen (February–April) and grass pollen (September–December).[14] Almost 77% of the Indian population is exposed to PM25 levels that exceed the 40 g/m^3 limit established by the National Air Quality Standards in India (the WHO standard is 10 g/m^3).[15] According to the third wave of the US National Health and Nutrition Examination Survey, which was conducted from 1988 to 1994, nasal symptoms were most common (30%) among people aged 17 to 29, and least common (10%) among people over 60. Since the 1960s, the global trends of atopy (the predisposition to develop allergies due to IgE antibodies produced by environmental or hereditary factors) and AR have shown a considerable increase.[16,17] While the global prevalence of allergy illnesses is claimed to be 20%–30%, statistics for the general population and working people in Hong Kong, Malaysia, the Philippines, Singapore, Thailand, and Vietnam (together referred to as Asia) are sparse. According to the World Allergy Association, the total prevalence rate of AR, the most prevalent allergy illness in Asia Pacific (APAC), is 10%–30%, which is similar to the global average.[18] This condition affects a vast number of people in Asia, ranging from 27% in South Korea to 32% in the United Arab Emirates.[19]

Pathophysiology

How rhinitis happens

Rhinitis is a problem that makes the nose inflamed and irritated. It can be caused by different things, such as allergies or other factors, or a combination of both. Depending on what causes rhinitis, the nose can have different symptoms. Each type of rhinitis has its own way of making the nose react. Allergic rhinitis only happens to people who have a genetic tendency to be allergic to some things.[20] When these people are exposed to things they are allergic to, such as dust or pollen, their immune system makes special cells called B cells and plasma cells. These cells produce specific IgE antibodies that can recognize the allergens. IgE antibodies attach to some receptors on other cells called basophils and mast cells. When the allergens come in contact with the IgE antibodies, they trigger the basophils and mast cells to release or make chemical substances that cause allergy symptoms.[8,12,20,21]

Early-phase reaction

The early-phase reaction is what happens when the mast cells break down and release a lot of substances after the IgE antibodies that are specific to an allergen, are made and linked together on the surface of the mast cells. One of these substances is histamine, which is the main cause of AR. Histamine activates some nerve endings in a nerve called the trigeminal nerve, which is part of the fifth cranial nerve. This makes the person sneeze a lot. Also, histamine and other substances make the blood vessels in the nose expand and leak fluid, which causes nasal congestion and runny nose.[22]

Late-phase reaction

The late-phase reaction is another thing that can happen after the person is exposed to the allergen. It usually starts 4–6 h later and can last for a whole day. It often comes after the early-phase reaction, but it can also happen by itself. The late-phase reaction makes the person have more symptoms, such as a long-lasting runny nose, sneezing, and stuffy nose. The late-phase reaction is mainly caused by inflammation, which is when the body tries to fight off the allergen. Inflammation brings more cells to the nose, such as basophils, eosinophils, and T lymphocytes. These are cells that are part of the immune system and help with allergies. Mast cells also make and release more substances, such as IL-4 and IL-13, which are types of cytokines and chemokines. These are molecules that help communicate between cells and coordinate the late-phase reaction [Figure 1 and Table 1].[23,24]

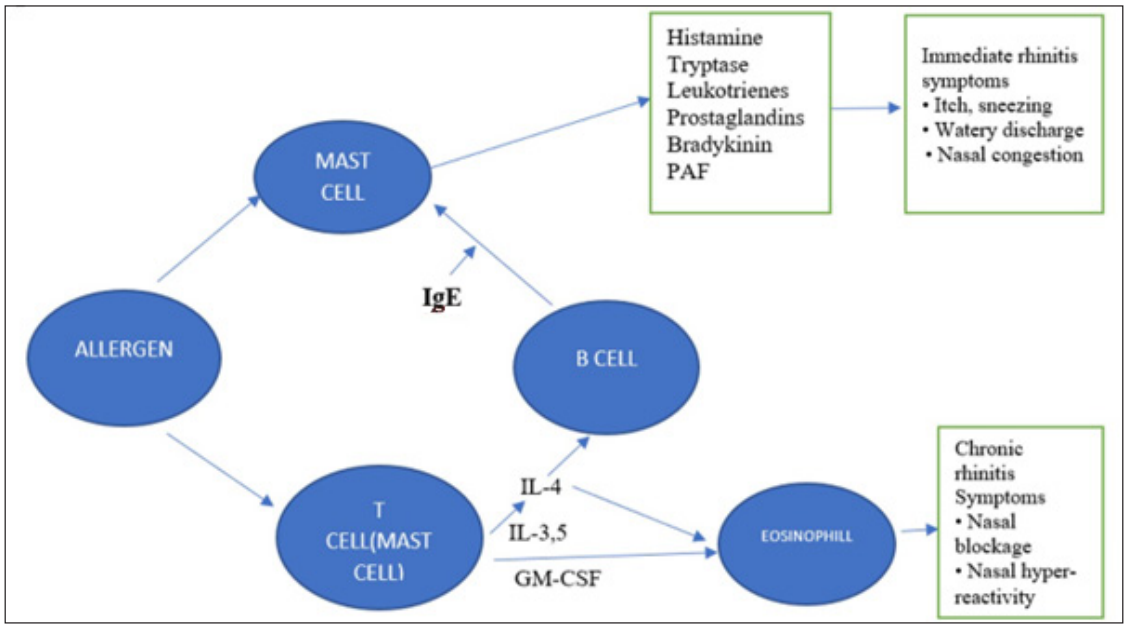

Figure 1: Mechanism of AR. PAF: Platelet Activating Factor, IgE: Immunoglobulin E, GM-CSF: Granulocytic-macrophage colony-stimulating factor, IL: Interleukins.

Table 1: Allergic triggers for rhinitis.

Trigger types	Origin/specific example of trigger	Type of rhinitis caused
Mites	House Dust Mite, Storage Mites, Allergen in Mite Fecal Pellets	Major Causes of Perennial Rhinitis
Pollens	Trees, Grasses, Shrubs, Weeds	Main Causes of Seasonal Rhinitis; Cross-Reactivity Among Pollens
Animals	Cats, Dogs, Horses, Mice Rats and Saliva	• Allergen In Sebaceous Glands • Allergen Mainly in Urine
Fungi	Altemaria, Cladosporium, Aspergillus	Seasonal And/or Perennial Symptoms • Reversible With Early Diagnosis and Avoidance but Becomes Chronic and Irreversible If Exposure Is Prolonged.
Occupational Induced	Flour, Latex, Laboratory Animals, Wood Dust, Enzymes, Other Airborne Proteins	• May Progress to Asthma.Diagnosis Based on Symptom Diary Cards and Provocation Tests.
Occupation Aggravated	Smoke, Cold Air, Formaldehyde, Sulphur Dioxide, Ammonia, Glues, Solvents, etc.	Pre-Existing Rhinitis Can Be Aggravated by Work-Place Irritants

Impact of allergic rhinitis on health-related quality of life

When a person has AR, it can affect their quality of life. This means how they feel about their health and happiness in different areas of their life.[25] Quality of life is not just about physical health but also about mental and emotional health and how the person interacts with others. The World Health Organization defines quality of life as "the way people perceive their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns". Both good and bad things that happen to a person's health might have an impact on their quality of life.[26,27] A person's quality of life may vary if they have an illness like AR. The term "health-related quality of life" (HRQOL) refers to this. It refers to how the patient believes their illness and its treatment impact their daily lives, well-being, and physical, mental, and social well-being. According to Settipane, AR might worsen a person's HRQOL if it is not adequately treated. Not getting enough sleep, feeling exhausted during the day, struggling to study, having difficulties thinking properly, and performing poorly at work or school are some of the symptoms that can cause this.[28] In a study of 3052 patients, the intensity and duration of AR were analyzed, and it was determined that 11%, 8%, 35%, and 46% of the patients, respectively, had intermittent mild forms, persistent mild forms, intermittent moderate-to-severe forms, and persistent moderate-to-severe forms.

Table 2: Definition of health concepts with the SF-36 questionnaire.		
Standard	**Count of things**	**Explanation**
Functional Status		
1. Physical Functioning	10	Extent To Which Health Interferes with A Variety of Activities (E.G., Sports, Carrying Groceries, Climbing of Stairs, and Walking)
2. Social Functioning	2	Extent To Which Health Interferes with Normal Social Activities (E.G., Visiting with Friends During Past Month)
3. Role Limitations Attributed To Physical Problems	4	Extent To Which Health Interferes with Usual Daily Activities (E.G., Accomplished Less Than Would Like)
4. Role Limitations Attributed To Emotional Problems	3	Extent To Which Health Interferes with Usual Daily Social Activities (E.G., Accomplished Less Than Would Like)
Wellbeing		
5. Mental Health	5	General Mood or Affect, Including Depression, Anxiety, and Psychologic Well-Being During the Past Month
6. Energy/Fatigue	4	Tiredness, Energy Level
7. Pain	2	Extent Of Bodily Pain in Past 4 Weeks
Overall evaluation of Health		
8. General Perception of Health	5	Overall Rating of Current Health in General
9. Change in Health	1	Evolution Of General Protection of Health

More than 80% of patients with more severe forms reported impairment in their activities as a result of the condition, compared to just 40% of those with moderate forms.[19]

Measurements of health-related quality of life

Utilizing questionnaires that probe the respondent on several facets of their health and well-being is one technique to gauge quality of life.[29] These questions can assist in determining how a person's social environment and way of life influence their daily activities. The questionnaires may include questions regarding the respondent's performance at work or school, their level of productivity, their self-esteem, and their health. When a person has rhinitis and receives therapy for it, some of these things may change. A variety of questionnaires are available for measuring HRQL. They range from being general to being specialized.[30,31] For various illnesses and health issues, general questionnaires might be employed. They can assist in comparing how various illnesses impact a person's quality of life. They can also assist in identifying the primary health problems in a community and providing solutions.[30] The Short-Form Health Survey (SF-36) is one of the most well-liked and trustworthy general questionnaires. It was created by a team of scientists known as the Medical Outcomes Study. A total of 36 questions are included, and they cover three crucial aspects of health: how the individual feels, how they function, and how they handle their medical issue. Numerous disorders, including AR, have been studied using it [Table 2].[26,30,31]

Specific questionnaires are created to assess a specific patient population (such as children), a specific function, or a specific condition, and they may be more sensitive because they include questions that are only applicable to certain diseases [Table 3].[32]

Burden of physical symptoms

In 2006, a big research project called Allergies in America was completed. It was the first time that a large group of people with nasal allergy symptoms were studied in detail. It was also the

Table 3: Health-related quality of life questionnaires used for AR.		
Instrument	**Type**	**Patient**
SF-36 Health Survey	Generic	Adults
Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ)	Disease-specific	Adults
Rhinitis Quality of Life Questionnaire	Disease-specific	Adults
Adolescent Rhinoconjunctivitis Quality of Life Questionnaire (ARQLQ)	Disease-specific	12-17 Y
Paediatric Rhinoconjunctivitis Quality of Life Questionnaire (PRQLQ)	Disease-specific	12-17 Y
Rhino sinusitis Disability Index	Disease-specific	Adults
PAR-ENT Quality of Life Questionnaire	Disease-specific	Adults

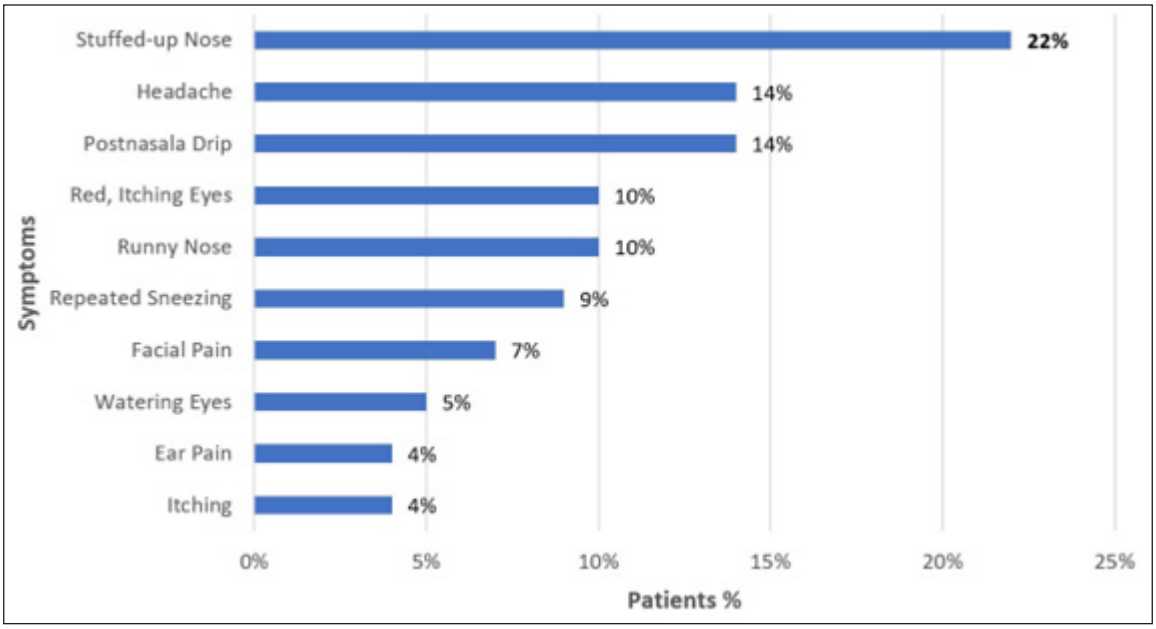

Figure 2: The most bothersome symptoms of nasal allergies.

biggest study of its kind ever done.[33] The results of the Allergies in America research showed new things about how AR affects the way people think and act. They also showed that AR is not a minor problem but a serious one. Most of the people who had nasal allergies (two out of three) said that AR made their daily lives harder in some way. And four out of 10 people said that AR had a big or medium impact on their lives [Figure 2].[33]

Burden on patient emotions

People with AR were questioned how they felt about their quality of life during allergy season as part of the Allergies in America study. They might respond with words that express their feelings.[33] Other research [34, 35] also investigated how AR impacts the mental health of those who have it. They discovered that adolescents with AR (aged 11 to 13) experienced higher emotional difficulties than those without AR. Additionally, they discovered that adolescents with AR were more likely to experience physical discomfort, worry, despair, and rage. They also struggled more with remaining calm under pressure, restraint, and impulse control. These negative feelings were stronger in young people than in adults who had AR.[35]

Burden on work activities

A person's performance at work or in school might also be impacted by AR.[26] People with AR are less able to work than they would be if they did not have it. Due to their symptoms, they can also skip work or school more frequently. The degree to which AR impairs academic or professional performance can be accessed via a questionnaire, which is named as Job Productivity and Activity Impairment-Allergy Specific Questionnaire. The individual is questioned about how their allergies impair their participation in job or school activities. Estimating the financial costs associated with AR and the types of therapies required can also be helpful.[26,36] Professional activities have been observed to be impaired in up to 60% of seasonal AR patients and 40% of permanent AR patients [*Allergic rhinitis: indicators of quality of* life*]. It has been estimated that 50% of people who use first-generation antihistamines (sedatives) to treat the condition operate at just 75% of their complete capacity for 14 days each year.[37]

