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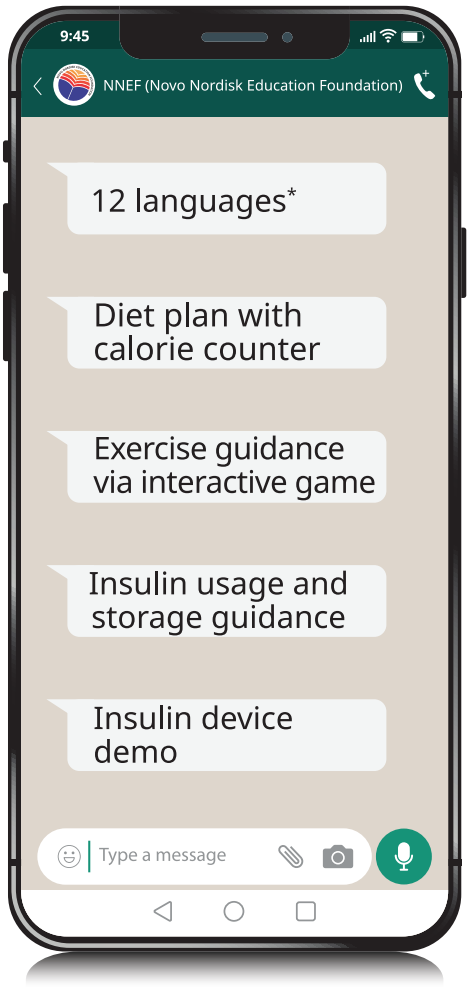

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EDITORIAL

Unveiling the Uncertainties: Exploring the Utility of Herpes Zoster Vaccines

Mangesh Tiwaskar[1*], Agam Vora[2]

Introduction

Herpes zoster, commonly known as shingles, is a viral infection caused by the reactivation of the varicella-zoster virus (VZV). After primary infection with VZV, which causes chickenpox, the virus remains dormant in the sensory ganglia. However, it can reactivate later in life, leading to the development of shingles. Shingles typically presents as a painful, unilateral, and vesicular rash along the distribution of sensory nerves. The condition can be associated with significant morbidity, including severe pain, postherpetic neuralgia (PHN), and other complications. In this editorial, we will delve into the global and Indian burden of herpes zoster, explore its complications, highlight the importance of prevention, shed light on the Shingrix vaccine, discuss its composition, and present the research on safety and efficacy, including the ZOE-50 and ZOE-70 studies. Furthermore, we will review the recommendations on the Shingrix vaccine by leading global medical societies, including the esteemed Association of Physicians of India (API).

The Global Burden of Herpes Zoster

Herpes zoster poses a significant burden on public health worldwide.[1] The World Health Organization estimates that over 90% of individuals worldwide are infected with VZV, indicating a high susceptibility to shingles. The risk of developing shingles increases with age and/or associated comorbid immunocompromising conditions like diabetes, chronic obstructive airway disease, senility, malignancies, chronic kidney disease, solid organ transplant recipients, patients on immunomodulators, etc. The global incidence of herpes zoster is expected to rise due to the aging population.[2] The burden of herpes zoster extends beyond the physical symptoms experienced by patients. It includes psychological distress, impaired quality of life, and substantial healthcare costs associated with the condition.

The Indian Scenario

In India, the burden of herpes zoster is substantial, with millions of cases reported each year. This becomes more relevant against the backdrop of India now being the most populous country in the world surpassing China with a very high incidence of both communicable and noncommunicable diseases. The country's majority population, largely unvaccinated against varicella during childhood, remains susceptible to VZV reactivation in adulthood. This has led to a higher incidence of shingles compared to countries with routine childhood varicella vaccination programs. According to a study published in the Indian Journal of Dermatology, Venereology and Leprology, the incidence rate of herpes zoster in India ranges from 1.2 to 5.4/1,000 person-years. Another Indian study also revealed a higher incidence of herpes zoster in the younger population of 31–40 years (24%) and 21–30 years (19%).[3] Moreover, factors such as underdiagnosis, lack of awareness, and limited access to healthcare services, particularly in rural areas, contribute to the increased burden of herpes zoster in India.

Complications of Shingles

Shingles can give rise to various complications, which significantly impact the health and well-being of affected individuals. PHN is one of the most common and distressing complications of shingles. It refers to persistent pain that continues beyond the resolution of the rash and can last for months or even years.[4] PHN can have a profound impact on the quality of life, resulting in sleep disturbances, depression, and limitations in daily activities. Other complications associated with shingles include bacterial superinfection of the rash, ocular involvement leading to corneal ulcers and vision loss, cardiovascular complications like acute myocardial infarction or ischemic heart disease, arrhythmias and/or heart failure, and neurological complications such as meningitis and encephalitis. The potential severity and long-term consequences of these complications emphasize the need for effective prevention strategies.

The Need to Prevent Shingles

Prevention is a critical component in addressing the burden of herpes zoster. Vaccination plays a pivotal role in reducing the incidence and severity of shingles. The Shingrix vaccine, developed by GlaxoSmithKline, has emerged as a breakthrough in shingles prevention. It is a nonlive recombinant vaccine designed to stimulate a robust immune response against VZV. Compared to the previously available Zostavax vaccine, Shingrix offers higher efficacy and longer-lasting protection.

The Importance of Herpes Zoster Vaccination in India

- Prevention of herpes zoster**:** The primary benefit of herpes zoster vaccination is the prevention of the disease itself. By stimulating an immune response, the vaccine reduces the risk of reactivation of the VZV, thereby preventing the onset of herpes zoster. This is particularly crucial for individuals with weakened immune systems and those suffering from chronic illnesses, as they are at a higher risk of developing severe complications from the disease.
- Reduction in PHN: Postherpetic neuralgia (PHN), a severe and often long-lasting complication of herpes zoster, can significantly impact the quality of life of affected individuals. By reducing the incidence and severity of herpes zoster, the vaccine plays a crucial role in preventing PHN and its associated pain and discomfort.
- Cost-effectiveness: While the initial cost of vaccination may seem high, it is essential to consider the long-term cost-effectiveness of the herpes zoster vaccine. By preventing the disease and its complications, the vaccine reduces the need for medical interventions, hospitalizations, and long-term management of PHN. This results in significant cost savings for both individuals and the healthcare system.

Shingrix Vaccine and Its Composition

The Shingrix vaccine consists of a purified VZV glycoprotein E (gE) antigen along with

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an innovative adjuvant system called $AS01_B$. The gE antigen is a key component of the VZV virus and stimulates the immune system to produce antibodies against VZV. The $AS01_B$ adjuvant is a novel adjuvant system comprising liposomes and monophosphoryl lipid A (MPL).[5]

The Role of Adjuvant $AS01_B$

The $AS01_B$ adjuvant in the Shingrix vaccine plays a crucial role in enhancing the immune response. It has been extensively studied and demonstrated to enhance vaccine immunogenicity and durability of protection. $AS01_B$ contains liposomes and MPL, which activate antigen-presenting cells, leading to a strong activation of the immune system. The adjuvant's ability to stimulate a robust immune response is particularly relevant in older adults, who typically have weaker immune systems. The inclusion of $AS01_B$ in Shingrix contributes to its superior efficacy, even in populations with diminished immune function.[5]

Research on Safety and Efficacy: ZOE-50 and ZOE-70 Studies

The safety and efficacy of the Shingrix vaccine have been rigorously evaluated in clinical trials, including the ZOE-50 and ZOE-70 studies. The ZOE-50 study involved individuals aged 50 years and older, while the ZOE-70 study focused specifically on individuals aged 70 years and older. In both studies, Shingrix demonstrated remarkable efficacy in preventing shingles and its complications. In the ZOE-50 study, Shingrix exhibited an overall vaccine efficacy of 97.2% against shingles and 89.8% against PHN. Similarly, in the ZOE-70 study, the vaccine showed an efficacy of 90% against shingles and 85.1% against PHN. These findings confirm the significant protective effect of Shingrix across different age groups.[6]

Recommendations by Global Medical Societies, Including the API

Global medical societies recognize the critical role of Shingrix in preventing shingles and its associated complications. The API acknowledges the burden of herpes zoster in the country and recommends the use of Shingrix in eligible individuals. The API emphasizes the importance of vaccination to reduce the incidence of shingles, especially in high-risk populations such as the elderly and immunocompromised individuals. Furthermore, the API underscores the necessity of comprehensive public awareness campaigns to educate healthcare professionals and the general population about the benefits of vaccination and the availability of Shingrix.