Sleep impairment

Sleeping issues are one of the issues that persons with AR may experience. They may experience breathing difficulties as a result of a plugged nose.[38] Numerous studies have demonstrated that those who have AR do not sleep as well as those who have not. They could struggle more to get to sleep, stay asleep, or feel relaxed. According to the Burden of Allergic Rhinitis study, those with AR had higher scores on the scales measuring sleep issues and adequate sleep than those without the condition. These are indicators of the quality of the sleep, either excellent or terrible. Microarousals during sleep may also be more frequent in those with AR. These are the fleeting moments when someone is somewhat startled out of sleep by anything. These disruptions may be brought on by breathing issues, increased nasal discharge, or increased upper airway resistance in persons with AR. In other circumstances, these microarousals might occur up to 10 times more frequently.[38,39] A person's thoughts and feelings may change if they are not getting enough sleep. They may

be less able to focus, recall information, or come up with solutions as a result. Additionally, it may increase their risk of developing mental health issues, including despair, anxiety, or alcohol abuse. In an AR survey, 68% of people with perennial AR and 48% of those with seasonal AR said that the condition interfered with their sleep.[37]

Burden of medication

The adverse effects of the medications AR patients use to treat their symptoms can also be an issue. In order to feel better, persons with AR occasionally need to take multiple medications at once. According to the Allergies in America survey, OTC treatments are used by people with AR more frequently than prescription drugs. You can purchase over-the-counter (OTC) drugs without a prescription. The drugs that require a prescription can only be purchased with a doctor's authorization. Intranasal corticosteroids are more effective than antihistamines for treating AR symptoms such as nasal obstruction, discharge, itching, and postnasal drip, according to clinical research. You spray intranasal corticosteroids into your nose to take them. Antihistamines are drugs that can be administered intravenously or orally. The individuals who take the prescription may get negative effects from both types of drugs, though. People with AR report that they feel dry (34%), have liquid leak down their throat (33%), feel tired (33%), experience headaches (25%), have a poor taste (22%), and feel burning (18%) are some of the side effects that they find extremely or very irritating. People with AR sometimes discontinue taking prescription drugs because they do not work as effectively (37%), work less over time (35%), do not last long enough (32%), or have bothersome side effects (25%).[33,38]

Burden of comorbidity

A person with AR may be dealing with other issues concurrently. Sinusitis, upper respiratory infections, otitis media with effusion (OME), and nasal polyposis are a few of these issues. When the sinuses swell up, it is called sinusitis. Allergy has also been considered a "contributing factor" in 40%–80% of the cases of chronic sinusitis.[37] When the nose, throat, or lungs become infected, it is known as an upper respiratory infection. When there is fluid in the middle ear, it is known as otitis media with effusion (OME). Nasal polyposis is a condition where the nose has growths. According to population studies, 38% of all AR patients also have asthma, and 78% of all asthma patients have rhinitis.[37] When the airways become constricted and difficult to breathe, it is called asthma. Numerous cases of chronic sinusitis can also be brought on by allergens. Allergens are things that make people allergic, like pollen or dust.[38]

Methodology

We searched for publications that were authored in English to conduct our investigation. We made use of certain websites with a large number of documents. These websites include Google Scholar, PubMed, ScienceDirect, the Directory of Open Access Journals, and Web of Science. We looked for documents that had certain words. These words were "Review OR Pathology" OR/AND "Allergic Rhinitis OR Hay Fever." To locate more publications, we combined these words in various ways. The articles in which the papers we found cited were also examined. In this manner, we tried to find other papers that deal with our subject. For this study, 30 publications were used.

DISCUSSION

Allergic rhinitis is a growing concern as it increasingly impacts a larger population. One of the most detrimental aspects of AR is its propensity to induce nasal congestion. This might potentially impede the quality of sleep. When individuals experience inadequate sleep, they may encounter a multitude of other issues. Individuals may experience sensations of drowsiness, fatigue, irritability, or cognitive disorientation during the day. Individuals may experience difficulties in cognitive processes such as thinking, memory, and learning. These issues have the potential to impact individuals of all ages, including both adults and children, albeit in distinct manners. Adult individuals may experience a decline in their work performance compared to their potential capacity. Additionally, it is plausible that they may have a higher incidence of workplace accidents or injuries. Children have the potential to exhibit increased levels of shyness or sadness. Individuals may also experience heightened levels of concern or fear. The efficacy of medications utilized for the treatment of AR may be insufficient for certain individuals. The duration of their efficacy may be insufficient to alleviate symptoms throughout the entire day. There is a demand for pharmaceutical interventions that provide extended relief from symptoms and enhance overall well-being.

Healthcare practitioners may occasionally lack a comprehensive understanding of the experiences and perspectives of individuals with AR. There is a possibility that individuals may have the belief that individuals are content with their treatment despite the presence of underlying dissatisfaction. This phenomenon has the potential to erode the faith individuals place in their healthcare providers, including physicians and nurses. Noncompliance with medication recommendations is a common occurrence among individuals. Individuals may consume either more or less than the recommended amount. This has the potential to exacerbate their symptoms related to AR or give rise to other

complications. Healthcare practitioners have access to several technologies that enable them to assess the impact of AR on individuals' health and overall well-being. The aforementioned tools are commonly referred to as health profile instruments. Individuals are queried on their emotional state and functional capabilities in relation to their AR experiences. Multiple researches have demonstrated that the utilization of these methods can facilitate the assessment of various therapies' efficacy in enhancing individuals' health and overall well-being. Several therapeutic interventions have been identified as potentially efficacious in managing AR, including non-sedating antihistamines, intranasal corticosteroids, and other pharmacological agents that have received approval for the treatment of this condition. Non-sedating antihistamines refer to pharmaceutical substances that do not induce drowsiness in individuals. Intranasal corticosteroids refer to pharmaceutical substances that individuals administer by nasal spraying. Healthcare practitioners have to employ these strategies and interventions in order to assist individuals suffering from AR. It is important to engage in more communication with individuals and actively attend to their apprehensions. In this manner, individuals may enhance their comprehension of augmented reality and effectively administer its use.

Current barriers

Education and training

There are significant gaps in allergy training and practice in India. In India, Allergy and Immunology (A&I) is not a recognized specialty. The Medical Council of India does not provide any postgraduate training courses in A&I. It has, however, provided a diploma program in A&I and established the objectives, skill sets, competencies, complete curriculum, training, and evaluation procedures, for which there have been few applicants.

A scarcity of India-specific scientific data and recommendations

Current Indian management methods are mostly based on guidelines taken from data gathered in Western nations, which might not be "suitable for purpose" in Indian medical practice. There are a few substantial and real-world research to back up these principles' applicability to the Indian populace.

CONCLUSION

Allergic diseases are a global health problem, and India is no exception. There is a necessity for raising awareness of this expanding disease within the medical community, in addition to among national policymakers and the general public.

Ethical approval

Institutional Review Board approval is not required.

Declaration of patient consent

Patient consent is not required as the patient's identity is not disclosed or compromised.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-Assisted Technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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Original Article

Granulomas co-occurring in malignancies – tale of etiological relationship

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ABSTRACT

Objectives: To determine the possible aetiopathogenesis of co-occurrence of granulomas with different malignancies in different body sites.

Material and methods: All cases with granuloma formation observed in draining lymph nodes or in the primary site of malignant tumors were included in the present study. After routine histopathology examination, modified Ziehl-Neelsen (ZN) staining for Mycobacteria was carried out in all the cases. Detailed history, especially of Tuberculosis, sarcoidosis, neoadjuvant chemotherapy, radiation, or previous procedure, was recorded.

Results: 11 out of 35 cases (31.4%) had granulomas within the primary tumor, while 24 cases out of 35 (68.6%) showed nodal granulomas. Of the 24 cases, 5 cases had nodal metastatic tumor deposits. Also, necrotizing granulomas with AFB were significantly more as compared to AFB in non-necrotizing granulomas (p value of 0.05). Of the total cases, 20% had an attributed risk factor. Three cases received neoadjuvant chemotherapy, and three had a history of systemic tuberculosis, while one case had associated Crohn's disease.

Conclusion: We recommend to characterize granulomas as necrotizing/nonnecrotizing, confluent/discrete, tumor deposit present/absent; prior history of systemic/local illnesses (like SS, TB, fungal infections, IBD, etc.); prior history of CT/RT; and to follow routine ZN staining in all cases of granulomas with malignancy.

Keywords: Granulomas, Malignancy, Sarcoidosis, Tuberculosis, ZN Staining

INTRODUCTION

Granulomatous inflammation is one of the most common diagnoses in pathology in India.[1] In patients with malignancy, granulomatous lesions may quite often be a fortuitous finding, especially in primary diagnostic biopsies.[2] These granulomas are included as a part of the primary cancer or are present in the lymph nodes that drain the tumor.[3] The exact etiopathogenesis or the therapeutic implications in patients with malignancy remain obscure.[1,3–5]

In a developing country like India, where the incidence of tuberculosis (TB) is high, it is sometimes difficult to distinguish between a concomitant TB and a nonspecific granulomatous response with focal necrosis.[4] Tuberculous etiology should mandatorily be ruled out before any other cause is considered.[3,6]

Granulomatous inflammation is a chronic inflammatory reaction characterized by microscopic aggregation of activated macrophages with an epithelioid appearance. These may or may not be associated with mononuclear cells, classical multinucleate Langhans' giant cells, or necrosis. The presence of granulomatous inflammation could be due to infective or noninfective causes, while granulomas can be necrotizing or nonnecrotizing.[3] A tubercular granuloma is discrete with the presence of Langhans' multinucleated giant cells, lymphocytes, epithelioid cells, and caseous necrosis, while the typical sarcoid granuloma is coalescent and noncaseating with the presence of plump epithelioid cells and giant cells (both foreign body or Langhans' type).[1,4]

Granulomas in the lymph nodes draining the primary tumor (with or without metastatic cancer) have been variously

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labeled as sarcoid-like or a sarcoid reaction.[1] Gorton and Linell were the first to report cases with sarcoid-like granulomatous inflammation in lymph nodes draining carcinomas.[7,8] Formation of a granuloma has also been observed in many malignancies treated with neoadjuvant chemotherapy or radiation therapy, especially in cancers of the breast, stomach, colonic, or larynx.[3,6]

Infections due to bacteria, mycobacteria, spirochaetes, viruses or fungi, local irritants, Crohn's disease, Whipple's disease, and cirrhosis have been found to be the different causes of granuloma formation in patients with malignancies. Amongst these, mycobacterial infection is probably one of the most frequently presenting infections asymptomatically.[2]

Several studies have demonstrated a relationship between systemic sarcoidosis (SS) and malignant tumors. Sarcoidosis can occur any time before, during, or after the cancer is diagnosed. Studies have shown that patients previously diagnosed with SS have a 2–3 times higher risk of developing malignancy in the near future, and the reason behind this is not welldefined.[9]

The present study was thus undertaken to describe the possible etiopathogenesis of co-occurrence of granulomas with different malignancies at different body sites.

MATERIAL AND METHODS

The present study was conducted at the Department of Pathology of a tertiary care center with a dedicated oncology unit. A retrospective observational study was planned. All cases with granuloma formation seen either in the draining lymph nodes or in the primary site of malignant tumors were included in the study. All benign tumors or inflammatory conditions with or without granulomatous inflammation were excluded from the present study. Institutional Ethics Committee (IEC) approval (Reference No. IEC/RMCH/86/2022/Aug) was obtained for this study with the waiver of consent.

Thirty-five cases met the inclusion criteria during the last four years. The required patients' data were retrieved from the medical records section and analyzed. In addition to histopathology reporting, the cases were re-analyzed for granuloma characteristics, and Ziehl–Neelsen (ZN) stain was performed on all the paraffin blocks. The granulomas observed were characterized under the following subtypes:

1. Necrotizing or nonnecrotizing granulomas
2. Lymph node granuloma with or without tumor deposits
3. Granuloma in the primary tumor and draining lymph nodes.

RESULTS

The site-wise distribution of cases of granuloma with malignancies is depicted in Figure 1–5.

Eleven out of the 35 cases (31.4%) had granulomas within the primary tumor while 24 out of 35 cases (68.6%) showed nodal granulomas in the tumor-draining lymph nodes [Table 1].

A comparison of different parameters with respect to the co-occurrence of granulomas in cases of malignancies is shown in Table 2. Correlation of adenocarcinoma and squamous cell carcinoma was done in patients presenting with concurrent granulomas. Cases of spindle cell sarcoma

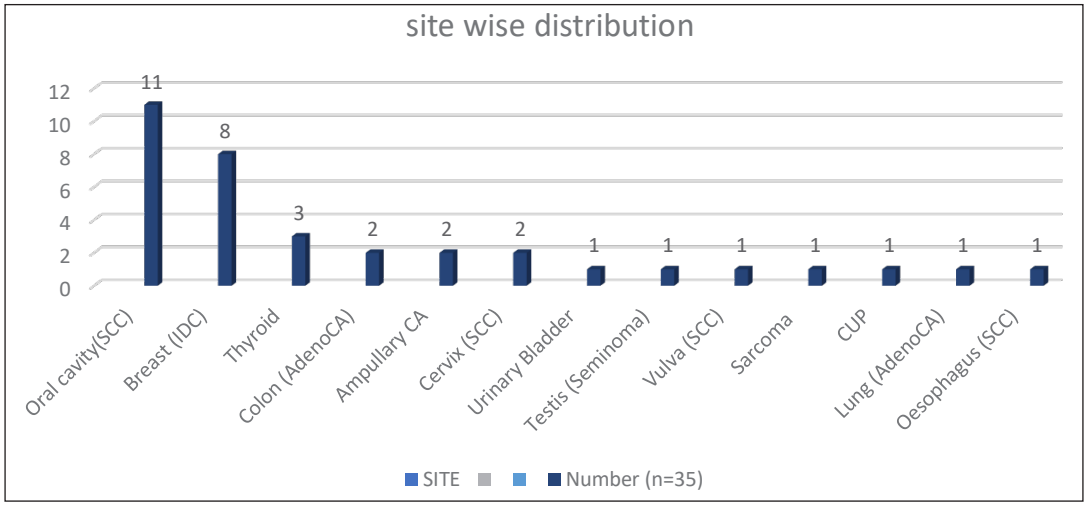

Figure 1: Site-wise distribution of cases of granuloma co-occurring with malignancies (SCC: Squamous Cell Carcinoma; IDC: Invasive Ductal Carcinoma; CUP: Carcinoma Unknown Primary); Peri/Ampullary CA (Adeno CA); Urinary bladder (High grade papillary CA); Sarcoma (Spindle cell).

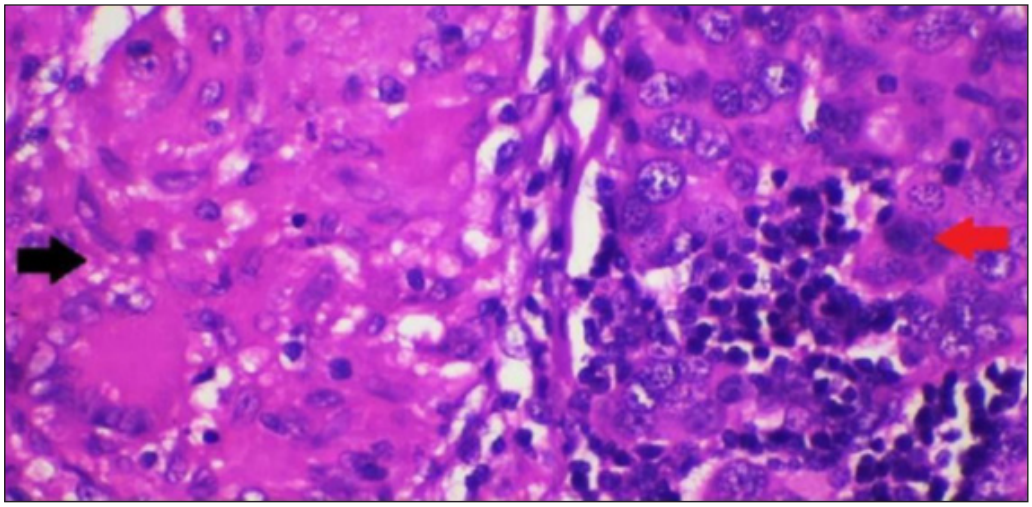

Figure 2a: Epithelioid granuloma (black arrow) with invasive breast carcinoma (red arrow) (Haematoxylin & Eosin ×400).