Conclusion

The burden of herpes zoster, encompassing the physical, psychological, and economic impact, necessitates a proactive approach to prevention. The Shingrix vaccine, with its unique composition and $AS01_B$ adjuvant system, represents a significant advancement in shingles prevention. Extensive research, including the ZOE-50 and ZOE-70 studies, has demonstrated the vaccine's safety, efficacy, and durability of protection. Global medical societies, including the API, recognize the importance of Shingrix and advocate for its integration into vaccination programs. It is incumbent upon healthcare professionals to actively promote and administer Shingrix to eligible individuals, thereby reducing the burden of herpes zoster and improving overall health outcomes. By embracing Shingrix and prioritizing prevention, healthcare professionals can play a vital role in mitigating the burden of herpes zoster and enhancing the well-being of individuals in India and worldwide.

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ORIGINAL ARTICLE

Impact of UG Training on Understanding the Severity of Antimicrobial Resistance

Anushka Deogaonkar[1*], Jayshree Dawane[2]

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Abstract

Background: Antibiotic resistance is increasing at an alarming rate. Many reasons are there which need urgent attention toward this problem. Efforts in all directions are required to address this serious issue. The irrational prescribing habits of physicians lead to increasing morbidity, mortality, and treatment costs. One of the key components of the system is the prescriber and their practices. In the present study, efforts were made to know the understanding of the medical students about the severity of the problem and to find out the focus area that needs to be modified.

Materials and methods: A cross-sectional, questionnaire-based survey was conducted among the second-year medical students of a teaching hospital after the ethical approval study was explained to the student and written consent was taken. The questionnaire was given before and after exposure to formal lectures on antimicrobial agents. The collected data was analyzed.

Results: It was clear that inappropriate use of antimicrobials causes antimicrobial resistance (AMR) (87.3%) and can harm patients (82%). Interactive patient-oriented problem-solving modules on the internet (35%) and small group problem-based learning are preferred by (32%) students. Preparedness about antimicrobial use was seen only in 50% of the students in a few essential aspects. Learning during the medical course only does the purpose of sensitization, for the thorough knowledge repeated exposure is essential.

Conclusion: Most of the students were partially aware of the AMR. The only concern was their casual attitude regarding antibiotic use. Further educational interventions are necessary to improve their understanding and perceptions of antibiotic resistance, as well as their attitude toward antibiotic use.

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Introduction

Antimicrobial resistance (AMR) poses a catastrophic threat to the entire world today. The increase in the incidence of the problem can be due to irrational use, liberal prescribing, lack of knowledge, self-medication, and poorly drawn-out clinical guidelines. The factors leading to AMR are well identified, like over and indiscriminate usage of antibiotics, easy availability of substandard antimicrobials, prescribing them on demand, and irrational use of newer and higher antibiotics, which are reserved drugs for severely infected patients.[1] New antimicrobial development is a herculean task in terms of manpower and economic burden. This evolving public health issue is driven by both the irrational use of antimicrobials for human health and the inadequate measures to control the spread of infections.[2]

Rational antibiotic prescribing can prevent the emergence of resistance.[3] Focus is needed on the development of novel antibiotics or modifications of the existing antibiotics and restricting the usage of available antimicrobials. AMR can be sufficiently delayed, or its onset can be manipulated. In 2016, the National Treatment Guidelines for Antimicrobial Use in Infectious Diseases were introduced. However, introducing rules alone is not sufficient to bring about the desired outcomes in the use of antimicrobial agents. For sustainable and effective adherence to best practices, prescribers need to be encouraged to internalize the principles that emphasize optimized antibiotic prescribing. Successful management of antibiotic therapy requires robust national and local information on the usage of antibiotics to enable a better understanding of the evolving relationship between antibiotic consumption, the emergence of resistance, and the prevalence of healthcare-associated infections.

Antimicrobial stewardship is an effort to educate antimicrobial prescribers and consumers on their appropriate and rational usage. The principles include accurately identifying patients who need antibiotic therapy, using local epidemiology to guide the selection of empiric therapy, avoiding agents with overlapping activity, adjusting antibiotics when culture results become available, monitoring for toxicity, and optimizing the dose, route, and duration of therapy.[4] According to the World Health Organization, education of healthcare workers and medical students on rational antimicrobial prescribing or "antimicrobial stewardship" is an integral part of all AMR containment activities.[5,6] Healthcare organizations need to work toward sustainable systems that promote shared knowledge and provide prescribers with a choice architecture that prompts and facilitates antibiotic prescribing.

Therefore, it is of utmost importance to assess understanding of the depth of knowledge about the antimicrobials and severity of resistance, awareness, and implications in the budding prescribers. The first step in this direction is to prepare our future prescribers to deal with the problem.[7] The present study was undertaken to assess the knowledge and perception of undergraduate (UG) medical students about AMR so that the appropriate remedies can be implemented in the medical curriculum to handle the problem of AMR.

Objectives

- Assessment of the understanding of UG students about AMR.
- To identify the gaps in knowledge to implement appropriate remedies for incorporating the culture of rational antimicrobial use.

Materials and Methods

This is a cross-sectional, unicentric, observational, descriptive study conducted in the Bharati Vidyapeeth (Deemed to be University) Medical College, Pune. Convenient samples, that is, the medical students of second year MBBS from Bharati Vidyapeeth (Deemed to be University) Medical College, Pune, were included in the study. Participation was voluntary, and responses were kept anonymous.

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Approval from the Institutional Ethics Committee was obtained before starting the study.

Necessary information like how it will be conducted, why we will be doing it, etc., regarding this study was given to the participants. Students are allowed to ask questions/queries regarding the study. We answered all the questions and cleared their doubts of the students.

Written consent was taken after giving complete information about the study to the participants.

No attempts were made to alter course material or teaching methods before or during the study.

The questionnaire was used pre and postexposure to antimicrobial teaching to obtain information about the knowledge and perception of UG students about AMR. Information included age, sex, attitudes, and perceptions about antimicrobials, awareness of the problem of antimicrobial-resistant diseases, sources of antimicrobial education, and self-confidence in antimicrobial prescribing. Students reported their perceptions about the quality of their education regarding the appropriate use of antimicrobials and their preparedness to prescribe antimicrobials upon graduation.

Statistical Analysis

Data generated from the questionnaire was analyzed using Statistical Package for the Social Sciences software and presented as mean and standard deviation. Categorical variables are presented as percentages (%). Nonparametric tests were used to assess differences between groups and correlations between variables.

Observations and Results

Perceptions about Antimicrobials

In the curriculum of MBBS, students are exposed in the second year to learn about antimicrobial agents and their use for different clinical conditions. This step is the foundation of gaining concrete knowledge of antimicrobials with rational use. In this study, we assessed the knowledge and understanding of use, adverse effects, resistance, and antimicrobial stewardship.

Most of them (84.6%) were of the opinion that antimicrobials are overused nationally. They have the understanding that better use of antimicrobials will reduce problems with antimicrobial-resistant organisms (85.2%). Only 4.6% in the posttest feel that the appropriate use of antimicrobials is not very useful in reducing AMR. It looks like there is still confusion about what exactly is the appropriateness of antimicrobial use. However, it was sure that a strong knowledge of antimicrobials is important in their medical career (89.3%), and more educational stress on the appropriate use of antimicrobials (82.6%) is required.

New antimicrobials will be developed in the future that will keep up with the problem of resistance (17.9%). A maximum of them are of the opinion that prescribing broad-spectrum antimicrobials when equally effective narrow-spectrum antimicrobials are available increases the problem of AMR (82.6%). Around 85.3% think that poor infection control practices by healthcare professionals cause the spread of AMR. Inappropriate use of antimicrobials causes AMR (87.3%) and can harm patients (82%).

Interactive patient-oriented problem-solving modules on the internet were the most (35%) preferred method to learn about antimicrobial prescribing and resistance. Problem-solving sessions (33%) attended by small groups of medical students by faculty was the next most common method. Grand rounds lectures (8%), lecture series for medical students (9%), interactive patient-oriented problem-solving modules on CD-ROM (7%), and roleplaying sessions (8%) dealing with patients demanding antimicrobial therapy are less preferred. There was minimal difference between the pre and posttest responses (Fig. 1).

Participants Rating the Sources to Learn about Antimicrobial Use and Resistance

Participants were given multiple choices for this question. The most common sources to obtain information about antimicrobial agents are textbooks (62%), peers (58%), Wikipedia (42%), smartphones (35.3%), medical journals (39.3%), infectious diseases specialists, noninfectious diseases physicians, hospital pharmacists, and pharmaceutical representatives are the less frequently used sources (Table 1).

Around 21.3% attended lectures on rational use, 21.3% learned the right duration of treatment for specific infections, 14.7% understood when to start antibiotics, and 6.6% got knowledge about the correct dosing from formal lectures (Table 2).

When we compared the education received for appropriate use of the antimicrobials. The maximum number (77.3%) of students expressed that they had minimal or no education about it in the pretest.

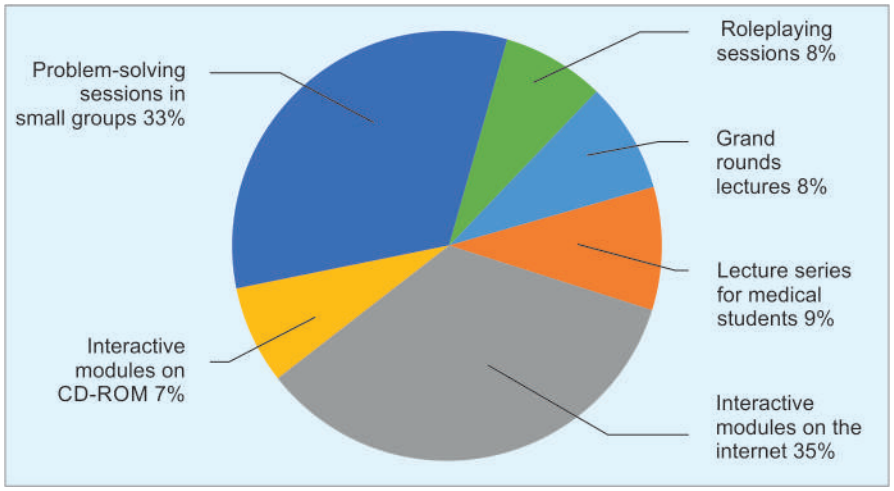

Fig. 1: Preferred method to learn about antimicrobial prescribing

Table 1: Sources preferred to learn about antimicrobial use and resistance

Sr. no.	*Items*		
1	Infectious diseases specialists	2.6%	4 (2.6%)
2	Noninfectious diseases physicians	1.3%	2(1.3%)
3	Hospital pharmacists	8.6%	13 (8.6%)
4	Pharmaceutical representatives	6.6%	3 (6.6%)
5	Medical journals	39.3%	59 (39.3%)
6	iPhone or smartphone apps	35.3%	53 (35.3%)
7	Wikipedia	42%	63 (42%)
8	Textbooks or study guides	62%	93 (62%)
9	Peers (other students)	58%	87 (58%)

Table 2: Formal lectures that address the following topics during medical curriculum

Attended any formal lecture(s) that address the following topics	*Yes*
Rational use of antibiotics in general	21.3%
When to start antibiotics?	14.7%
How to select the correct dosing?	6.6%
How to select the right duration of treatment for specific infections?	21.3%

Table 3: Use of medical education for preparedness upon graduation

To know when to start antimicrobial therapy	41.3%
How to select the best antimicrobial for a specific infection?	50.6%
To describe the correct spectrum of antimicrobial therapy for different antimicrobials	25.9%
Understand the basic mechanisms of AMR	43.3%
How to streamline or de-escalate antimicrobial therapy?	14.0%
How to find reliable sources of information to treat infections?	13.9%
How to transition from IV to oral antibiotics (IV to PO switch)?	24.0%
How to handle a patient who demands antimicrobial therapy that is not indicated?	26.6%

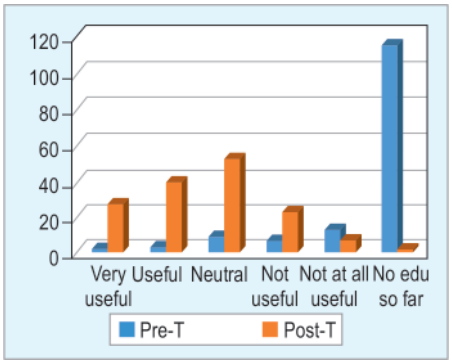

Fig. 2: Education regarding the appropriate use of antimicrobials so far

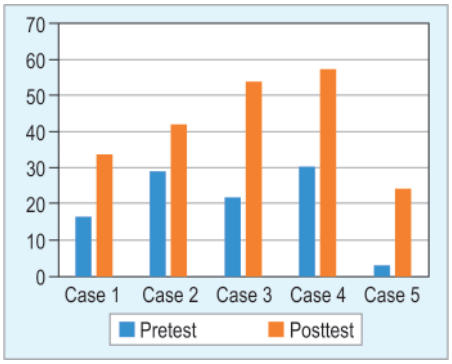

Fig. 4: Clinical scenario questions correctly answered by the participants

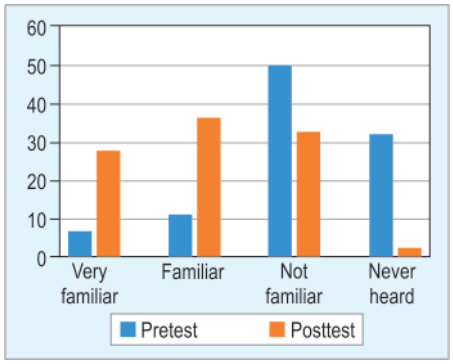

Fig. 5: Participants familiarity with the term "antimicrobial stewardship"

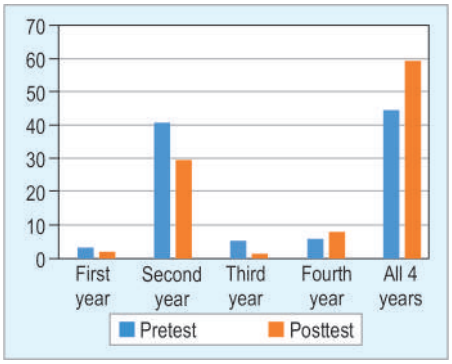

Fig. 3: Preferred academic year for learning the appropriate use of antimicrobials

However, after the exposure to the lectures, half of the students (78.6%) mentioned that they now have very useful knowledge about the appropriate use of antimicrobials (Fig. 2).

At this level of exposure (50.6%), students were confident about the selection of antibiotics for specific infections and (43.3%) knew the basic mechanism of AMR. A total of 41.3% were sure about when to start antimicrobial therapy (Table 3). Knowledge of the correct spectrum of antimicrobial therapy for different antimicrobials (25.9%), the transition from intravenous (IV) to oral antibiotics (25.9%), and (26.6%) handling a patient who demands antimicrobial therapy that is not indicated. Overall, 50% of the students said that on completion of the course, they are prepared to deal with the various aspects of antimicrobial use. Some remedial measures are required to increase the preparedness of all students (Table 3).

Most of them would like to get the learning about the appropriate use of antimicrobials in the second and fourth, that is, the final year of MBBS. They feel that learning in the second year and revision in the fourth year will help them to perfect their understanding and practical knowledge of it (Fig. 3).

Students were given four clinical conditions for the comment on diagnosis and use of medicines. A significantly high number of students were able to diagnose it correctly and advised treatment after the lecture series on chemotherapy (Fig. 4).

In the pretest, most of them were unaware of the term antimicrobial stewardship, but in the posttest, 60% of the students responded positively (Fig. 5).

Discussion

The discovery of antibiotics was a boon to human life. Antibiotics have been used successfully to treat various infections for many decades. The problem of antibiotic resistance started to step in with irrational use, self-medication and easy availability. AMR is a massive, persistent threat at present. There are many contributing factors to AMR which need to be addressed immediately. Lack of provider knowledge, particularly with regard to prescribers who are insufficiently qualified, supervised or supported, prescriber habit, and poor availability of independent medicines information such as clinical guidelines and drug bulletins.[8]

Poor knowledge and lack of training about antibiotic resistance during UG medical studies may lead to a change in behaviour among future doctors.[9] The present study is a small effort to know the shortcomings in knowledge delivery and create awareness of the problem for future prescribers. Second-year medical students participated in this study, and they were evaluated based on a pre and posttraining designed test questionnaire considering them as future prescribers. Knowledge about the current practices, rational use of antibiotics, and adverse effects arising due to the use of antimicrobial agents were assessed.