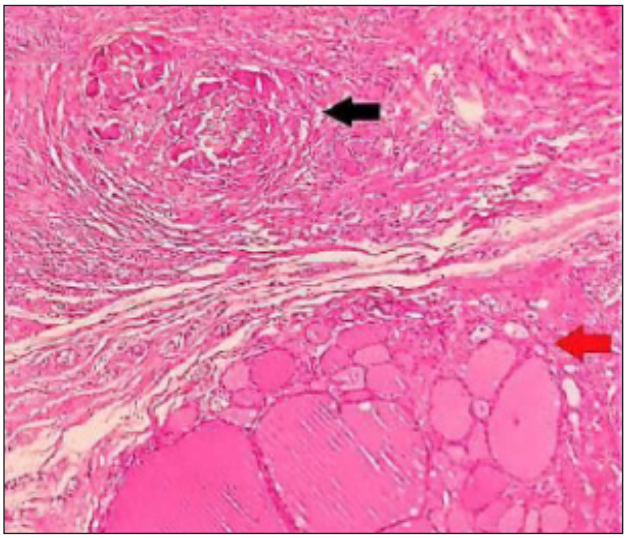

Figure 2b: Epithelioid granuloma (black arrow) with encapsulated follicular patterned neoplasm thyroid (red arrow) (Haematoxylin & Eosin ×100).

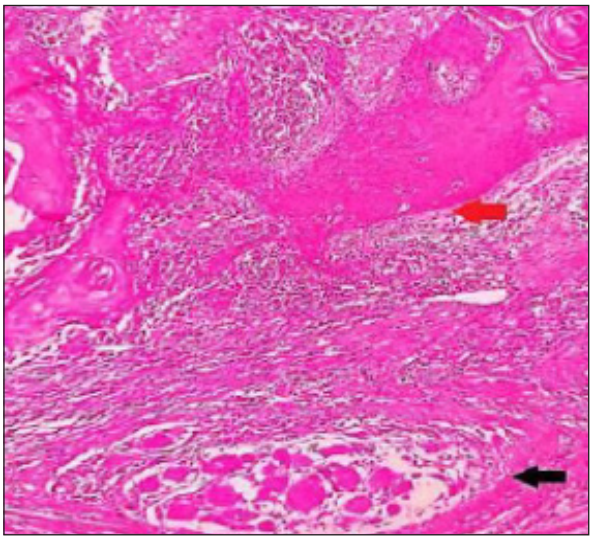

Figure 2c: Epithelioid granuloma (black arrow) with oral squamous cell carcinoma (red arrow) (Haematoxylin & Eosin ×100).

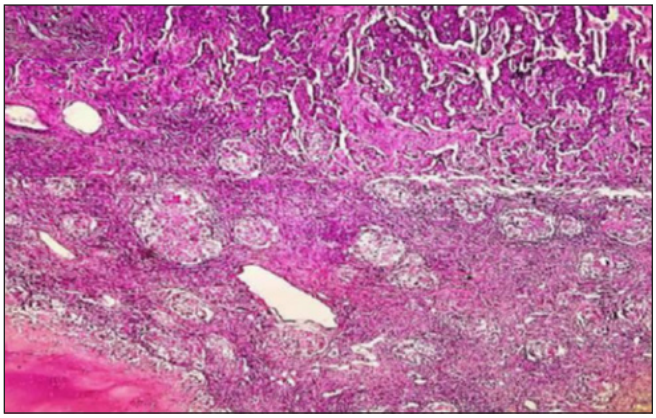

Figure 3a: Necrotizing granuloma in lymph nodes with metastatic deposits from invasive breast carcinoma (Haematoxylin & Eosin ×40).

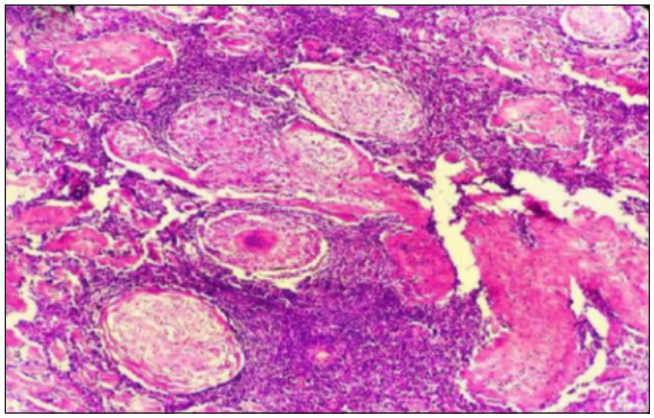

Figure 3b: Necrotizing granuloma in lymph node without metastatic deposits in a case of oral squamous cell carcinoma (Haematoxylin & Eosin ×100).

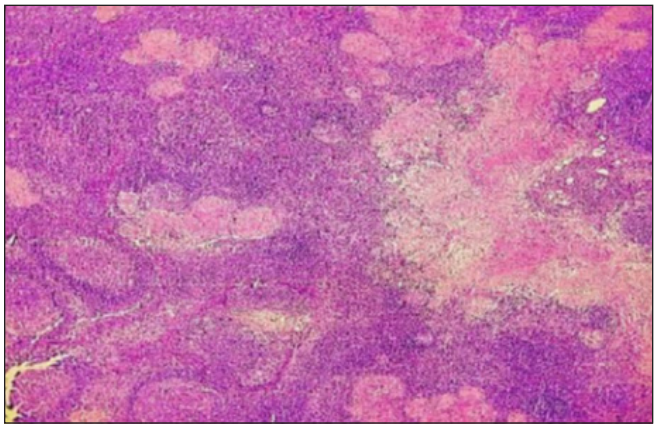

Figure 4a: Necrotizing discrete tuberculoid-like granuloma in lymph nodes (Haematoxylin & Eosin ×40).

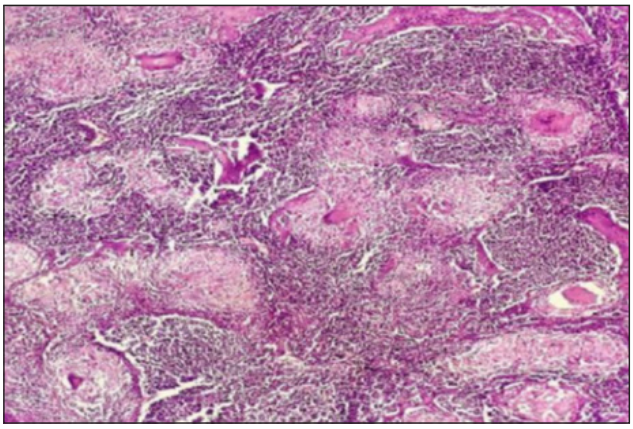

Figure 4b: Nonnecrotizing coalescent sarcoid-like granuloma in lymph nodes (Haematoxylin & Eosin ×100).

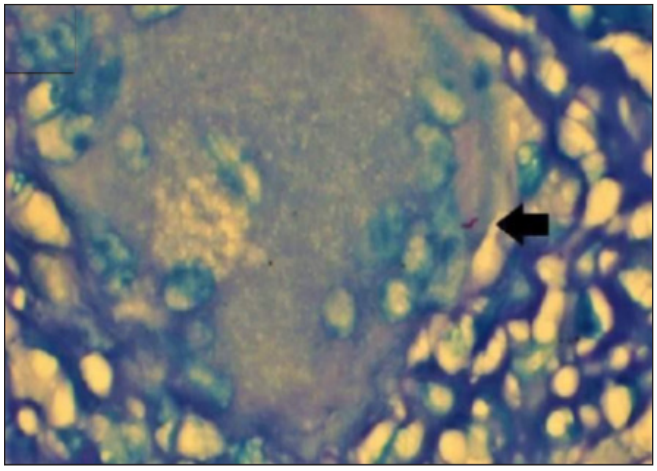

Figure 5a: AFB positivity in a necrotizing granuloma in a case of invasive breast cancer (ZN stain ×1000; oil immersion). Pink, rod shaped acid fast bacilli , depicting AFB positivity (Black arrow). (ZN: Ziehl-Neelsen; AFB: Acid Fast Bacilli)

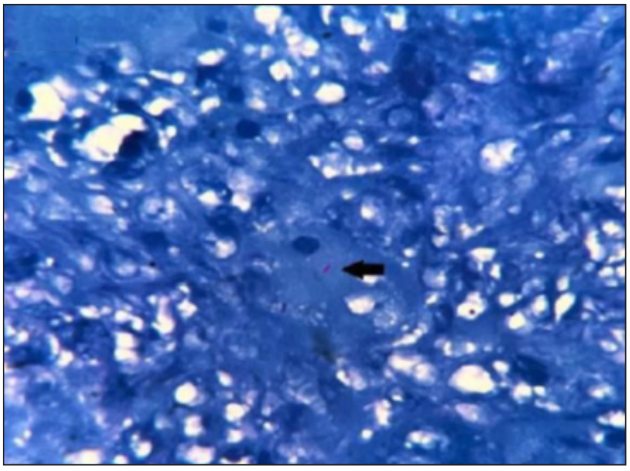

Figure 5b: AFB positivity in a necrotizing granuloma in a case of carcinoma cervix (ZN stain ×400). Pink, rod shaped acid fast bacilli , depicting AFB positivity (Black arrow). (ZN: Ziehl-Neelsen; AFB: Acid Fast Bacilli)

(hand), seminoma testis, metastatic carcinoma, carcinoma of unknown primary in lymph node, and high-grade papillary urothelial carcinoma urinary bladder were not included in the comparative analysis. Table 2 also shows ZN staining results, distribution of necrotizing and nonnecrotizing granulomas, etc. History of neoadjuvant chemotherapy, past history of systemic TB, and history of IBD in a case of colon adenocarcinoma were taken, none of the cases had any history of SS or prior Fine needle aspiration cytology (FNAC)/Biopsy procedure done.

Furthermore, in the present study, we observed that acid fast bacilli (AFB) positivity was higher in necrotizing granulomas as compared to AFB in nonnecrotizing granulomas (p-value = 0.05). Of the total 35 cases, 2 cases showing positivity for AFB by ZN staining also had granulomas in the adjoining draining lymph nodes. The comparison of nodal versus extranodal AFB positivity was statistically insignificant (p-value = 0.84).

In our study, of the total 35 cases, 11 (31.43%) cases had the presence of granulomas in the primary tumor, of which 3 (8.57%) had necrotizing tuberculoid-like granulomas while 8 had nonnecrotizing sarcoid-like granulomas. Among the 24 cases with nodal granulomas, 14 (58.33%) cases had, nonnecrotizing sarcoid-like granulomas, and 10 (41.67%) cases had necrotizing tuberculoid-like granulomas. The p-value (0.65) for this distribution was statistically insignificant.

DISCUSSION

The presence of granuloma and malignancy at the same time is a rare occurrence, and it carries with it a diagnostic as well as a therapeutic dilemma. In a TB endemic and resource-limited nation like ours, a big question is whether to further

Table 1: Comparison of cases with the presence of granuloma in lymph nodes versus primary tumor with different malignancy sites.

Sites	Cases with granuloma in lymph nodes	Cases with granuloma in the primary tumor	Total
Oral cavity	9	2	11
Breast	7	1	8
Thyroid	0	3	3
Colon	1	1	2
Periampullary/ ampullary	2	0	2
Cervix	1	1	2
Urinary bladder	0	1	1
Testis	0	1	1
Vulva	0	1	1
Hand	1	0	1
Carcinoma unknown primary (lymph nodes)	1	0	1
Lung	1	0	1
Esophagus	1	0	1
Total	24 (68.6%)	11 (31.4%)	35 (100%)

investigate these cases or take these granulomas (especially the necrotizing ones) as sufficient to diagnose TB unless proven otherwise. There have been several case reports and studies describing granulomas at different cancer sites in the body. Most studies have described single cancer sites. In our study, the common three sites were oral squamous cell carcinoma (31.4%), breast carcinoma (22.8%), and papillary thyroid carcinoma (8.5%). Overall, a total of 51.6% cases of adenocarcinoma (breast, thyroid, colon, GIT, lung, etc.) and 48.4% cases of squamous cell carcinomas (oral and cervix) were included.

In one study, squamous cell carcinoma was considered to be more commonly associated with granulomas, possibly owing to more number of head and neck surgeries in the given institute.[3] To date, we have not been able to find any other study in English literature that has discussed such a comparison.

Another study showed one case of papillary carcinoma thyroid having coexistence of metastatic deposits and TB in the cervical lymph nodes.[10] While in our study, we reported two such cases with no history of TB along with AFB negativity.

The etiopathogenesis of these granuloma formations is multifactorial, and the following causes have been found in

Table 2: Distribution of cases according to different parameters.

Parameters		Distribution/frequency	Percentage	Total
1) Histological type	Adenocarcinoma	16	51.6	100% (n = 31)*
	Squamous cell carcinoma	15	48.4	
2) ZN staining	AFB positive	2	5.7	100% (n = 35)
	AFB negative	33	94.3	
3) Types of granuloma	Necrotizing tuberculoid-like granulomas	13	37.1	100% (n = 35)
	Nonnecrotizing sarcoid-like granulomas	22	62.9	
4) Sites of granuloma	Presence of granuloma in primary tumor	11	31.4	100% (n = 35)
	Presence of granuloma in lymph node	24	68.6	
5) Presence of granuloma in lymph node	Lymph node with tumor deposits	5	20.8	100% (n = 24)**
	Lymph node without tumor deposits	19	79.2	
6) Post-neoadjuvant or chemotherapy related	Cases with prior neoadjuvant or chemotherapy	3	8.6	100% (n = 35)
	Cases without prior chemo or neoadjuvant therapy	32	91.4	
7) Past history	Tuberculosis	3	8.6	100% (n = 35)
	Inflammatory bowel disease	1	2.9	
	Others (Any systemic sarcoidosis or prior FNAC/Biopsy procedure done)	0	88.5	

*Four cases: Spindle cell sarcoma (hand), seminoma testis, metastatic carcinoma, carcinoma unknown primary in lymph nodes, and high-grade papillary urothelial carcinoma urinary bladder were not included as they could not be categorized into SCC/adenocarcinoma.