Students were sure about the fact that antimicrobials are overused; better use will help to deal with the problem of resistance. In-depth knowledge is important, and acquiring it during the UG course will be of great value. Problem-solving sessions in small groups and interactive modules on the internet will provide a chance to interact and create more impact. They think that journals and textbooks are the authentic sources to obtain information about AMR, but this alone is not sufficient. After attending the formal lectures on antimicrobials, there was definitely an increase in awareness about the problem, but repeated exposure to the topic is required by using different methods to incorporate it into the practice. Till now, they were unaware of the resistance, but lectures were definitely useful to them in improving their understanding of the severity of the problem. They are sure that they will be able to use it rationally at graduation. In the study, the perception of antimicrobials

increased considerably in a positive light after the posttraining analysis compared to the pretraining. Results obtained in the study[10] by Higuita-Gutiérrez et al. showed a discrepancy where limited knowledge was reflected in the selection of antibiotic treatment for respiratory, urinary tract, and skin and soft tissue infections after completion of the curriculum. However, in the study conducted by Khan et al., most of the students were aware of AMR and its consequences,[11] the only concern being their casual attitude regarding antibiotic use. In the posttraining analysis, most of them preferred the second and fourth years of the UG course for learning the appropriate use of antimicrobials.

When to start, for specific conditions, knowing the spectrum of activity, and when to de-escalate are the important points to be remembered in antimicrobial therapy. Antimicrobial de-escalation has been largely ignored as an important operational component and should earn its rightful place alongside guidelines and clinical pathways. These are the highly concerned aspects in preventing AMR. Similarly, completing the prescribed course of antibiotics is equally important. It has been observed that many times, the patients abruptly stop the antibiotic once symptoms disappear.[12,13]

Information to find out the source of infection needs to be obtained for prescribing the correct antibiotic, depending on the organisms. Once started to transfer the patient from parenteral (IV) to oral formulation is equally important.

Patient demand for antimicrobials is commonly seen in the population with the belief of prompt relief from the disease; this has been shown to increase unnecessary prescriptions and the problem of AMR.[14] Knowledge of handling these patients who demand antimicrobial agents where not needed and proper training to communicate with them has to be imparted thoroughly in the students. Chandy suggested in his editorial that effective communication and insight about the problem would help to develop relevant strategies to contain AMR.[15]

In the study, opinions about educational interventions in the current curriculum favored interactive patient-oriented problem-solving modules on the internet, while the least favored were grand rounds of lecture and roleplaying sessions. The study conducted by Ross et al. showed that only one intervention from the World Health Organization good prescribing guide proved useful.[16] Another study by Davenport et al. features an outcome-based approach for teaching and introduces a key strategy in facilitating prudent prescribing,[17] where the early introduction of the relevant knowledge concepts and skills into the UG medical curriculum. In the present study in posttraining analysis, a large number of students feel that to create a long-term impact of the rational use; it should be taken twice in the course in the second and in the final year of MBBS. Another research carried out by Sikkens et al. showed an alternative wherein E-learning during a limited period of time can significantly improve medical students' performance of an antimicrobial therapeutic consultation in a situation simulating clinical practice 6 months later.[18]

They were given four clinical conditions for the correct diagnosis and the drug of choice. Posttest, there was a significant increase in the number of students with correct answers. A study conducted by Okedo-Alex et al. suggested that there was good knowledge of antibiotic use and resistance; however, practice levels were poor.[19] Our results appeal to various interventions to improve the clinical knowledge and rational application base of UGs.

Antimicrobial stewardship needs high-level executive commitment.[20] Infection control and antimicrobial stewardship teams need to be introduced during the course and allowed to interact with the students. Antimicrobial stewardship must be seen as part of patient care and not a management-led cost-saving exercise. No structured provision about it currently exists in India.[21] The study also determined awareness regarding antimicrobial stewardship, with only almost 37% deeming it fully familiar and 33% familiar but unaware of the meaning. An article published by Hsu showed that approximately 150 students who participated in this module have been highly engaged in identifying antibiotic stewardship interventions in their early practice and creating potential solutions.[22] Incorporation of antimicrobial stewardship is a must in the graduating course time to improve the practices toward the rational use of antimicrobials.

Conclusion

The perception of rational antimicrobial use was seen to be definitely improved after the training. Although the curriculum equipped the students with a theory base, a lack of clinical navigation and knowledge was observed. It was deemed necessary and important to include learning of antimicrobial use for all years of UG medical training. Moreover, educational interventional studies should continue to be carried out to understand what curriculum works best for equipping UG students for rational antimicrobial usage in clinical practice. There is also a need to enrich existing courses and training about antibiotic use in the curriculum of the medical course with more emphasis on antimicrobial stewardship.

Ethical Clearance

The study was conducted after approval from the Institutional Ethics Committee.

Source of Support

ICMR-STS approved.

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ORIGINAL ARTICLE

Utility of Atypical Lymphocytes and Large Immature Cells in Prediction of Dengue Severity

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Abstract

Background: There is a clinical imperative to devise metrics to prognosticate dengue severity. Our objective was to determine the association between longitudinal trends in atypical lymphocytes and large immature cell count with platelet count and dengue severity.

Materials and methods: Retrospective analysis of longitudinally measured clinical and hematological data from (n = 79) hospitalized dengue patients was done.

Results: The cohort consisted of patients with dengue fever without warning signs (DFWOWS) (n = 40, females = 14, and age = 19.9 ± 14.6 years), dengue fever with warning signs (DFWWS) (n = 36, females = 13, and age = 16.1 ± 14.1 years) and severe dengue (n = 3, females = 2, and age = 5.3 ± 4 years). Platelet count increased at a rate of 11,524 cells/mm^3/day, with a slower rate of rise as the severity increased (p = 0.001***). Concurrently hematocrit and neutrophil percentage decreased, while the lymphocyte percentage and white blood cell (WBC) count increased during the hospital stay. Every 1% increase in atypical lymphocyte count (ATY) was associated with a fall in platelet count by 16,963 cells/mm^3 (p = 0.001***). A similar but weaker trend was found for large immature cells (LICs).

Conclusion: The data support the usefulness of longitudinal tracking of atypical lymphocyte and large immature cell count for dengue prognosis. The time trends of the hematological parameters indicate the progression of patients from the critical to the recovery phase.

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Introduction

Dengue is a mosquito-borne viral hemorrhagic fever caused by the *Dengue virus*, a flavivirus. It is transmitted by the bite of infected Aedes mosquitoes. This disease is principally seen in tropical and subtropical regions between the latitudes 3° N and 35° S.[1] It is estimated that there are 390 million dengue virus infections globally every year, of which 96 million show clinical manifestations and approximately 40,000 succumb to the disease annually.[2] In India, 7,95,211 cases were reported, with 1,151 fatalities for 2017–2022, with an average yearly of 1,32,535 cases and 192 deaths.[3] Dengue virus comprises four predominant serotypes (DEN-1, 2, 3, 4) with positive-sense single-stranded RNA as the genetic material and host membrane-derived lipid envelope. The virus is endowed with three structural proteins (capsid C, membrane M, and envelope E) and seven nonstructural (NS) proteins.[1]

Any of the four virus serotypes can cause illness, but most infections are asymptomatic. Primary infection induces lifelong immunity specific to the causal serotype. Second infection by a different serotype is thought to increase the risk for severe dengue. Antibodies induced by primary infection cross-react with the virus during the secondary infection in a nonneutralizing manner. Instead of clearing the virus, these antibodies facilitate the entry of the virus into cells that bear antibody receptors. The virus thrives inside the cell, creating an inflammatory cascade that culminates in the cardinal features of severe dengue, *viz*, vascular leakage, fluid accumulation, thrombocytopenia, shock, and potential death. Vascular leakage due to endothelial damage causes a rise in hematocrit, and fluid accumulation in the lungs and peritoneum, with severe leakage resulting in hemodynamic collapse and multiple organ dysfunction. Shock can also progress to disseminated intravascular coagulation, which, in the background of thrombocytopenia, can cause life-threatening hemorrhage. In addition to the antibody-medicated enhancement, cross-reactive T cells, NS1 antigen, antidengue virus NS1 antibodies, and autoimmunity contribute to the pathophysiology of dengue complications.[1,4] The natural history of dengue fever has three stages/phases[1,5]—(1) the acute febrile phase, (2) the critical phase following the defervescence, and (3) the recovery phase. Severe dengue with all attendant complications generally occurs after the febrile phase in the critical stage when the hematocrit rises and the platelets fall. After the peak/nadir, hematocrit and platelet return to the baseline values in the latter part of the critical phase as the patient progresses into recovery.

A clinical imperative is to devise metrics to predict the onset and extent of severe dengue and its underlying pathophysiology. Several markers have been explored for their prognostic purpose.[4,6–9] Atypical lymphocyte has been proposed as a helpful metric in dengue and other viral infections for assessment of severity and prognostication.[10–12] In the present work, we focus on atypical lymphocytes and large immature cells (LICs), which are the research parameters returned by modern five-part colters. The present study is an exploratory study based on retrospective analysis of clinical and hematological data routinely collected in hospitalized dengue patients serially overtime during their hospital stay. By longitudinal tracking of such data, we aim to—(1) determine time trends in hematological parameters indicative of pathophysiological features of severe dengue, *viz*, plasma leakage (rising hematocrit) and thrombocytopenia, (2) determine the association of the longitudinal patterns in atypical lymphocyte and large immature cell with time trends in platelet counts and hematocrit.

Materials and Methods

Retrospective data analysis was done on (n = 79) consecutive dengue patients hospitalized between October 2019 to 2022 for whom complete medical records were available. Enzyme-linked immunosorbent assay confirmed the diagnosis in all the cases

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for both NS1 antigen and immunoglobulin M antibody. We anonymized the data before the statistical analysis. The Institutional Ethics Committee approved the study (EC/NEW/INST/1527/2022/11/37). The data collected included clinical features from case sheets and hematological parameters from medical records from admission to discharge. Dengue severity is stratified into three categories according to the World Health Organization (WHO) 2009 classification, *viz*, dengue fever without warning signs (DFWOWS), dengue fever with warning signs (DFWWS), and severe dengue. The hematological parameters were measured using Mindray BC-5300 5-part coulter. Hematological parameters that were longitudinally tracked during the hospital stay included platelet parameters (platelet count, mean platelet volume (MPV), platelet distribution width (PDW), leukocyte parameters (atypical lymphocyte, LICs, differential leukocyte count—neutrophil, lymphocyte, total leukocyte count, and red blood cells (RBC) parameters (RBC count, hematocrit, and hemoglobin concentration).

Table 1: Demographic and clinical features

No. of patients	*DFWOWS*[†]	*DFWWS*[‡]	*Severe dengue*
N = 79 (females = 29)	*N = 40 (females = 14)*	*N = 36 (females =13)*	*N = 3 (females = 2)*
Age	19.9 ± 14.6	16.1 ± 14.1	5.3 ± 4.0
Age composition			
Age <12 years	13	17	3
Age ≥12 years	27	19	–
Clinical features			
Fever	39	35	3
Nausea/vomiting	19	24	3
Rash	3	7	0
Headache	10	3	1
Body pains	10	7	0
Abdominal pain	1	22	2
Edematous gall bladder wall	0	12	2
Abdominal tenderness	0	3	0
Mucosal bleeding	0	3	1
Ascites	0	14	2
Pleural effusion	0	13	2
Hepatomegaly/ splenomegaly	0	14	3
AST/ ALT >1000	0	0	3

[†]DFWOWS, dengue fever without warning signs; [‡]DFWWS, dengue fever with warning signs

The data consisted of repeated measures of hematological parameters. Linear mixed models were used for the regression analysis. A linear mixed model was fit for every parameter to assess the time trend and its interaction with the disease severity. Regression coefficients from the model output were used to estimate the parameters' daily rise/fall trends. A parsimonious mixed model was also fit to predict platelet count from the hematological parameters. Statistical analysis was done using RStudio software, and mixed models were fit using the LME4 package. A false discovery rate was used to adjust the *p*-values for multiple comparisons, and the adjusted *p*-value of 0.05 was used for statistical significance. We also report 95% confidence intervals (CI) for the regression slopes to indicate the precision of the model results.

Results

The clinical and demographic features of the participant cohort are summarized in Table 1. Of the total sample size of 79, only three were in the severe dengue category. Hence, the severe group was excluded from the inferential statistics because of the small sample. All the inferential statistics reported in the present work reflect the trends in the nonsevere dengue only, that is, DFWOWS and DFWWS. Table 2 shows the summary statistics of the hematological parameters. For continuous variables, mean ± standard deviation (SD) is shown, while for categorical data, frequencies are presented.

The longitudinal trends in the hematological parameters of dengue patients during their hospital stay are discussed below.

Table 2: Laboratory hematology parameters

	DFWOWS[†]	*DFWWS*[‡]	*Severe dengue*
Platelet count (×10^3 cells/mm^3)	121.1 ± 102.4 (minutes = 15)	111.0 ± 105.7 (minutes = 10)	102.3 ± 77.2 (minutes = 15)
MPV (fL)	9.4 ± 1.2 (minutes = 6.2)	9.2 ± 1.1 (minutes = 6.1)	9.5 ± 1.2 (minutes = 6.6)
PDW	16.6 ± 0.7 (minutes = 14.9)	16.7 ± 0.8 (minutes = 15.1)	16.9 ± 0.7 (minutes = 15.8)
Total WBC count (×10^3 cells/mm^3)	6.1 ± 3.8 (minutes = 1.55)	5.4 ± 3.3 (minutes = 0.95)	7.2 ± 3.3 (minutes = 2.59)
Differential count			
Neutrophil	47.1 ± 17.4 (minutes = 11)	43.3 ± 17.9 (minutes = 4)	33.3 ± 12.3 (minutes = 15)
Lymphocyte	43.1 ± 17.1 (minutes =4)	46.7 ± 18.1 (minutes = 8)	55.2 ± 10.7 (minutes = 30)
NLR	1.7 ± 2.3 (minutes = 0.13)	1.4 ±1 .6 (minutes = 0.09)	0.7 ± 0.5 (minutes = 0.2)
Monocyte	7.7 ± 3.1 (minutes = 0)	7.4 ± 3.1 (minutes = 1)	10.4 ± 4.0 (minutes = 5)
Atypical lymphocytes	1.7 ± 1.5 (minutes = 0)	1.9 ± 1.7 (minutes = 0.1)	2.6 ± 1.4 (minutes = 0.6)
LIC	0.9 ± 1.3 (minutes = 0)	1.6 ± 2.3 (minutes = 0)	1.0 ± 2.3 (minutes = 0)
Hematocrit	40.3 ± 6.4 (minutes = 26.9)	38.3 ± 5.9 (minutes = 23.4)	35.7 ± 4.6 (minutes = 29.7)
Hemoglobin (gm/dL)	13.3 ± 2.0 (minutes = 8.1)	12.7 ± 2.1 (minutes = 6.5)	11.9 ± 1.6 (minutes = 9.8)
RBC count (×10^6 cells/mm^3)	5.0 ± 0.7 (minutes = 2.94)	4.7 ± 0.8 (minutes = 2.47)	4.3 ± 0.6 (minutes = 3.53)

[†]DFWOWS, dengue fever without warning signs; [‡]DFWWS, dengue fever with warning signs. For each parameter, mean and standard deviation are shown (mean ± SD). The lowest/nadir value (minute) for the same is also shown in the parenthesis