**Remaining 11 out of 35 cases had the presence of granulomas in the primary tumor. AFB: Acid Fast Bacilli; FNAC: Fine needle aspiration cytology; ZN: Ziehl-Neelsen

different studies like: neoadjuvant therapy-related, foreign body reaction owing to the necrotic tumor, or previous procedure like FNAC/biopsy; an associated systemic or local illness like TB, sarcoidosis, inflammatory bowel disease, or fungal infection; idiopathic causes, etc.[1,3,4]

In majority of the cases, no definite cause has been found by researchers, and the etiology remains obscure. In these cases, granuloma formation is due to T-cell-mediated (type IV hypersensitivity) immunological reaction to soluble antigens shed by the tumor or tumor-derived debris,[11] which leads to a granulomatous response, whereas others attribute it to the persistence of a non-degradable product like keratin/psammoma bodies/mucin, etc.[12]

Diligent history taking and routine use of ZN stain were done in all cases. Owing to this, we observed 20% of patients with one or the other etiopathogenic factor to which a coexisting granuloma formation could be attributed. Three out of 35 cases (8.6%) had a prior history of neoadjuvant chemotherapy, which may have induced granuloma formation in the residual tumor. Studies by other researchers have substantiated the occurrence of granulomatous inflammation in post-neoadjuvant chemo/radiotherapy/radioisotope therapies for various carcinomas soon after or later in the course of the disease.[2-4,6] Three out of 35 (8.6%) cases in the present study had a history of TB, and two of these showed the presence of AFB within the associated necrotizing granulomas. Previous authors from India performed ZN staining in few cases of granulomas with malignancy, but they could not completely rule out tuberculosis as a cause of the primary malignancy associated with granulomas in spite of negative ZN stain result.[3,6] In a study from India, three out of seven cases showed ZN positivity for AFB.[13] The specificity of ZN microscopy is high (78.5%) but sensitivity is imperfect (22.2%).[14]

Thus, although negative cases do not rule out TB, there is a possibility of exacerbation of TB due to immune suppression and cancer therapy in such patients. Hence, a prior history, inexpensive ZN stain, and chest radiograph may be carried out in all patients of granuloma with malignancies to pick up smoldering TB cases.

One case of colonic adenocarcinoma in our study had coexisting Crohn's disease, which is a well-known risk factor for adenocarcinoma.[15] SS is another described risk factor in some studies.[2,9,16] However, we couldn't elicit a prior history of SS in any of our cases.

Based on histomorphology, granulomas were classified as necrotizing (TB like) and nonnecrotizing ones (sarcoid-like). In our study, out of the total 35 cases, 22 cases (62.9%) were nonnecrotizing sarcoid-like granulomas, while 13 cases (37.1%) were necrotizing tuberculoid-like granulomas. This is in contrast to a study on lung cancers where necrotizing granulomas were found more frequently with 8 of 19 patients (42%), whereas 6 (31.6%) had nonnecrotizing granulomas.[6]

Granulomas in TB have been reported to be caseating in 58.7%, non caseating in 23.8% and atypical in 17.5% cases in a previously conducteds autopsy study;[17] however, in the present study both the AFB positive cases were necrotizing granulomas. Similarly, there have been case reports and studies with ZN stain positivity in necrotizing granulomas.

One study recommended that in cases of ZN positivity, a mastectomy should be performed for operable breast cancer followed by 18 months of anti- TB therapy. To avoid the effect of immunosuppression, chemotherapy is recommended after 4 weeks of anti-TB therapy.[18] Hence, treatment guidelines are amenable to modifications in cases of AFB-positive granulomas associated with malignancies.

In our study, out of the total 35 cases, 11 cases (31.4%) had a presence of granuloma in the primary tumor with or without the involvement of lymph nodes and were negative for AFB. Similarly, Dagaonkar *et al*. found 19 out of 127 cases (14.9%) of lung carcinoma with the presence of granuloma in the primary tumor and were negative for AFB.[6] Also, studies conducted by Alalshee *et al*., Daroca PJ. and Bässler R, Birke F. observed two, three, and five such cases, respectively, of breast carcinoma with primary stromal granulomas.[4,19,20]

In our study, out of the total 35 cases, 24 cases (68.6%) had a presence of granulomas in draining lymph nodes, of which 5 cases (20.8%) had tumor metastatic deposits, while 19 cases (79.2%) were negative for metastasis. In another study, of all the consecutive lymph node dissections done for cancer patients during one-and-a-half years, 27 cases showed granulomatous inflammation in lymph nodes, of which 2 cases (7.4%) had metastatic deposits.[3]

In a study done by Krvavac A *et al.* of 40 lung carcinoma patients, 3 (7.5%) had nodal granulomas without metastatic deposits.[21]

Several queries, therefore need to be addressed regarding this interesting observation, especially whether a history of prior systemic TB or sarcoidosis needs to be evaluated in all patients having cancer. The presence of necrotizing versus nonnecrotizing granulomas should be further evaluated using ancillary tests like ZN stain for AFB, Polymerase chain reaction (PCR) testing, or Gene-Expert testing. Whether these findings could have any implications in the treatment modifications in cancer patients need to be evaluated.

Some of the older reports have also shown an association of epithelioid granulomas with tumor metastasis in the lymph nodes. A close scrutiny of such granulomas is therefore necessary to avoid underdiagnosis of the metastatic disease. However, it may not be easy to identify the tumor cells in the

lymph node, and immunostaining with cytokeratin may be required to rule out occult metastasis, as recommended in some studies.[3,4]

Pathologists often describe the granulomas in malignancies, but mostly, they are neither specifically mentioned nor elaborated on in the final diagnosis. This is important because clinicians focus on the final pathologic diagnosis, and many microscopic descriptions are not read. This synchronous occurrence also leads to a state of confusion for the treating physician.[22] Conscientious data recording in routine synoptic reporting formats for all cases of granulomas coexisting with malignancies may prove an exemplar for larger model multisystemic studies in the future. A large series on this phenomenon may perhaps help to identify the true incidence and prognostic importance of granulomas in draining lymph nodes of a carcinoma.

CONCLUSION

Granulomatous reactions in the stroma usually indicate a T-cell-mediated immunological response to the antigens present on the cell surface. There are only a handful of multisystemic studies on this topic available in the English literature. With cases of TB showing both caseating and noncaseating granulomas and lack of a confirmatory gold standard test available for TB, a careful search for AFB on routine ZN stain should be carried out in all the cases of granulomas with or without necrosis. Other ancillary tests such as TB culture, molecular-based PCR, Gene Expert, and regular clinical examination for an increase in the size of the lymph node, production of sputum, etc., should be carried out for all patients with granulomas in malignancies, especially in countries endemic for TB. Presence of occult metastatic tumor cells within necrotic granulomas in the draining regional group of nodes should be identified by immunohistochemistry wherever possible. Granuloma characteristics like necrotizing/nonnecrotizing, confluent/discrete, and tumor deposit present/absent; prior history of systemic/local illnesses (like SS, TB, fungal infections, Inflammatory Bowel Disease (IBD), Chemotherapy (CT)/Radiotherapy (RT)) should be documented in all cases with dual granulomas as well as malignancies.

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Ethical approval

The authors declare that they have taken the Institutional Ethics Committee approval and the approval number is IEC/RMCH/86/2022/Aug.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

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Original Article

Utility of CBNAAT (GeneXpert MTB/RIF assay) in rapid diagnosis of extrapulmonary tuberculosis in a hepatobiliary tertiary center

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ABSTRACT

Objectives: Newer diagnostic techniques like cartridge-based nucleic acid amplification techniques (CB NAAT) need to be evaluated for extrapulmonary tuberculosis (EPTB), as being a paucibacillary condition, it is often underdiagnosed with conventional methods. We conducted this study to assess the utility of CB NAAT (GeneXpert MTB/RIF assay) in rapid diagnosis of extrapulmonary tuberculosis.

Material and Methods: Liver disease patients admitted from June 2019 to June 2020 were investigated for EPTB based on clinical and radiological suspicion. EPTB was diagnosed based on one of the following: (i) histological evidence of caseating granulomas; (ii) smear positivity for acid-fast bacilli; (iii) CB NAAT (GeneXpert MTB/RIF assay, Cepheid, USA).

Results: A total of 290 EPTB specimens received in the laboratory were included. The extrapulmonary samples that were received included body fluids (n = 143) which included pleural fluid, ascitic fluid, drain fluids, and pus aspirates, followed by biopsies (n = 82), lymph nodes (n = 43), urine (n = 19), and CSF (n = 3). GeneXpert MTB/RIF assay was positive in 10.3% (n = 30) samples, whereas negative in 89.7% (n = 260) samples. The overall sensitivity of GeneXpert MTB/RIF assay was 61.36% (95% CI 46.62%–74.28%), specificity 89.29% (95% CI 72.8%–96.29%), positive predictive value (PPV) 90% (95% CI 74.38%–96.54%), and negative predictive value (NPV) 59.52% (95% CI 44.49%–72.96%).

Conclusion: The GeneXpert MTB/RIF assay is a valuable tool for extrapulmonary tuberculosis. In addition to other tests like smear, culture GeneXpert MTB/RIF assay helps in the confirmation of diagnosis. Rapid diagnosis of tuberculosis with overall good sensitivity and specificity makes it a beneficial test.

Keywords: Extrapulmonary Tuberculosis, GeneXpert, Histopathology, Paucibacillary, Radiology

INTRODUCTION

Diagnosing extrapulmonary tuberculosis (EPTB), an entity distinct from pulmonary TB is a challenge. EPTB has a high burden comprising 1/5th of all TB cases, which consists of 15%–20% of all TB cases in HIV negative patients, and 40%–50% of new TB cases which are HIV positive.[1] A wide spectrum of disease with commonly involved sites, which include lymph node, urogenital tract, central nervous system, bone and joints, gastrointestinal tract, and cardiovascular system.[2] Being a paucibacillary condition there needs to be a strong diagnostic armamentarium. While most studies have been performed on respiratory samples, we lack literature on extrapulmonary samples. With the advent of recent techniques, nucleic acid amplification techniques (NAATs) play a definitive role in diagnosis of TB.[3] Literature states the use of cartridge-based nucleic acid amplification techniques (CB NAAT) (GeneXpert MTB/RIF assay, Cepheid, USA) in the diagnosis of pulmonary tuberculosis, but there is lesser documentation of its use in EPTB.[4] As compared to smear microscopy and culture, GeneXpert Mycobacterium tuberculosis complex/resistance to Rifampin (MTB/RIF) assay has a low detection limit of 131 CFU/mL, making it beneficial for conditions like paucibacillary EPTB.[5] In a previous study from our center, the prevalence of EPTB was found to be 15.65% among liver disease patients.[6] Thus with this in mind, the study was conducted to assess the role of GeneXpert MTB/RIF assay as a rapid diagnostic tool in EPTB.

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MATERIAL AND METHODS

Statement of Institutional Review Board Approval

Research of the following retrospective study has been approved and recommended by the Institutional Ethics Committee (IEC/2020/56/MA05).

Study population

A total of 290 consecutive patients admitted to our tertiary care center for hepatobiliary disease from June 2019 to June 2020 were investigated. A retrospective analysis of extrapulmonary samples of suspected EPTB patients was done. Patients were investigated for EPTB based on clinical and radiological suspicion. Extrapulmonary samples included lymph node samples, tissue biopsy, urine, Cerebrospinal fluid (CSF), and body fluids such as ascitic fluid, pleural fluid, pus, and drain fluids.

Processing of samples

Samples were processed and subjected to Ziehl–Neelsen (ZN) staining and molecular assay CB NAAT (GeneXpert MTB/RIF assay, Cepheid, USA). Simultaneously samples were sent for cytologic analysis and histopathology. EPTB was diagnosed based on one of the following: (i) histological evidence of caseating granulomas; (ii) smear positivity for acid-fast bacilli; (iii) CB NAAT (GeneXpert MTB/RIF assay, Cepheid, USA).

Statistical data analyses were performed using SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of GeneXpert MTB/RIF assay were calculated using histopathology and response to therapy as the gold standard.

RESULTS

Based on clinical suspicion, patients were sent for radiological examination and patient samples for histopathological, cytological, and microbiological examination. A total of 290 EPTB specimens received in the laboratory within the one year as categorized in Table 1 were included. Analyses were done on a per-sample basis and not on a per-patient basis. Gender distribution included males (n = 206) and females (n = 84). Table 2 shows the results of radiological and histopathological examination along with GenXpert results.

Table1: Description of routine EPTB specimens received and tested using GeneXpert MTB/RIF assay.

Specimen type	Samples received in laboratory, n (%)
Body fluids	143 (49.3%)
Biopsies	82 (28.3%)
Fine-needle aspirates [lymph node]	43 (14.8%)
Urine	19 (6.6%)
CSF	3 (1%)

EPTB: Exptrapulmonary tuberculosis, MTB/RIF: Mycobacterium tuberculosis complex/resistance to Rifampin, CSF: Cerebrospinal fluid

Table 2: Results of radiological, histopathological, and GeneXpert examination.

Examination	TB Suggestive/ detected	Not suggestive/ not detected
Radiology	64 (22.1%)	226 (77.9%)
Histopathology	32 (11%)	258 (89%)
GeneXpert	30 (10.3%)	260 (89.7%)

Results of microbiological examination of samples mainly included ZN staining and GeneXpert MTB/RIF assay. There were few samples received for culture hence combined histopathology and response to therapy were considered the gold standard. GeneXpert MTB/RIF assay was positive in 10.3% (n = 30) samples, whereas negative in 89.7% (n = 260) samples. Histopathology was suggestive of tubercular etiology in 11% (N = 32) samples. Seventy two percent patients received antituberculosis treatment (ATT) based on the results of laboratory or radiological examination or clinical diagnosis in case of negative reports. 44 (61.11%) patients responded to therapy with clinical improvement. To evaluate the performance of the GeneXpert test for lymph node samples we compared the results by combining histopathology and response to therapy. For body fluid samples, response to therapy was considered the gold standard. Table 3 shows the GeneXpert MTB/RIF assay-positive samples. The results were recorded according to the critical threshold values which included very low, low, medium, and high as described in Table 4.

Table 3: Results of GeneXpert MTB/RIF assay positive samples.

Samples	GeneXpert MTB/RIF assay Positive
Lymph node	15/43
Biopsy	2/82
Body fluids	13/143
Urine	0/19
CSF	0/3

MTB/RIF: Mycobacterium tuberculosis complex/resistance to Rifampin, CSF: Cerebrospinal fluid

Table 4: Interpretation of GeneXpert MTB/RIF assay results.

GeneXpert MTB/RIF assay result	%
Very Low	1.7%
Low	5.2%
Medium	2.8%
High	0.7%

MTB/RIF: Mycobacterium tuberculosis complex/resistance to Rifampin

Table 5: Sensitivity, specificity, PPV, and NPV of GeneXpert MTB/RIF assay in EPTB samples.

Sample	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV% (95% CI)
GeneXpert compared to histopathology and response to therapy	61.36 (46.62–74.28)	89.29 (72.8–96.29)	90 (74.38–96.54)	59.52 (44.49–72.96)

EPTB: extrapulmonary tuberculosis, PPV: positive predictive value, NPV: negative predictive value, CI: confidence interval, MTB/RIF: Mycobacterium tuberculosis complex/resistance to Rifampin.

In our study no rifampicin resistance was detected. Using histopathology and response to therapy as the gold standard, the sensitivity, specificity, PPV, and NPV for GeneXpert MTB/RIF assay are given in Table 5.

DISCUSSION

Extrapulmonary TB (EPTB), a paucibacillary disease and a more neglected diagnosis needs to be investigated with as much concern as pulmonary TB. The causes responsible for a difficult diagnosis of EPTB include a neglected clinical suspicion, problems in sample collection, paucibacillary condition, and lack of data on EPTB.[1] Laboratory diagnostic techniques such as microscopy and culture techniques have less sensitivity. The scanty literature on the utility of GeneXpert MTB/RIF assay for extrapulmonary samples makes this study a very important one. Histopathological examination is the gold standard for the diagnosis of EPTB from tissue samples, however, the sampling is often difficult, laboratory set up, is time consuming and needs expertise for diagnosis.[7] The study was conducted in a hepatobiliary tertiary care center, which even further makes the diagnosis important as liver cirrhosis has been implicated as a risk factor for extrapulmonary TB.[8] From our previous study, out of a total of 816 samples which included 260 pulmonary and 556 extrapulmonary samples, the positivity rate by Mycobacterial Growth Indicator Tube culture (MGIT) or MTB qPCR (Cobas TaqMan MTB assay) included pulmonary 31/260 (11.92%) and extrapulmonary 87/556 (15.65%) samples.[6] Thus, at our institute, there is a higher prevalence of EPTB, as opposed to pulmonary TB mandating the need for the establishment of a proper diagnostic protocol. The chances of recovery or detection of organisms from the extrapulmonary samples are less because of the lower load of bacilli present, as opposed to pulmonary samples like sputum in pulmonary TB.[9] Also an invasive sampling technique, lesser quantity of sample mounts difficulties in diagnosis. The diagnostic techniques available are smear microscopy (ZN staining), culture, GeneXpert MTB/RIF assay, and histopathology.[10] The diagnostic value of ZN staining is 0%–40%. In our previous study, in extrapulmonary disease smear positivity (14.9%) was much lower than the pulmonary disease (61.2%).[6] Mycobacterial culture sensitivity varies from 30% to 80%, but because it is a time-consuming method (2–6 weeks) it is not useful in the initiation of an early therapy.[6] Thus it becomes important to have a more rapid test for diagnosis.

Nucleic acid amplification techniques (NAATs) are a substantial aid in the diagnosis. [11] GeneXpert MTB/RIF assay, a real-time PCR is a robust assay with a shortened turnaround time (2 h) with a lower bacterial load (131 Colony forming unit/mL (CFU/mL)) required for detection. An automated method wholly integrated with an individual cartridge reduces the hands-on time and chances of cross-over contamination.[12] In various studies done, sensitivity of GeneXpert MTB/RIF assay ranged from 25% to 95%, with most of the studies exceeding 50%.[13] In our study, the overall sensitivity of GeneXpert MTB/RIF assay was 61.36% (95% CI 46.62%–74.28%), specificity 89.29% (95% CI 72.8%–96.29%), positive predictive value (PPV) 90% (95% CI 74.38%–96.54%), and negative predictive value (NPV) 59.52% (95% CI 44.49%–72.96%). The sensitivity is higher as compared to other diagnostic modalities like ZN stain and radiology. The recent Index TB guidelines for the diagnosis of EPTB categorize its use in various presentations, including lymph node TB, TB meningitis, abdominal TB, and TB pericarditis, and showed a pooled sensitivity and specificity of 83.1% and 93.6%, respectively.[14] Thus along with cytology, smear, and culture, GeneXpert MTB/RIF assay helps in confirmation of the diagnosis of EPTB. Also being a rapid test in comparison with other diagnostic modalities, a quicker diagnosis helps in the early initiation of therapy. The guidelines state that GeneXpert should not be used to diagnose pleural TB because of the low sensitivity of 46.4%, which is like our study where only 1/47 pleural fluid samples were positive.[14] Whereas, in other samples like urine and CSF none of the samples were positive. Amongst the GeneXpert MTB/RIF assay positive samples, being a paucibacillary condition the load of organisms was low (5.2%) followed by medium (2.8%), very low (1.7%), and high (0.7%) based on the critical threshold values. Thus, this study reinforces the fact that GeneXpert MTB/RIF assay is a test which is of great value in EPTB.

A strong clinical suspicion and a better and more rapid diagnostic armamentarium substantiate a quicker and confirmative diagnosis of EPTB. Thus, we tried to analyze different rapid diagnostic modalities to construct a well-formed protocol. In a patient with a clinical suspicion of EPTB histopathology and GeneXpert MTB/RIF assay

remain the two most important diagnostic tests for a rapid diagnosis. A recent modification of GeneXpert MTB/RIF assay is the GeneXpert Ultra which has a lower limit of detection of 10 CFU/mL and thus appears to be extremely useful in such paucibacillary samples.[15] Based on the cycle threshold (CT) value, GeneXpert Ultra has an additional result interpretation called Mycobacterium tuberculosis complex (MTB) trace, further strengthening the diagnostic modality. WHO recommends the use of Xpert Ultra as the initial diagnostic test for suspected tuberculous meningitis with a higher sensitivity of 95%, as compared to either Xpert/culture both having a sensitivity of 45%.[16] The limitation of this study is that very few samples were received for culture, so comparison with culture could not be done.

CONCLUSION

The CBNAAT assay (GeneXpert MTB/RIF)is a valuable tool for rapid diagnosis of extrapulmonary tuberculosis. It is an important tool for diagnosis and can be supplementary to other tests like smear microscopy, mycobacterial culture and histopathology. The test shows comparable sensitivity and specificity and can be used for diagnosis of various extrapulmonary tuberculosis samples.

Author's contribution

All authors have substantive intellectual contributions to this study.

Ethical approval

The authors declare that they have taken the Institutional Ethics Committee approval and the approval number is IEC/2020/56/MA05.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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Original Article

Quality of life outcomes in patients of genitourinary tuberculosis undergoing major surgical procedures: A single center experience

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ABSTRACT

Objectives: In India, an estimated 18% of patients suffering from tuberculosis (TB), have Genitourinary tuberculosis (GUTB). Understanding the effect of GUTB on overall health and QoL is essential for providing better patient care and modifying health programs. The present study was designed to provide insight into the change in QoL in GUTB patients following surgical management using the World Health Organisation Quality of Life Brief Version (WHOQOL-BREF) questionnaire.

Material and Methods: A total of 35 patients, who underwent extirpative or reconstructive surgery at our center from January 2016 to December 2021 were included. Demographic profiles, clinical details, laboratory data, radiological imaging findings, and microbiological data were recorded from our database. To assess the impact of the disease and the subsequent surgical treatment, patients themselves completed the WHOQOL-BREF Hindi questionnaires twice, based on the recall method.

Results: The mean age of the study cohort was 36.89±12.64 years. Lower urinary tract symptoms (LUTS) were the most common (68.6%) presenting symptoms. Kidney alone or in combination with other organs was involved in all but six cases, amongst them three cases each exclusively involved the ureter and bladder only. Isolated kidney was involved in 7/35 (20%) patients. Preoperative diversion of the upper tract was needed in 27 patients. Nephrectomy was the most commonly performed surgery (48.6%) overall. Ileal conduit with cystectomy was the most frequently performed reconstructive surgery (14.3%). Amongst all the domains, preoperative mean physical and environmental domain scores improved from 51.08 ± 12.39 to 57.71 ± 14.53 and 55.37 ± 13.41 to 64.02 ± 16.59 after surgery, respectively.

Conclusion: GUTB significantly affects all domains, psychological and physical being the worst affected facets. With surgical treatment, improvement in overall QoL and its domains could be achieved.

Keywords: Genito-urinary Tuberculosis, Nephrectomy, Quality of Life, Tuberculosis, WHOQOL-BREF Questionnaire

INTRODUCTION

India has one of the highest burdens of tuberculosis (TB) worldwide. As per the Global World Health Organization-Tuberculosis (WHO TB) Report 2020, the incidence is 193 per 100,000 population.[1] At present, a microbiological cure remains the litmus indicator for successful TB control services all over the world. Even though a gradually declining trend was noted, still around 10 million people were suffering from symptomatic TB globally in 2019.[1] In India, of all the patients suffering from TB, an estimated 18% of patients have genitourinary tuberculosis (GUTB).[2] GUTB causes significant morbidity as it causes multi-organ involvement. The disease and the treatment both cause considerable morbidity and consequently affect the Quality of Life (QoL) of these patients. Patient perceptions about the disease, sequelae of the disease, and its subsequent effect on QoL remained largely undetermined. It is important to understand the effect of GUTB on health and QoL to improve patient care and formulate health policies related to extrapulmonary tuberculosis.

Assessment of QoL in TB survivors is uniquely tricky as there is a lack of a validated TB-specific QoL calculator. There is difficulty in choosing pertinent control populations and a lack of descriptive and analytical data on the health status of the general population in many TB endemic areas. Therefore, self-reported health-related QoL is an important adjunct measure to understand and subsequently quantify the actual impact of GUTB on affected individuals.

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The WHOQOL-BREF is a person-focused, multilingual tool for the subjective assessment of QoL. Its unique multi-dimensional profile enables it to be used in a wide range of diseases. It has four domains: physical, psychological, social, and environmental. The Hindi version of WHO QoL has been validated in the Indian healthcare setting, making it a valuable tool for assessing QoL in GUTB.[3–5] The present study was designed to look into the changes in QoL in GUTB patients who have undergone major surgical procedures using the WHOQOL-BREF questionnaire.

MATERIAL AND METHODS

In this ambispective study, the electronic database was accessed for details of GUTB patients. Institutional ethics committee approval was obtained (IEC). Patients diagnosed with GUTB and undergoing extirpative or reconstructive surgery from January 2016 to December 2021 were included. Informed written consent was obtained from all the patients. All patients were diagnosed with GUTB based on one or more of the following criteria: microbiological, radiological, or clinical.

Clinical details, demographic details, laboratory parameters, radiological imaging findings, and microbiological data were recorded from our computer database. Any of the positive suggestive findings for GUTB from imaging such as intravenous pyelogram (IVP), micturating cystourethrogram (MCU), nephrostogram, non-contrast computerized tomogram (NCCT), or contrast-enhanced computerized tomogram of kidney ureter and bladder (CECT KUB) were noted. For microbiological diagnosis, acid-fast bacillus (AFB) smear positivity, Cartridge-based nucleic acid amplification test (CB-NAAT), TB- polymerase chain reaction (TB-PCR), or culture positivity in voided urine or urine from percutaneous nephrostomy (PCN) along with typical constellations of symptoms were considered. In the absence of any radiological or microbiological criteria, a history of symptom complex of GUTB was sought and further confirmation with histopathological evidence was done.

Quality of life was evaluated using the WHOQOL-BREF (Hindi variant) questionnaire (Annexure I). It is a truncated 26-item adaptation of WHOQOL-100, which initially had 100 questions. The Likert-type scale was used for scoring in the following four domains:

Domain 1: Physical health

Domain 2: Psychological health

Domain 3: Social relationships

Domain 4: Environment

Patients were called and given all necessary instructions. The change in QoL was assessed by administering the WHOQOL-BREF (Supplementary File 1) questionnaire to the patients on two occasions based on the recall method. The raw scores obtained from the patients on the questionnaire were changed to the transformed score out of 100 in each domain as per the equations for computing domain scores provided by WHO (Supplementary File 2). The first entry corresponding with the QoL before undergoing the surgical treatment, and the second one corresponding to the post-surgery period were noted. The median time to administration of the questionnaire was six months (range 3 months–12 months). Patients with missing values of more than 20% were excluded from the analysis according to the standard rule of the WHOQOL Group (the WHOQOL Group, 1995; World Health Organization, 1996).

Measures of central tendency like mean and median were used with measures of dispersion like standard deviation and interquartile range for continuous data. For qualitative data, frequency and percentage were analyzed. The normality of data was checked with Kolmogorov–Smirnov test and Shapiro–Wilk test. We then compared pre-treatment and post-treatment scores of all questionnaires with Student's paired t-test and compared two means. All the data were analyzed by SPSS version 23 (SPSS Inc, Chicago, IL, USA). The raw scores were converted to transformed domain scores by using SPSS syntax (the scores are converted on a scale from 0 to 100 to enable comparisons between domains composed of unequal numbers of items). P-value ≤ 0.05 was considered significant.

RESULTS

Thirty-nine GUTB patients who met the inclusion criteria were screened, and four patients were excluded from the study because of the unavailability of adequate data. The pre-surgery questionnaire was filled prospectively in 21 patients, whereas 14 patients filled it using the recall method. Of the 35 patients interviewed, six (17.1%) patients had a history of pulmonary TB in the past and none had any evidence of active pulmonary Koch's at the time of seeking treatment for GUTB. The mean age of the study cohort was 36.89 ± 12.64 years. More than 50% 18/35 (51.4%) of the patients were females. Lower urinary tract symptoms (LUTS) were the most common (68.6%) presenting symptom, followed by flank pain and hematuria [Table 1].

Sterile Pyuria was present in 17 (48.5%) patients. Simple microscopy of voided urine identified AFB in 37.1% of patients. Simple microscopy, along with TB-PCR of voided urine, successfully diagnosed 62.8% of GUTB patients. PCN urine was exclusively positive for AFB in 11 (31.4%) patients. Eight (22.8%) patients had concomitant positive bacterial urine culture at the time of presentation. *Escherichia coli* is the most common (11.4%) organism responsible for concurrent

Table 1: Baseline demographic data of enrolled GUTB patients.

Sl. No.	Parameter	Value (n = 35)
1.	Age (years) (Mean ± SD)	36.89 ± 12.64
2.	Sex	Male 17(48.6%), Female 18 (51.4%)
3.	Weight (kg) (Mean ± SD)	55.17 ± 13.13
4.	Height (cm) (Mean ± SD)	159.74 ± 9.91
5.	Fever	7 (20%)
6.	Flank pain	17 (48.6%)
7.	Hematuria	12 (34.3%)
8.	Lower urinary tract symptoms	24 (68.6%)
9.	History of pulmonary tuberculosis	6 (17.1%)
10.	Comorbidity	DM - 2 (5.8%), HTN - 1 (2.9%), CKD - 1 (2.9%)

SD: Standard deviation, DM: diabetes mellitus, CKD: chronic kidney disease, HTN: Hypertension, GUTB: Genitourinary tuberculosis.

Table 2: Imaging investigations.

Sl. No.	Imaging	Finding with number (n = 35)
1.	Organ involved	Kidney only - 7 (20%) Kidney + Ureter - 10 (28.6%) Kidney + Ureter + Bladder - 11 (31.4%) Ureter only - 3 (8.6%) Urinary Bladder only - 3 (8.6%) Kidney + Bladder - 1 (2.9%)

urinary tract infection, followed by *Enterococcus, Klebsiella*, and *Pseudomonas* sp.

Isolated kidney was involved in 7/35 (20%) patients. The rest of the involved organ patterns are depicted in Table 2. A diuretic renogram revealed 18 of 35 patients having unilateral non-functioning kidneys (NFK) (non-visualized on a radionuclide scan) and another eight having poorly functioning kidneys (<10%).

Preoperative diversion of the upper tract was needed in 27 patients. PCN was the most commonly performed procedure (62.8%). Double J Stent was placed in 5/35 patients. Various surgical procedures performed are depicted in Figure 1. Nephrectomy with excision of the affected ureter was the most commonly performed surgery (48.6%). Ileal conduit with simple cystectomy was the most frequently performed reconstructive surgery (14.3%). Clavien-Dindo grade 3 and 4 complications were not seen in any of the patients, whereas grades 1 and 2 were seen in three (5.7%) and four (11.4%) patients.