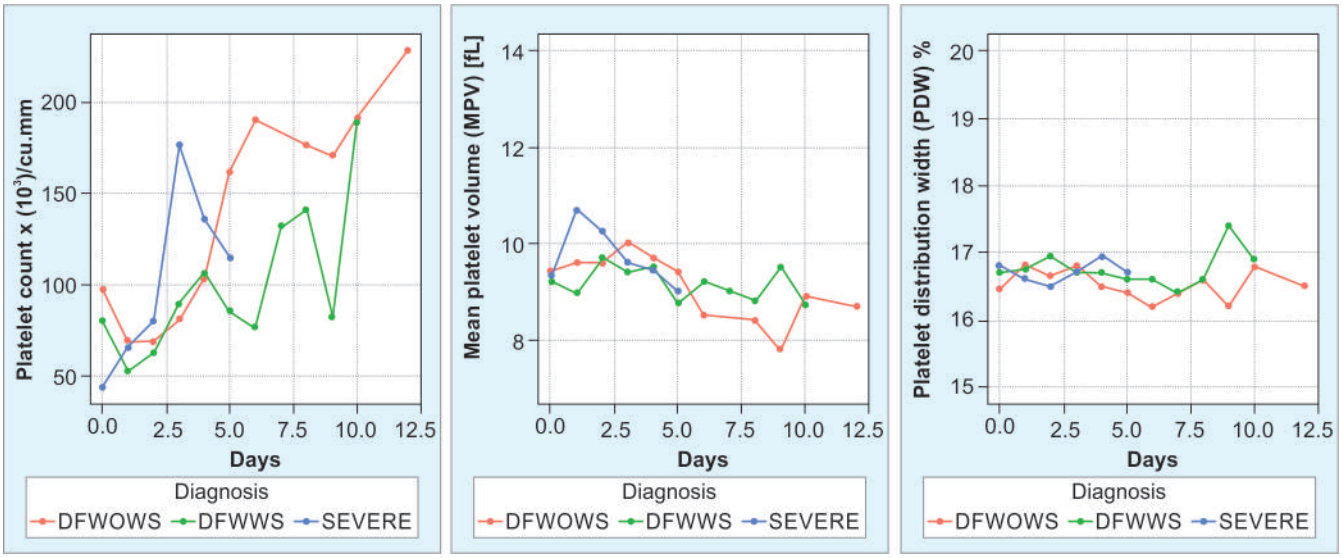

Fig. 1: Platelet parameters

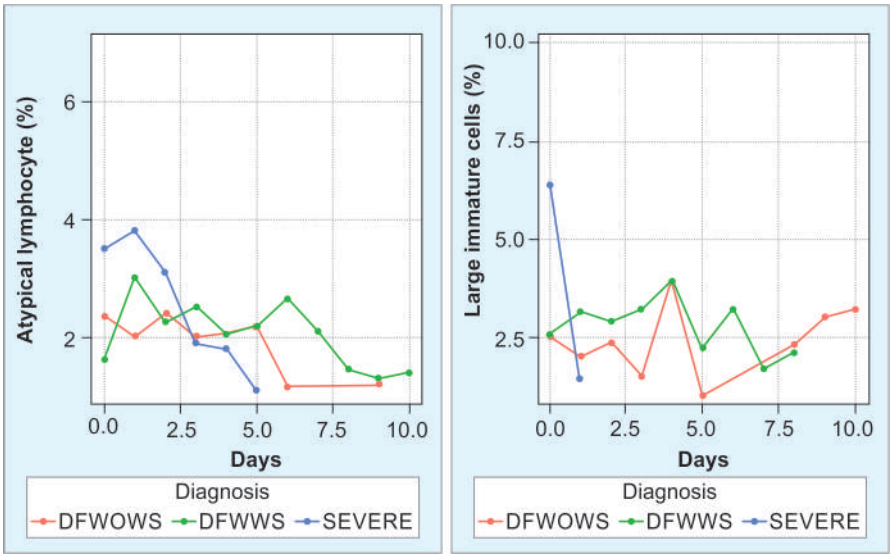

Fig. 2: Atypical lymphocyte and large immature cells

Platelet Parameters

The time trends of platelet parameters are shown in Figure 1. The platelet counts increased in the aggregated participant cohort ($p < 0.001$***) at a rate of 11524 cells/mm^3/day [(95% CI) = (5573, 17115) cells/mm^3] approximately. The platelet count increase over time is significantly less in DFWWS when compared to DFWOWS ($p = 0.001$***). The rate of increase in platelet count is lesser in the DFWWS group by 21262 cells/mm^3/day [95% CI = (–29502, –13080)]. In contrast, the average platelet count is not significantly different between the groups [$p = 0.157$|95% CI = (–10796, 68659)].

Mean platelet volume (MPV) has no significant trend over time [($p = 0.609$|95% CI = (–0.04, 0.06)]. MPV is not different between the groups [($p = 0.181$|95% CI = (–0.79, 0.14)]. In contrast, there is a significant interaction between the duration of hospital stay and the severity of dengue in affecting the value of MPV, with a difference of 0.08 femtolitre (fL) between the groups [($p = 0.04$**, 95% CI = (0.012,0.16)].

Platelet distribution width (PDW) is a stable parameter with no significant trend over time [($p = 0.553$|95% CI = (–0.016,0.061)], groups [($p = 0.571$|95% CI = (–0.21, 0.41)] or interaction effect [($p = 0.571$|95% CI = (–0.033, 0.074)].

Atypical Lymphocytes, LICs, and Other White Blood Cell (WBC) Parameters

Atypical lymphocyte count (ATY) has no significant linear trend over time [$p = 0.933$|95% CI = (–0.112, 0.085)], between the groups [$p = 0.933$|95% CI = (–0.586, 0.557)] or interaction effect [$p = 0.763$|95% CI = (–0.078, 0.185)]. LIC have no significant change over time [$p = 0.202$|95% CI = (–0.199, 0.048)], while LIC is higher in DFWWS [$p < 0.001$***|95% CI = (0.63, 1.94)] and has significant interaction effect between the time of hospital stay and severity [$p = 0.015$**|95% CI = (–0.377, –0.044)]. Atypical lymphocytes and LIC changes over time are shown in Figure 2.

Lymphocyte significantly increased over time [$p < 0.001$***|95% CI = (2.076, 3.626)]. The increase was greater in DFWWS when compared to DFWOWS [$p = 0.007$***|95% CI = (0.525, 2.737)]. Mirroring the lymphocyte, neutrophil declined over time [$p = 0.002$***|95% CI = (–4.213, –2.582)], with the decline greater in DFWWS when compared to DFWOWS [$p = 0.033$**|95% CI = (–2.315, –0.097)]. Total WBC count also increased with time [$p = 0.008$***|95% CI = (0.083, 0.411)], while the trend was nonsignificant over the group ($p = 0.839$|95% CI = (–1.621, 1 .446)] and interaction effects [$p = 0.183$|95% CI = (–0.414, 0.06)]. The neutrophil-lymphocyte ratio (NLR) decreased over time [$p = 0.002$***|95% CI = (–0.561,–0.334)], with nonsignificant difference between the groups [$p = 0.379$|95% CI = (–1.063, 0.39)] and interaction effect ($p = 0.105$|95% CI = (–0.018, 0.293)]. Other WBC time trends are shown in Figure 3.

RBC Parameters

The time trends in RBC parameters are shown in Figure 4. With multivariate analysis, hematocrit was found to have no significant trend over time [$p = 0.172$|95% CI = (–0.42, 0.03)], groups [$p = 0.189$|95% CI = (–4.132, 0.643)] or interaction effect [$p = 0.189$|95% CI = (–0.5, 0.116)]. In contrast, univariate analysis revealed a significant fall in hematocrit over