All the domains in the WHOQOL-BREF score showed improvement following surgery. Physical and environmental

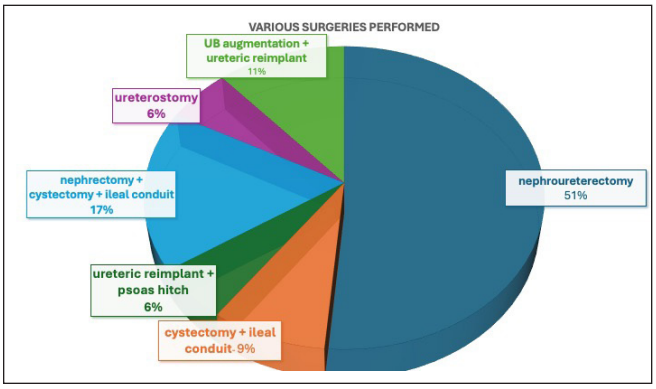

Figure 1: Various surgeries performed in genitourinary tuberculosis patients.

domain scores significantly improved from 51.08 to 57.71 and 55.37 to 64.02 after surgery, respectively. Psychological and social domains also showed improvement in the postoperative period, but the changes were not statistically significant [Table 3]. The change of QoL scoring following surgery was analyzed with respect to organ involvement. Patients with bladder involvement showed statistically significant improvement in physical domain scores ($p = 0.012$) following surgery compared to patients with other genitourinary organ involvement. Change of score following surgery in psychological ($p = 0.55$), social (0.078), and environmental (0.414) domains did not show any significant difference with respect to organ involvement.

Surgery-wise distribution of QoL scores is provided in Table 4. Nine patients underwent cystectomy and urinary diversion (seven patients ileal conduit, one patient continent cutaneous diversion, and one orthotopic neobladder). The mean scores for all the domains pre- and post-operatively, respectively, are as follows: physical (45.6 vs. 73.7), psychological (48 vs. 56.2), social (52.8 vs. 75), and environmental (54.3 vs. 66). There was a statistically significant difference between the pre-and

Table 3: Results of the WHOQOL-BREF Hindi questionnaire.

Sl. No.	Questionnaire	Pre-treatment value (mean ± SD)	Post-treatment value (mean ± SD)	P-value
1.	Physical domain score	51.08 ± 12.39	57.71 ± 14.53	0.021
2.	Psychological domain score	49.34 ± 15.68	54.31 ± 15.25	0.057
3.	Social domain score	54.42 ± 18.54	61.08 ± 18.34	0.051
4.	Environmental domain score	55.37 ± 13.41	64.02 ± 16.59	0.001

SD: Standard deviation.

Table 4: Surgery-wise average scores distribution.

Surgery	Physical (Pre vs. Post)	Psychological (Pre vs. Post)	Social (Pre vs. Post)	Environmental (Pre vs. Post)
Nephrectomy	54.8 vs. 53.3	52.2 vs. 53	53.2 vs. 53.7	57.9 vs. 64.8
Ureteric reimplantation/Ureteroureterostomy	49.17 vs. 49	42.83 vs. 49	59.5 vs. 60.33	51.33 vs. 59/5
Cystecomy + Urinary diversion	45.6 vs. 73.7	48 vs. 56.2	52.8 vs. 75	54.3 vs. 66
Augmentation cystoplasty	47.25 vs. 53.25	45.25 vs. 51.5	48.25 vs. 68.75	48.75 vs. 67.25

Pre: pre-surgery scores, post: post-surgery scores.

post-surgical scores for all the domains. On the contrary, the patients who underwent nephrectomy alone did not show a significant difference in pre-and post-surgical scores: physical (54.8 vs. 53.3), psychological (52.2 vs. 53), social (53.2 vs. 53.7), and environmental (57.9 vs. 64.8). Similar scores were observed for patients undergoing ureteric reimplantation [Table 4]. It is to be noted that many patients underwent more than one surgical procedure.

DISCUSSION

The focus of TB management in individuals with multi-organ involvement is targeted at giving a better QoL. Individuals afflicted with GUTB undergo significant changes in QoL due to the disease itself, treatment-related adverse effects and sequelae of the disease. Long-duration treatment schedules for months with multiple drugs, the need for urinary diversion, and reconstructive and extirpative surgical procedures affect patients in all domains of health and carry a lasting impact on the individual. Health-related quality of life (HRQoL) measures the repercussions of a disease on a patient's day-to-day activities, functional state, behavior, and perceived health. HRQoL recently became more relevant in widening the concept of measuring health status beyond conventional indicators such as mortality and morbidity.[6, 7]

In this context, our findings highlight the improvement in post-treatment HRQoL of GUTB patients as measured by the WHOQOL-BREF questionnaire. In this study, we tried to find the effect of surgical intervention for GUTB on QoL. In two separate studies from Asian countries, it was seen that females were more affected in social domains,[8] but in our study, patients of both genders were affected equally and improvement in QoL scores post-surgical intervention was comparable in both the sexes.

All the domains in the WHO-QoL BREF questionnaire revealed a set of low baseline scores. This could be attributed to the affliction by the disease itself and the delay in seeking treatment in our part of the world. End organ damage requiring nephrectomy in 51% of the patients reflects the delayed presentation on the part of the patients. This could be attributed to the poor socioeconomic status of the patients and lack of education. Extirpative and reconstructive surgeries were almost equally performed, simple nephrectomy being the most common surgery among all. The surgery was performed by Urology Consultants along with the senior residents with good experience in handling these cases. Most of the nephrectomy and nephroureterectomy were performed laparoscopically, while ureteric reimplantation, cystectomies with urinary diversion, and augmentation procedures were performed by an open approach. PCN was a more commonly used method of diversion as compared to Double J stent. This probably stems from the fact that these patients presented to emergency with infected hydronephrosis and PCN drainage was thought to be a better mode of diversion. Double J stenting was attempted in the majority of these patients. However, these patients had either obliterative strictures, renal pelvic scarring, or small capacity bladder, which precluded the retrograde or antegrade stent placement. Three of these patients had more than one PCN. Removal of these tubes itself is contributory to the improvement in QoL scores.

Even though the kidney was the most commonly involved organ, the QoL improvement following surgery was better seen with patients with bladder involvement. On further analysis, cystectomy and urinary diversion were found to be the surgery with the most profound effect. The same effect was observed in patients undergoing augmentation cystoplasty. Our findings imply that LUTS, the presence of catheters interferes with the QoL aspects of life significantly more than the symptoms arising from upper tract involvement. Rushing to the bathroom and the inability to travel and work due to LUTS or small capacity bladder leads to a significant impact on the QoL of these patients. The same is reflected in Table 4. Patients who underwent cystectomies or augmentation showed significant improvement in all the domains of QoL as compared to other surgical procedures. Although it is perceived that the application of a Conduit bag may lead to body dysmorphism, it was not noted in our cohort, as seen in Table 4. Also, it is notable that for surgeries such as nephrectomy and ureteric reimplantation, not much difference was found between the pre-surgery and post-surgery scores. Gender-wise, no difference was seen in terms of sexual function between males and females after cystectomy (73.8 vs. 76.5).

We found that the worst affected domain was the psychological domain. A wide arena of psychological reactions has been observed in patients with TB. Denial, worry about the diagnosis, depression, reduced sexual desire, tiredness, sleep cycle abnormalities, and anorexia are pretty common following diagnosis, and they mostly stem from fear of social retreat.[9–12] More significantly, hospitalization and isolation of patients have been seen to have substantial emotional and psychological offshoots.[11,13] Early identification and adequate treatment can dampen these psychological issues.

A study from South Africa on patients with pulmonary Koch's used the Hospital Anxiety and Depression Scale (HADS) and showed that there was an improvement by +95% in both anxiety and depression domains, which led to a change from "moderate problems" to a state of "no problems".[14] Also, in our study there was an improvement of QoL in the psychological domain, but it did not reach statistical significance. Good empathetic communication and attention to minor details should be an inherent part of management, especially during diagnosis and treatment initiation. Adequate social and psychological support from family, friends, and community has been seen to give a better QoL to these patients.[15] This social support and out-of-hospital care, which is usually lacking in our part of the world, needs to be strengthened further.

Physical functioning is a state which describes an individual's capability to carry out daily activities self-care, and to fulfill the entitled and pre-desired roles at home, work, and in society. The diagnosis of tuberculosis in the family increases the workload on the family, especially on first-degree relatives, which further diminishes the caregiver's capability.[16] Physical domain scores in the preoperative period were also low in our study population, as mentioned previously, which subsequently improved in the postoperative period.

The environmental domain, which relates to the sense of safety, home environment, transport, and financial security, also showed significant improvement following treatment. Particular attention should be paid to culturally relevant and targeted interventions for TB-affected persons, especially in the early months of treatment, to integrate patients back into their communities as quickly and effectively as possible.

One of the essential dimensions affecting QoL in TB patients is secondary to the social blemish, which comes from family and the community.[11,17] This stigma is even more prevalent in third-world countries. In one of the studies from urban Zambia, 82% of TB patients reported the fear of social seclusion at some point of the disease.[18] Even though a lesser number was seen in a study from southern India, about 51% of patients felt the repercussions.[19] In our study, we saw that the mean social domain score was affected, and even though it showed improvement in the postoperative period, it could not reach statistical significance. The appointed health personnel should be categorically trained to the special needs of these patients so that they can identify specific mental and physical health components and offer help. Primordial prevention can only come from promoting awareness, which can bring down the severity of social stigma. There is an urgent need to understand the origins of misbeliefs about TB and its curability with early interventions.

Patient-reported outcomes (PROMS) are the outcome of interest in most disease conditions. One such outcome is QoL, besides the physical health of an individual. Our study is the first of its kind that explored the QoL in a systematic way in patients suffering from GUTB. The limitation of our study is a component of recall bias in patients reporting pre-surgical QoL scores. Nevertheless, the study highlights the importance of patient-reported outcomes, especially of QoL, in patients suffering from GUTB as this is a prevalent disease in our part of the world and taking a step forward for out-of-hospital care for these patients.

Disease eradication is the latest goal of the National Tuberculosis Elimination Program (NTEP), but the disease and its treatment leave sequelae far outreaching inpatient care. Only bacteriological cure should not be the goal, but the ongoing programs should follow the participants till they fully recover health in all dimensions, allowing their complete return to society. At a higher level, policy strengthening, environmental protection, and livelihood interventions should be made.[20] Outreach programs to educate and support TB patients, at-risk community members, and healthcare providers may prove useful.

CONCLUSION

Genitourinary tuberculosis significantly affects the QoL of the patients in all the domains with psychological and physical being the worst affected facets. The surgical management of GUTB improves both domain-wise and overall QoL. Bladder involvement causes the maximum deterioration of QoL and surgical procedures for the same have a maximum impact on QoL.

Ethical approval

Institutional ethics committee approval was obtained From Ethics Committee at PGIMER, Chandigarh. INT/IEC/2021/SPL-1194 dated 12/08/21.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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Original Article

Attention Deficit Hyperactivity Disorder and dopamine D4 receptor (DRD4) polymorphisms in South Indian population

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ABSTRACT

Objectives: Four decades of research have found that Dopamine D4 Receptor (DRD4) is the major candidate gene however, few studies have supported the association between the DRD4 exon III long seven repeat allele and Attention Deficit Hyperactive Disorder (ADHD). Two Indian studies had shown there is an association between DRD4 7 repeat allele; hence, we investigated in the south Indian population. AIMS: To study the association of DRD4-EXON-3-7R long allele and minor physical anomalies with ADHD in comparison to age & sex-controlled normal subjects with no evidence of ADHD. settings and design-cross-sectional case-control study for two years at National Institute of Mental Health And NeuroSciences, Bangalore.

Material and Methods: 60 children with ADHD and 60 healthy children of 4-16 years of age group were recruited after informed consent. Assessed by DSMIV-TR, ADHD RS IV HOME VERSION 18 items, comorbidities by detailed interview of child and parents using Mini-International Neuropsychiatric Interview for Children & Adolescents (M.I.N.I). Kid for minor congenital anomalies modified waldrop scale & for the perinatal complications, Lewis Murray Obstetrics Complication Scales were applied. For the family history family interview for genetic study, global functioning was measured by children global assessment scale, neuropsychological tests of response inhibition test were used and blood samples was collected for genotyping.

Results: The genotype 2 2,2 4 ,4 4,4 5,4 7 repeat allele has shown equal distribution between cases and controls with p-value 0.492 with no significance.

Conclusion: There is no association between DRD4 EXON-3-7R long allele gene polymorphism and ADHD in South Indian population. DRD4 7R could be having influence on minor physical anomalies in ADHD.

Keywords: ADHD-Attention Deficit Hyperactivity Disorder, DRD4 7R – Dopamine D4 Receptor 7Repeat allele, VNTR – Variable Number Tandem Repeats

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric problem with onset in early childhood. ADHD is a condition characterized by inattention, impulsivity or both, and hyperactivity with onset before the age of seven years, giving rise to significant academic, social, and emotional problems at home and at school.[1]

ADHD is a polygenic disorder with more than 30 dopaminergic, noradrenergic, serotonergic, and gamma-aminobutyric acid neurotransmitter genes known to contribute to its susceptibility. Evidence of genetic susceptibility comes from several studies on a repertoire of genes, including Dopamine D4 Receptor (DRD4), dopamine receptor, DRD5, dopamine beta-hydroxylase gene, Serotonin transporter Gene (5HTT), 5HydroxyTryptamine Receptor 1B Gene (HTR1B), and Synaptosomal Associated Protein of 25kDa (SNAP25).[2] In India, there is very little systematic research on ADHD in children.[3]

Meta-analysis has shown a statistically significant association between ADHD and dopamine system genes, especially DRD4 and DRD5.[4] Four decades of research have found that DRD4 is the major candidate gene, however, few studies failed to support the association between the DRD4 exon III long seven repeat allele and ADHD.[3,4,5]

Dopamine is the key neurotransmitter in the development of ADHD. Evidence to support dopaminergic dysfunction in ADHD derives from three research areas: the neuropharmacology of stimulant medication[4,6] the behavior and biochemistry of animal models, and neuroimaging studies in ADHD adults.[4] Various meta-analyses have shown

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consistent evidence of the association of many candidate genes with childhood ADHD. Significant associations were identified for several candidate genes including DAT1, DRD4, DRD5, 5HTT, HTR1B, SNAP25, and also significant heterogeneity was observed for DBH, ADRA2A, 5HTT, TPH2, and MAO-A.[4,6,7,8] From 1991 to 2004, including three genome-wide linkage studies and association studies of 94 polymorphisms in 33 candidate genes.Evidence for association exists for four genes in ADHD: the dopamine D4 and D5 receptors and the dopamine and serotonin transporters.[9] Family association studies examined genetic components in the etiology of ADHD by using the Transmission Equilibrium test for the association between ADHD and DRD4 7R allele.[4]

As heritability is high, there is a 2–8-fold increase in risk for ADHD in children whose parents had ADHD, while twin studies attribute 80% of the etiology to genetic factors. The mean heritability estimates of 76% amongst twins indicate ADHD is the most heritable psychiatric disorder.[4]

Minor physical anomalies (MPAs) are congenital abnormalities of body structure as they develop from the same ectoderm layer in the embryo, which reflect fetal maldevelopment. MPAs are markers of central nervous system anomalies. High MPA counts have been associated with hyperactive behavior in normal boys and with inhibited behavior in normal girls.[10]

DRD4 STRUCTURE AND FUNCTION

The dopamine D4 receptor structurally and pharmacologically resembles the dopamine D2 and D3 receptors. The dopamine D4 receptor gene is located on chromosome 11p15.5[11,12] and contains a remarkable number of polymorphic regions. There is a hypervariable region in the third cytoplasmic loop of the dopamine D4 receptor gene consisting of 2–10 imperfect 48 base pair repeats.[13] Therefore, the D4 receptor isoforms differ in the length of the third cytoplasmic loop and have 1, 4, 7, or 11 times the same insert of a stretch of 16 amino acid residues in their protein structure. The dopamine D4.2, D4.4, and D4.7 receptor alleles occur the most frequently, the ancient polymorphisms[14] but there is considerable variation in the distribution of alleles depending on ethnicity.[14,15] The universality of the polymorphism with only three common repeat-number alleles (4, 7, and 2) indicates that the polymorphism is ancient and arose before the global dispersion of modern humans. Various meta-analysis studies have shown varying genetic associations of DRD4 with ADHD.