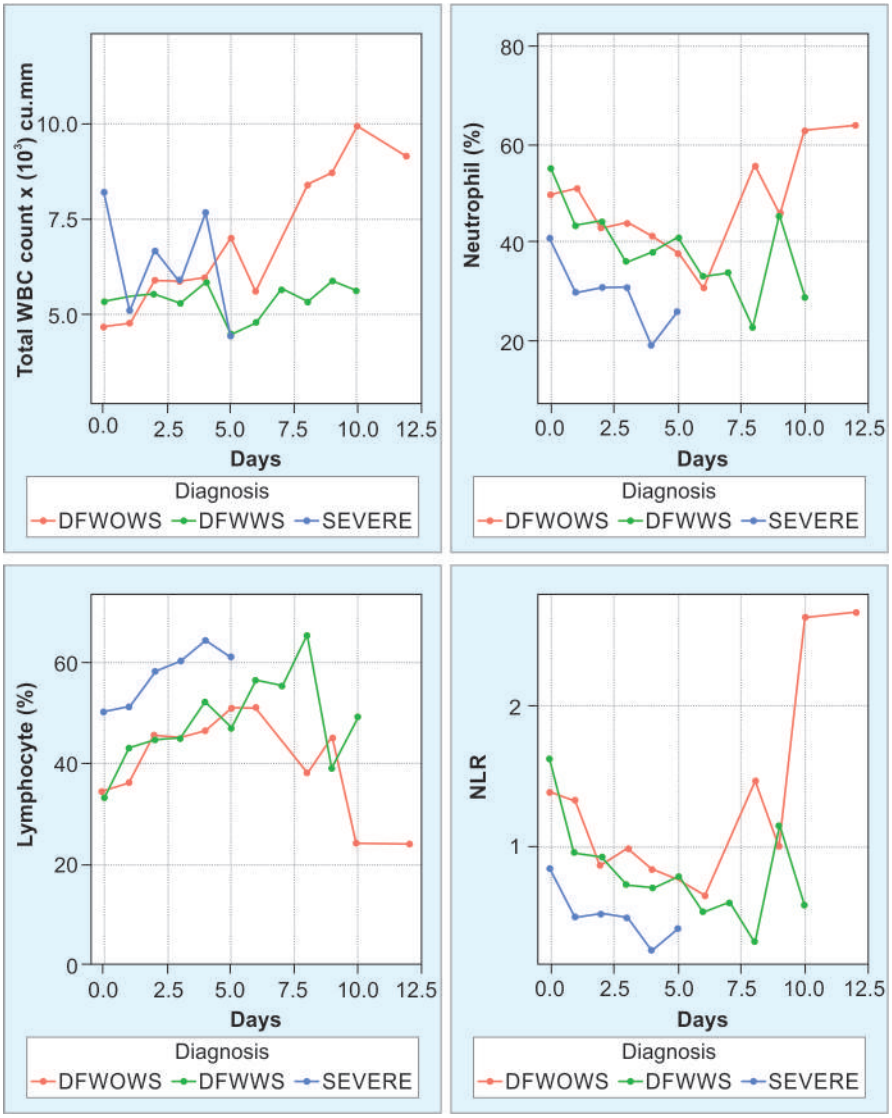

Fig. 3: WBC parameters

time [p = 0.001***|95% CI = (−0.43, −0.14)]. Subgroup analysis revealed a greater fall of hematocrit in DFWWS [p = 0.044**, −0.185, 95% CI = (−0.35, −0.003)] when compared to DFWOWS [p = 0.006***, −0.398|95% CI = (−0.643, −0.181)].

On multivariate analysis, RBC count fell significantly over time [p = 0.015**|95% CI = (−0.058, −0.006)], while there was no significant difference between the groups [p = 0.644 | 95% CI = (−0.35, 0.21)] or interaction effect [p = 0.243|95% CI = (−0.057, 0.015)]. The univariate analysis also revealed a fall in RBC count over time [p = 0.001***|95% CI = (−0.062, −0.023)]. Subgroup analysis revealed a more remarkable fall in RBC count in DFWWS [p = 0.006***, −0.032|95% CI = (−0.055, −0.008)] when compared to DFWOWS [p = 0.001***, −0.053|95% CI = (−0.081, −0.027)].

Hemoglobin decreased significantly with time [p = 0.001*** | 95% CI = (−0.162, −0.025)], while there was no significant trend for severity [p = 0.241|95% CI = (−1.374, 0.313)] or interaction effect [p = 0.436|95% CI = (−0.14, 0.059)]. Similar results were found on univariate analysis with a fall in hemoglobin over time [p = 0.001***|95% CI = (−0.165,−0.064)]. Subgroup analysis revealed a fall in hemoglobin in both the groups DFWOWS [p = 0.009***,−0.093|95% CI = (−0.154, −0.032)] and DFWWS [p = 0.001***, −0.137|95% CI = (−0.215, −0.059)].

Association between Platelet Counts and Atypical Lymphocytes and Other Hematological Parameters

The fall and rise in the platelet count was closely mirrored by the rise and fall of ATY with higher values of ATY associated with

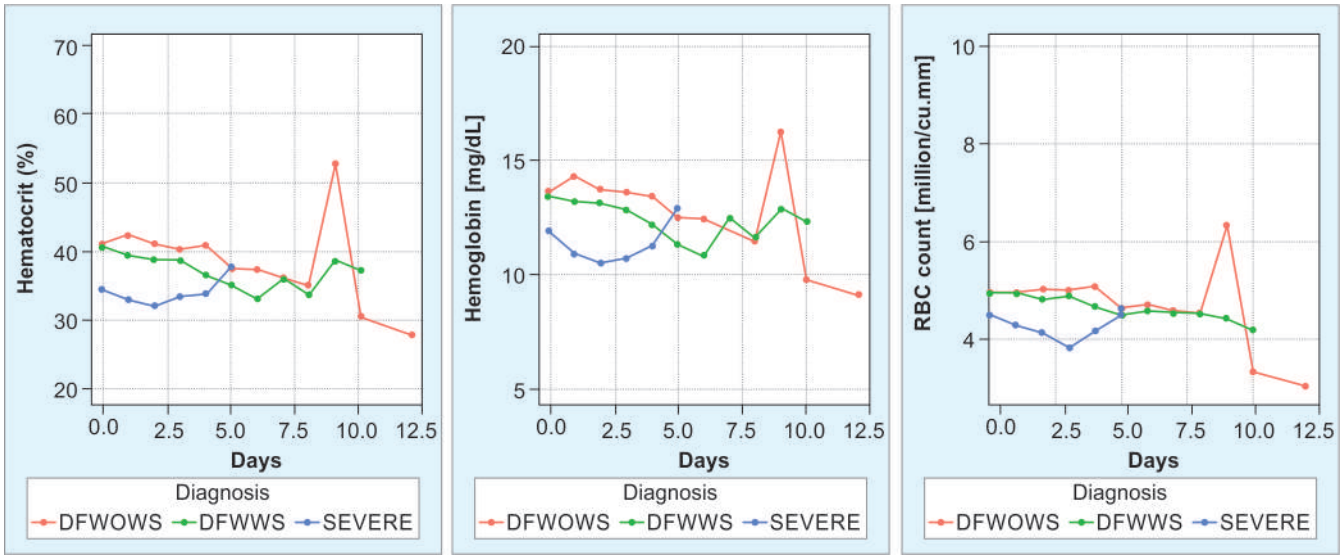

Fig. 4: RBC parameters

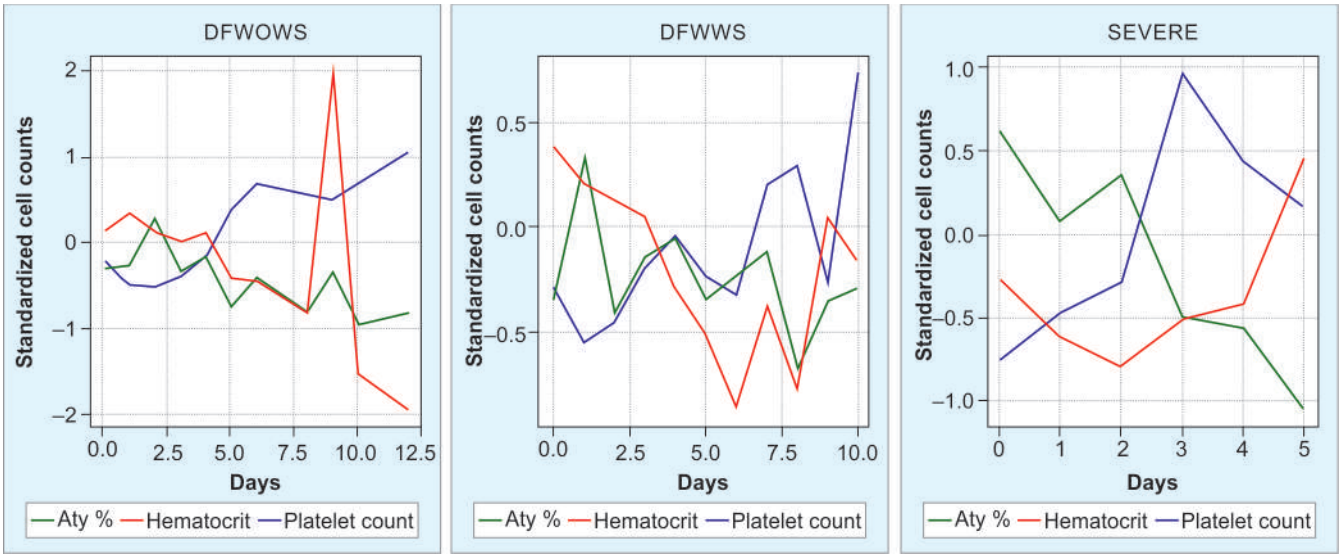

Fig. 5: Temporal association of atypical lymphocyte with platelet count and hematocrit. Cell counts have been standardized** (zero mean and unit variance) to depict all the counts on the same scale. It can be seen that platelet counts increase as the ATY decreases and vice versa

lower values of platelets as shown in Figure 5 [$p = 0.001$***, regression coefficient = –14.64, 95% CI = (–20.747, –8.742)]. The regression coefficient value indicates that every 1% increase in atypical lymphocyte percentage is associated with a decrease in platelet count by 16,963 cells/mm^3. This suggests that the ATY may play a causal role in the platelet count fluctuations in dengue. A similar albeit weaker relation was found between platelet count and LIC, with a platelet count fall by 6,680 cells/mm^3 for every 1% rise in LICs as indicated by the regression coefficient [p = 0.006***, regression coefficient = –6.679|95% CI = (–11.4, –2.096)].