INDIAN CONTRIBUTION

An Indian study has found that ADHD transmission of different polymorphisms of the DRD4 in different ethnic groups. Bhaduri *et al.* study in 2006 is the first report on the transmission of different polymorphisms of DRD4 in Indian subjects.The transmissions of 6 and 7 repeat alleles of exon 3 48-bp Variable Number Tandem Repeats (VNTR) showed a significant association with ADHD.[16] Das M *et al.* study in 2011 showed significant preferential transmission of the 7R-T (DRD4 exon3 VNTR-rs1800955) and 3R-T (MAOA-u VNTR-rs6323).[17] Haplotypes were noticed from parents to probands of East Indian population. However, Stanley *et al.* (2017)'s Mumbai-based study has failed to support the association between the DRD4 exon III long seven repeat allele.[18] There is a paucity of Indian studies to generalize the association between DRD4 and ADHD in Indian population, hence this study was conducted.

MATERIAL AND METHODS

After getting approval from the Institutional Ethics Committee of National Institute of Mental Health and Neurosciences Bangalore, assent from children with written informed consent from parents was taken.

Study design: Cross-sectional case–control study.

Inclusion criteria: Cases of 60 children aged 4–16 years who were diagnosed to have ADHD as per DSM-IV TR from inpatient & outpatient of child and adolescent psychiatry services. sixty controls of children aged 4–16 years with no ADHD were recruited from Indira Gandhi Institute of Child Hospital, Bangalore.

Exclusion criteria: *Children* with identifiable dysmorphic syndrome, pervasive developmental disorders, any form of mental retardation including Fragile-X syndrome, and any serious systemic illness like cardiac, renal, or liver failure were excluded.

Phenotype assessment

1. **Mini Kid** developed by David Sheehan *et al.* was used for brief structured diagnostic interviews for children and adolescents within 15 min. Children under 13 years of age were interviewed in the presence of their parents.[19]
2. **Modified Waldrop Minor Congenital Anomaly Scale:** This scale assesses MPAs of the head, eyes, ears, mouth, hands, and feet, as it takes only 15 min with very minimal removal of clothing. It's a simple instrument with good inter-rater reliability and inter-scorer agreement. The coefficient of correlation of the scale has been found to be +0.84.[20]
3. **Lewis Murray Obstetrics Complication Scale** (LMOCS) rates 15 obstetric complications as absent or definitely present; 9 items of the exposure can also be rated as equivocally present. It provides a measure of perinatal insults that may affect brain development from case notes, birth records, and maternal interviews.[21]
4. **Family Interview for Genetic Study** (FIGS) is a guide for gathering genetic diagnostic information about relatives in the pedigrees being studied.[22]

5. **ADHD-Rating Scale IV Home Version** (ADHD-RSIV) is a revised version that can be completed by either parents (home form) or teachers (school form). There are two subscales, Inattentive and Hyperactive/Impulsive subscale. It can be administered to 4–20 years old. The test has good psychometric properties, particularly reliability and discriminant validity, making it especially useful for clinical samples. The ADHD RS-IV has high utility for multiple applications due to its quick completion, easy scoring, and sensitivity to treatment that was used to measure Inattentivity and Hyperactivity/Impulsivity in children.[23,24] Response Inhibition Test.
   a) **Stroop Color Word Interference Test:** These measures selective attention, cognitive flexibility, and processing speed. In this test, subjects have to read the names of colors, naming colors, and naming color names that are printed in the color chart. The score is the amount of time needed by the subject to correctly identify the items per page and the number of errors committed. The last task has an interference component because it requires the subject to override or inhibit a reading response. It is a measure of executive functioning, cognitive flexibility, and the ease with which a person can shift his or her perceptual set to conform to changing demands and inhibit usual response from interfering with the unusual one.[25] An increase in Stroop interference is seen in ADHD.
   b) **Go/No – Go TEST** is a measure of one's ability to suppress reflexes motor impulses and is done in two parts. First, the subject has to get into the set with contrasting movements, i.e., when the examiner shows one finger, the subject has to show two, and when the examiner shows two fingers, the subject has to show one. The sequence of movements is performed with the dominant hand. The subject is instructed that the rules would be changed, i.e., when the examiner shows two fingers, the subject has to show one finger, and when the examiner shows one finger, the subject has to do nothing. The correct responses, as well as the errors, are noted and form the score, which was used to measure a child's ability to suppress reflexes and motor impulses.[26]

Children's Global Assessment Scale Score (CGAS) (Schaffer *et al*. 1983)[27] an instrument that provides a global measure of level of functioning in children and adolescents. It is designed to reflect the lowest level of functioning for a child or adolescent during a specific time period. The measure provides a single global rating, on a scale of 0–100, where scores above 70 indicate normal function. In rating, the clinician makes use of the glossary details to determine the meaning of the points on the scale. This assesses current functioning and retrospective measures of the highest past functioning and the worst past functioning for a period of 1 month. The reliability has been reported to be kappa = 0.61 (Hanssen-Bauer *et al.* 2007).[28]

METHODOLOGY

Genotyping

Five (5) ml of venous blood drawn from subjects under aseptic conditions & DNA was isolated from leukocytes nuclei by salting out method (Miller *et al.* 1988).[29]

Principle

Detergents can solubilize lipids in the cell and nuclear membranes, thus releasing DNA into solution. High salt helps in the precipitation of the excess protein in the solution. The residual protein is further degraded by the addition of a special kind of protease called Proteinase K. High-molecular weight DNA is precipitated using cold absolute ethanol.

DRD4 gene is extremely polymorphic. It has one polymorphism located in the third exon coding for the third cytoplasmic loop of the receptor and consisting of a variable number of copies ofa 48 base pair sequence, from 2 to 10.

Primers flanking the third cytoplasmic loop repeat region were used for Polymerase Chain Reaction (PCR).

Forward primer -- 5′TGTGGTGTAGGGAACGGCCTGAG 3′

Reverse primer -- 5′CTTCCTGGAGGTCACGGCTCAAGG3′[30]

Polymerase chain reaction (PCR) was carried out for 30 cycles along with thermostable DNA polymerase enzyme, TAQ POLYMERASE, at an annealing temperature of 61°C, and then products were resolved on 2.5% agarose gel, fragment sizes were determined by comparison with molecular weight, standards, and tested for 2, 4, 5, and 7 repeat alleles of DRD4 exon 3–48 base pairs.

Statistical Analysis

The data sheets were coded and analyzed using descriptive statistics such as means, frequency distributions, percentages, and standard deviation. For continuous variables, the parametric Student's '*t*' test was used to compare the means between the two groups andnon-parametric. There is an increase in MPAs and DRD4 present ADHD cases.

Tests such as chi-square, and Mann–Whitney *U* test were used for categorical variables through Statistical Package for Social Sciences (SPSS13.0).

Hardy–Wienberg equilibrium states that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors ($p^2 + q^2 + 2pq = 1$). It was checked in all sample distribution of genotypic & allelic frequencies and were compared in cases and controls using

chi-square test. The group was in Hardy -Weinberg equilibrium. The data does not follow the Test of Normality (Kolmogorov -Smirnov Z), so the non-parametric tests were used.

RESULTS

Table 1: Age of onset & severity of ADHD.

	Mean (in months) & SD	Results (%)
Age of onset ADHD	31.05 (13.73)	Male 49 (81) Female 11 (19)
Age of 1st assessment	70.87 (32.71)	
ADHD RS-IV severity	34.95 (10.23)	p = 0.0001
CGA score	59.83 (11.57)	

ADHD RS-IV: Attention Deficit Hyperactive Disorder Rating Scale IV; CGA score: Children's Global Assessment Scale Score; SD: Standard deviation.

Table 2: Case parameters.

Scales	Cases Mean & SD	Controls Mean & SD	Results
Waldrop minor congenital anomaly scale	3.67 (1.90)	2.0 (1.27)	p = 0.0001*
Lewis-Murray obstetric complication scale	0.45 (1.26)	0.02 (0.13)	p = 0.010*

* p value is significant at 0.05; SD: Standard Deviation.

Table 3: Stroop test.

Cases Mean (s) & SD	Controls Mean (s) & SD	Results
139.75 (39.15)	106.64 (24.19)	0.009*

SD: Standard Deviation. * p value significant <0.05.

Table 4: Genotype frequency data of DRD4.

Genotype alleles	Cases		Controls	
	Frequency	Percentage (%)	Frequency	Percentage (%)
2 2	0	0	3	5
2 4	6	10	4	6.7
4 4	49	81.7	48	80
4 5	1	1.7	1	1.7
4 7	4	6.7	4	6.7
Pearson chi-square test	*p*-value = 0.492. No significance.			

DRD4: Dopamine Receptor D4.

Table 5: Minor physical anomalies and obstetric data.

	Case	Controls	Mann–Whitney *U*	*p*-value significance
Waldrop score	75.48	45.52	907	0.0001*
LMOC score	67.54	53.46	1377	0.0001*

* p value significant < 0.05; LMOC: Lewis Murray Obstetrics Complication.

Table 6: ADHD RS-IV score and DRD4 7R in cases.

DRD4 7 repeat	ADHD RS-IV score	Mean ranks	Mann–Whitney u	Significance
Present	4	38.75	79	0.349
Absent	56	29.91		

ADHD RS-IV: Attention Deficit Hyperactive Disorder Rating Scale IV; DRD4 7R – Dopamine D4 Receptor 7Repeat allele.

Table 7: MPA score and DRD4 7R in cases.

DRD4 7 repeat	Waldrop score	Mean ranks	Mann–Whitney *U*	Significance
Present	4	29.63	63.5	0.143
Absent	56	42.62		

MPA: Minor physical anomalies; DRD4 7R – Dopamine D4 Receptor 7Repeat allele.

DISCUSSION

There is a gap between the onset and first consultations of 32 months, which is consistent with Malhi *et al.*'s study, as shown in Table 1.[3] ADHD subtypes—combined were 76.6%, 18.3% of inattentive, 1.7% of hyperactive-impulsive, and 3.3% of residual type, among which males were higher in numbers which is consistent with Malhi *et al.*'s study.[3] It showed higher rates of ADHD in males as compared to females, which varies between 3 and 7:1, in our study, the M:F is 4:1 which is consistent with the Mukhopadhyay *et al.* study.[31] The ADHD RS IV score in DRD4 7R cases present was 38.75, whereas it was 29.91 in the DRD4 7R absent cases. Though this was not statistically significant, but there appears to be a trend of greater severity of ADHD in the presence of DRD 4 7R allele.

The Child Global Assessment Score (CGAS) in cases had shown a Mean of 59.83 in variable functioning with sporadic difficulties or symptoms in several but not in all areas. Disturbance would be apparent to those who encounter the child in a dysfunctional setting or time but not to those who see the child in other settings.

The majority of studies on ADHD have supported a strong familial nature of this disorder as 2–8-fold increase in the risk for ADHD in parents and siblings of children with ADHD. In fact, the mean heritability was shown to be 76% (Bovincini *et al.*, 2020),[4] which is comparable to other neuropsychiatric

disorders such as schizophrenia or bipolar disorder. Butin our study, we had 11.6% of cases with a family history of any psychiatric illnesses, among which 3.3% had ADHD, which is the least, as we have not done the familial genetic analysis.

In ADHD, it is shown that there are higher rates of MPAs and obstetrical complications, which have been reported in those with inattention and hyperactive behaviors. In our study, Waldrop minor congenital anomaly score ($p = 0.0001$) and Lewis Murray Obstetric Complication score ($p = 0.01$) showed significant differences between cases and controls in Table 2 and consistent with Fogel CA *et al.*'s study.[10]

An increase in stroop interference shown by meta-analysis which was tested by using stroop color word interference test. In the neuropsychological test, 81.7% of cases completed the Stroop test, and 19.3% (those who could not do the Stroop test) were administered the go-no-go test. In the control group, 90.0% could complete the Stroop test, and the remaining 10% did the go-no-go test. There was a statistically significant difference between the cases and controls [Table 3]. 28.8% had specific learning disorders, 26.6% had expressive language disorder, and seizure disorders were 21%. This is consistent with the studies by Bush, Lansbergen, and Lopez *et al.*[32-34]

In our study [Table 4, Figure 1], the VNTR alleles of the DRD4 gene exon 3 found include 2-repeat (R), 4R, 5R, and 7R alleles. We have found 4R allele is the highest which is 81% and is consistent with the worldwide prevalence of 64.3% (Polanczyk G *et al.* 2007).[1] The second highest is longer 7R with about 6.7% but the global prevalence is 20.6% and it differs in Americans with 48.3%, and least in Southeast Asia with 1.9%. The third most common is the shorter 2R allele which is 5% in our study, as compared to global prevalence of 8.2%, and it has shown to be highest in Southeast Asia (18.1%). None of the alleles were significantly associated with ADHD cases, which is consistent with one Mumbai-based study (Stanley *et al.* 2017) and 42 similar studies shown in the meta-analysis of Bonvicini, Gizer *et al.*'s study.[4,35-37] However, 34 studies and 2 Indian studies (Bhaduri, Das M *et al.*) on subjects from Eastern India has shown an association of DRD4 Exon3-7R and ADHD (Bonvicini, Wang, Gizer, Sánchez-Soto, Li *et al.*).[4,16,17,35-40] These results indicate a possible difference in the allele frequencies of the DRD4 VNTR's across different ethnic groups.

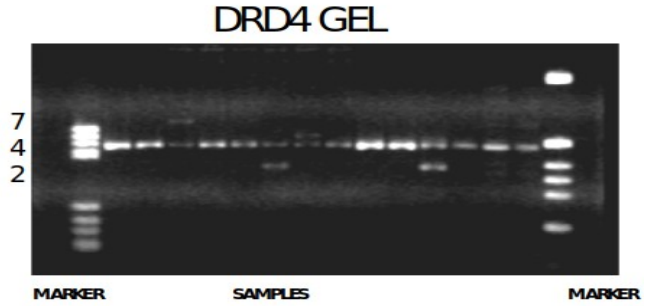

Figure 1: Variable Number Tandem Repeats (VNTR) polymorphism fragments of DRD4 7repeat allele gene photograph.

Lanes 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

This is the photograph of the 2.5% agarose gel showing the DRD4 7R gene alleles under ultraviolet illumination, after the completion of gel electrophoresis. The sizes were determined after comparing the allele size with standard marker which was loaded in the agarose gel, along with the amplified polymerase chain reaction (PCR) products of the subjects. In the above photograph, the marker is present on the left- and right-hand side. Lanes 1,16- Bands were compared with standard DNA marker 1-PBR, 16- Haelll Digest

Lanes 2,3,5,6,9,10,11,13,14,15- genotype of 4 4

Lane 4 ---------- genotype of 4 7

Lanes 7, 12 ---------- genotype of 2 4

Lane 8 ---------- genotype of 2 2

In Table 5, the mean Waldrop score ($p = 0.0001$*) and mean Lewis Murray Obstetrics Complication Scale (LMOCS) score ($p = 0.0001$*) was higher in the cases as compared to controls, which is statistically significant. Table 6 shows mean ranks between the DRD4 7R present and absent group in cases with ADHD RS-IV score. On Mann–Whitney U non-parametric test, there is no significant difference, which is consistent with Fogel CA *et al.*[10] There was no association between DRD4 7 repeat allele and ADHD. In Table 7, there is no significant association between MPA and DRD4 7R allele. The MPAs are indicating that some early embryonic either genetic or non-genetic had played a role in the genesis of ADHD in this population.

STRENGTHS

The researchers were blind to the genotyping of the sample, and the accuracy was 99.52%. There was no ethnicity problem, as all subjects in the study were South Indians. The current study adds to the knowledge of the present status of DRD4 7R allele and the ADHD, given the fact that ADHD is a polygenic disorder with variable environmental influence.

LIMITATIONS

In view of the low sample size, the influence of predictor variables, like psychosocial adversities, could not be analyzed. Chances of type II error are high due to the small sample size. Hence, the generalizability of the finding to our population is difficult and needs further validation with a larger diverse sample size and family-based studies to find any significant association of the DRD4 7 R allele with ADHD.

CONCLUSION

Cases with DRD4 7R allele had a non-significantly higher mean rank of ADHD RS-IV score but no association.

In our study, there is no association between DRD4 7R and ADHD.

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Ethical approval

The research/study approved by the Institutional Ethics Committee of National Institute of Mental Health and Neurosciences Bangalore, number NIMHANS 59th 59 IEC/8.09/21-05-2008.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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Brief Report

Comparison of hematologic parameters as predictors of severity in COVID-19: A single-center cross-sectional study

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ABSTRACT

COVID-19 disease was a global pandemic that marred humanity globally from 2019 end till early 2023. The diseases caused by the novel SARS cov2 virus had a wide array of presentations, from asymptomatic to mild illness to severe acute respiratory illness, with patients presenting with 'happy hypoxia and sudden deterioration of clinical condition to multiorgan involvement and proven to be fatal in many cases. There was an effort to identify a point-of-care test to screen patients so as to predict the disease course; therefore, this study was designed to compare hematologic parameters to predict the severity of the disease. This study showed that TLC,dNLR, and NLR can be effectively used in diagnosing severe COVID-19.

Keywords: COVID-19, haematologic parameters, severity

INTRODUCTION

The emergence of SARS-CoV-2, widely recognized as COVID-19, originated in December 2019 in Wuhan, China. Within no time, it evolved into a highly contagious pandemic, and its rapid spread and severity led to its classification as a global health emergency.[1] SARS-CoV-2 belongs to the novel coronavirus family, Coronaviridae (subfamily Coronavirinae), with a host range spanning from bats to humans.[2] COVID-19 is a complex ailment affecting multiple systems, but it primarily impacts the respiratory system.[3] Individuals with mild-to moderate cases often exhibit flu-like symptoms—fever, dry cough, and breathlessness. Many recover through isolation and medical care, yet a subset experiences rapid deterioration or enters a critical state early on, indicating severe disease. This severe manifestation often results from an immune response imbalance leading to an excessive release of cytokines, termed the "cytokine storm" syndrome. Elevated cytokine levels trigger swift leukocyte recruitment to organs, particularly the lungs, culminating in acute respiratory distress syndrome (ARDS). The progression involves monocytes, macrophages, dendritic cells, and lymphocytes, collectively contributing to the cytokine storm. Critically ill patients deteriorate into nonresponsive respiratory failure, ARDS, and multiple organ dysfunction syndrome (MODS), posing significant challenges for medical professionals.

Prompt identification of high-risk patients and early intervention using point-of-care tests can facilitate targeted allocation of resources and categorization into mild, moderate, and severe groups. This strategic approach optimizes healthcare delivery, potentially leading to reduced mortality rates through specialized critical care. In addition, this approach aids in recognizing potential severity within mild to moderate cases, enabling intensified monitoring and timely intervention.

Essential to this approach are readily accessible biomarkers predicting systemic inflammation, potentially serving as prognostic indicators. Parameters like peripheral white blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR), derived neutrophil-to-lymphocyte ratio (d-NLR, calculated by dividing neutrophil count by the result of WBC count minus neutrophil count), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR) have established roles in predicting systemic inflammation across diverse diseases. Elevated NLR correlates with COVID-19 severity and mortality.[4] Though studies have explored NLR, d-NLR, PLR, and LMR as prognostic indicators for COVID-19,

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a consensus on their relationship with clinical prognosis remains elusive. Consequently, our study aims to evaluate these hematological parameters in both mild to moderate and severe COVID-19 cases at our center. This study seeks to compare complete blood count (CBC) findings, NLR, d-NLR, PLR, and LMR between mild to moderate and severe COVID-19 cases.

MATERIAL AND METHODS

This cross-sectional study received ethical approval from the Institutional Ethical Committee for Human Research. The study involved a sample of 27 COVID-19 cases who were admitted to the center. Among these, 12 cases were categorized as mild-to-moderate COVID-19, and 15 cases fell under the severe category. The inclusion criteria included admitted patients with confirmed SARS-CoV-2 virus infection, verified by a positive reverse transcriptase polymerase chain reaction (RT-PCR) test. Both mild-to-moderate and severe cases were considered for inclusion, while pediatric COVID-19 cases were excluded from the study.

The categorization of cases into mild, moderate, and severe was based on the clinical criteria established by the Ministry of Health and Family Welfare (MOHFW), Government of India (Clinical Management Protocol COVID-19 Version 6). Mild COVID-19 cases exhibited symptoms of upper respiratory tract infection without shortness of breath and with normal SpO_2 levels. Moderate cases showed signs of difficulty in breathing, fever, cough, SPO_2 levels between 90 and 93% on room air, and a respiratory rate of ≥24/min. Severe cases displayed clinical pneumonia symptoms with SPO_2 levels below 90% on room air, a respiratory rate exceeding 30/min, and indications of ARDS, sepsis, or septic shock.

Each case underwent detailed clinical history and examination recording. Venous blood (2 mL) was collected and stored in Ethylene diamine tetra acetic acid (EDTA) vials. Using an Automated Hematology Analyzer (Mindray BC 6800), various CBCs (including hemoglobin, total red blood cell (RBC) count, RBC indices, total leukocyte count (TLC), differential leukocyte count, and platelet count) were measured. NLR, dNLR, PLR, and LMR were manually calculated from CBC values. dNLR was computed as the absolute neutrophil count divided by the difference between the total leukocyte count and absolute neutrophil count.

Statistical analysis was conducted using SPSS v.26. For comparison between the two patient categories, the independent *t*-test was employed when data exhibited normal distribution and equal variances. In cases of skewed and kurtotic data, the Mann–Whitney U test was utilized. The receiver operator characteristic (ROC) curve, along with Youden J statistics, were employed to identify cutoff values. In addition, binary logistic regression analysis was performed to assess variables as prognostic factors, determining odds ratios. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 presents an overview of the collected data. Our study found statistically significant differences in several parameters between severe and mild COVID-19 patients. Specifically, the TLC exhibited a significant increase ($p < 0.05$) in severe COVID-19 cases (Mean ± SD: 19840 ± 8305) compared to mild cases (Mean ± SD: 7508 ± 2227). NLR and dNLR also showed substantial elevation ($p < 0.05$) in severe cases (NLR Mean ± SD: 11.2 ± 6.8, dNLRMean ± SD: 6.7 ± 3.3) in contrast to mild cases (NLR Mean ± SD: 5.4 ± 4.2, dNLRMean ± SD: 3.5 ± 2.7). However, no statistically significant difference was observed between the two groups for the remaining parameters, including platelet count, PLR, and LMR.

Table 1: Depicts TLC, NLR and dNLR in recognizing severe COVID 19 infections.

Parameter	Senstivity	Specificity
TLC	93.3%	91.7%
NLR	73.3%	72.7%
dNLR	80%	81.8%

TLC: Total leukocyte count, NLR: Neutrophil-to-lymphocyte ratio, d-NLR: derived neutrophil-to-lymphocyte ratio.

To assess the diagnostic utility of these parameters, an ROC curve analysis was conducted, yielding the corresponding area under the curve (AUC) values. Figure 1 shows the ROC curve which has been attached as a jpg attachement highlighted by the icon. For the total leukocyte count (TLC), the AUC was 0.976. The AUCs for NLR and dNLR were 0.770 and 0.800, respectively. Utilizing Youden J statistics (Sensitivity + Specificity − 1), optimal cutoff values were identified: 10,950 for TLC, 6.19 for NLR, and 4.13 for dNLR. These values were then evaluated against the clinical diagnosis as the gold standard. As a result, the total leukocyte count (TLC) demonstrated a sensitivity of 93.3% and specificity of 91.7%; neutrophil-to-lymphocyte ratio (NLR) exhibited a sensitivity of 73.3% and specificity of 72.7%; dNLR showed a sensitivity of 80% and specificity of 81.8% in identifying severe COVID-19 infections.

Furthermore, a binary logistic regression analysis was performed to ascertain the prognostic significance of TLC, NLR, and dNLR for severe disease and unfavorable clinical outcomes. The calculated odds ratio was 1.001 for TLC ($P < 0.05$), 1.463 for dNLR ($p < 0.05$), and 1.221 for NLR ($p < 0.05$). These findings underscore the potential of these parameters as indicators of poor prognosis in severe COVID-19 cases.

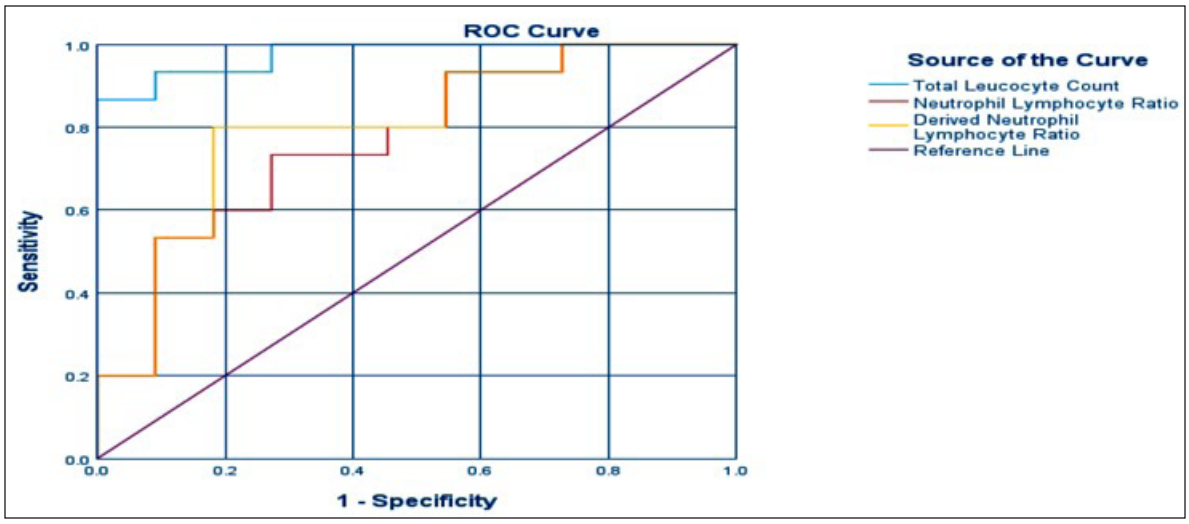

Figure 1: ROC: Receiver operator characteristic curve.

DISCUSSION

The phenomenon of the cytokine storm, an acute hyperinflammatory response, has been implicated in the pathogenesis of severe illnesses associated with viral infections, cancer, sepsis, and similar conditions. This immune system dysregulation is also believed to contribute to the severity of COVID-19, caused by the novel coronavirus SARS-CoV-2.[5] This phenomenon underlies several severe manifestations of COVID-19, including ARDS, thromboembolic events, acute kidney injury (AKI), and vasculitis. Studies have consistently demonstrated elevated levels of various inflammatory markers such as granulocyte-macrophage colony stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), tumor necrosis protein alpha (TNF), interleukin 6, C-reactive protein, ferritin, and procalcitonin in severe COVID-19 cases, suggesting a state of hyperinflammation.[5]

Our study revealed a significant elevation in TLC values among severe COVID-19 patients compared with mild cases. This aligns with previous research indicating that severe COVID-19 infection is associated with an increased neutrophil count and decreased lymphocyte count.[6] We also observed significantly higher NLR and dNLR values ($p < 0.05$) in severe cases, reflective of the prevailing imbalance between elevated neutrophil and diminished lymphocyte counts—a hallmark of COVID-19 infection. The exaggerated immune response in severe COVID-19 triggers the release of cytokines such as GM-CSF, interleukin 6, interleukin 8, and tumor necrosis factor alpha, contributing to heightened neutrophil production. Concurrently, secondary bacterial infections can also lead to neutrophilia, while lymphopenia may arise from cytokine-induced inhibition, increased apoptosis, and lymphocyte redistribution within the lymphatic system. These observations are in concurrence with previous studies.

Our study demonstrated that TLC, NLR, and dNLR possess significant clinical relevance in diagnosing severe COVID-19 infections. ROC curve analysis highlighted that TLC exhibited the largest AUC at 0.976, followed by dNLR with AUC 0.800, and NLR with AUC 0.770. This indicates that, according to our findings, TLC holds the most diagnostic value, followed by dNLR, which surpassed NLR in diagnosing severe COVID-19 infections. This contrasts with some previous studies that emphasized NLR's higher sensitivity and specificity than dNLR.[7] Through Youden's J statistic, optimal cutoff values were determined: 10,950 for TLC, 6.19 for NLR, and 4.13 for dNLR. By applying these cutoff values, TLC emerged as the most sensitive and specific indicator, followed by dNLR, for identifying severe COVID-19 cases. The cutoff value for NLR in our study resonates with that in the study by Prozan *et al.*,[8] albeit slightly deviating from a few other studies. Binary logistic regression analysis further corroborated these findings, revealing statistically significant results and reinforcing the variables as indicators of poor prognostic factors for severe disease, consistent with prior research. While we anticipated differences in platelet count, PLR, and LMR between the two groups based on previous studies, our analysis did not yield statistically significant differences. A possible explanation for this observation could be the limited sample size in our study.

CONCLUSION

Severe complications stemming from COVID-19 demand immediate attention due to their potential life-threatening nature. Identifying poor prognostic factors early on and intervening promptly can yield significant benefits. Our study underscores the effective utilization of easily accessible circulatory biomarkers—TLC, dNLR, and NLR—in diagnosing severe COVID-19 infections. These biomarkers

indirectly reflect the hyperinflammatory response associated with severe disease. With vigilant monitoring, we stand to identify high-risk individuals promptly, enabling timely interventions that can reduce mortality rates

Ethical approval

The research/study approved by the Guru Teg Bahadur Hospital Ethics Committee, GTBHEC Protocol number - GTBHEC2021/P-141, dated 12-04-2021.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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