Multivariate regression analysis using linear mixed-effects models indicates that the following parameters are significantly associated with platelet count in dengue patients in the best parsimonious model—ATY ($p = 0.007$***), hematocrit ($p = 0.004$***), neutrophil% ($p = 0.002$***), lymphocyte ($p = 0.002$***), and total WBC count ($p < 0.001$***), the interaction effect between neutrophil and severity ($p = 0.017$**) and lymphocyte and severity ($p = 0.002$***).

Discussion

The present study was conducted to longitudinally track the hematological parameters in hospitalized dengue patients and explore the predictors of platelet counts, focusing on the ATY and large immature cell fraction for which the data from 79 patients were analyzed. The clinical profile and complications of dengue are thought to be induced by several mechanisms,[1,4] involving cross-reacting adaptive immunity best illustrated by the "antibody-dependent enhancement" pathway. It is caused by nonneutralizing cross-reactive antibodies produced during a prior dengue infection by a different virus serotype. These antibodies are thought to promote the entry of the virus into FC-receptor-bearing cells wherein the virus propagates unimpeded, away from the fury of host immunity, with high viral load and the consequent inflammation provoked by disorganized cytokine response, vascular damage occurs, resulting in many pathological features. The vascular damage is known to compromise capillary integrity and cause plasma leakage from the capillaries into the tissues. The plasma leakage results in the rise of hematocrit. Antibody-mediated peripheral destruction and diminished production of platelets cause thrombocytopenia. The hematocrit peaks in the critical phase and falls as the patient progresses into recovery, and leaked fluids are resorbed back into the circulation.

Similarly, the platelets attain the nadir in the critical phase and rise afterward.[1,5] We found that platelet count increased during the hospital stay, with the increase slower in DFWWS compared to DFWOWS. At the same time, the hematocrit (and other RBC parameters) decreased concurrently, suggesting that most of the patients are past the peak severity and are progressing from the critical stage to the recovery stage of dengue. Greater changes in the parameters in the DFWWS group are consistent with more severe disease in this group compared to the DFWOWS group.

Atypical lymphocytes fluctuated in lock-step with the platelet count but in an anticorrelated manner with lower platelet counts whenever the atypical lymphocytes increased and vice versa. We interpret this finding to suggest that atypical lymphocytes play a potentially causal role in the decline of platelet counts in dengue. The anticorrelated nature of the longitudinal trend strengthens the case for our conjecture regarding the causal association between them. Jampangern et al. 2007[13] made an implicit suggestion regarding the identity of the atypical lymphocytes as they reported a significant positive correlation between atypical lymphocytes and a cluster of differentiation 19 (CD19) + B cells. In contrast, other studies[14] have reported the presence of T cell markers (CD2 and CD7) on atypical lymphocytes. We conjecture that atypical lymphocytosis reflects the heightened and misdirected adaptive immune response to dengue characterized by the triad of cross-reacting B and T cells, cytokine storm, and autoimmunity leading to tissue damage causing dengue pathophysiology.

Large immature cells (LICs) have a qualitatively similar but quantitatively weaker relationship with platelet count. A rise in LIC indicates increased release from the bone marrow of immature cells of both lymphoid/myeloid lineage and can occur in infections and systemic inflammatory response conditions. We conjecture that fluctuations of humoral response (atypical lymphocytes) on the way to recovery induce corresponding changes in platelet counts and LIC, accounting for the anticorrelated nature of the relationship between platelets/hematocrit and atypical lymphocytes/LIC we show. Total WBC count increased over time consistent with recovery from critical phase induced fall in total WBC count. Virus-induced inhibition of myeloid progenitor cells has been proposed to explain the leucopenia in dengue. We interpret the

rising WBC count in our data to indicate the recovery from the leucopenic dengue response. The neutrophil decreased while the lymphocyte increased, explaining the fall in the NLR in the cohort. The pattern of changes in neutrophils and lymphocytes (therefore, the NLR) we report is consistent with what is usually seen in viral infections.

Conclusion

The data from the present study offers a snapshot of changes in hematological parameters in the recovery phase following the peak of the rise/fall of cell counts. Nevertheless, by tracking their longitudinal trends, the data support the association between atypical lymphocytes and the pathognomonic hematological changes of dengue, *viz*, platelet count and hematocrit. Our study also supports the predictive potential of ATY and LIC in dengue. However, there is a paucity of effective prognostic markers for the cardinal pathophysiological feature of dengue, *viz*, vascular leakage and its severity. Future studies could address this deficit in our current understanding. Early prediction of the cardinal pathology can open new avenues for intervention to mitigate and reverse the incipient pathology of the disease in its tracks.

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Authorship Statement

Ramadevi Peraka—conceptualization of the study, collection of data, critical review, and Editing of the manuscript; Aditya Koppula—analysis of the data, interpretation of results, and manuscript writing; Shalini MB—collection of data, critical review of the manuscript; Mani mekhala Parsa—conceptualization of the study and critical review of the manuscript.

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ORIGINAL ARTICLE

Correlation of Clinical Parameters with Findings of High-resolution Computed Tomography Chest in COVID-19 Patients

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Abstract

Background: Studies have shown that high-resolution computed tomography (HRCT) of the chest along with clinical parameters is useful in determining the clinical progression, extent of disease and severity of illness in patients with COVID-19. Hence, the present study was done to assess HRCT chest features in patients with COVID-19 infection and to find its association with clinical status in these cases.

Materials and methods: This was an observational study over the period of 18 months in patients diagnosed with COVID-19 disease following reverse transcription polymerase chain reaction (RT-PCR). Demographic details, history, clinical parameters, blood and imaging details of enrolled patients were recorded in the study proforma and analyzed using Statistical Package for the Social Sciences (SPSS) software for Windows.

Results: The study included 150 COVID-19 patients. HRCT chest severity score was mild in the majority of patients (46.7%), moderate in 24.7%, severe in 5.3%, and negative in 23.3% of cases. HRCT chest severity score was directly correlated with fever, dyspnea, cough, sore throat, reduced appetite, tachypnea, tachycardia, heart rate, respiratory rate, systolic blood pressure, peripheral oxygen saturation, and Glasgow Coma Scale (GCS) score ($p < 0.05$).

Conclusion: The HRCT chest severity score is directly correlated with clinical symptoms, clinical parameters, coexisting comorbidities, and radiological findings in patients with COVID-19 disease. Hence, HRCT chest plays an important role in assessing the severity of COVID-19 disease and predicting the outcome in these patients.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was recently found to be responsible for a pneumonia outbreak that began in Wuhan (China), around the end of December 2019. First reported on 30th January 2020 in India, >11 million cases of COVID-19 cases were confirmed attributing to 158,856 deaths in India as of 16th March 2020. The cumulative incidence was reported to be 23.422/million people as of 31st October 2020; males reported a higher incidence (30.223/million) than females (20.705/million). People aged 61–80 had the highest cumulative incidence (41.064/million), whereas those under 20 had the lowest.[1] Epidemiological research suggests that these viruses can spread swiftly amongst humans and are extremely contagious once they infect a host.[2] The severity of infection varies from asymptomatic illness to severe and critical illness.[3]

Reverse transcriptase polymerase chain reaction (RT-PCR) is one of the most accurate and reliable methods to identify viruses.[4] Although the detection which is based on PCR has some drawbacks, including the need for a very pure sample, significant costs associated with laboratory equipment and training for specialists as well as a lengthy response time. Interleukin-6 (IL-6), glucose, D-dimer, fibrinogen, thrombin time, and C-reactive protein (CRP) were all significantly different between moderate and severe COVID-19 cases.[5] Chest X-rays may show opacities in one or both lungs, sometimes in a basilar and markedly peripheral distribution. Nevertheless, chest X-ray is quite sensitive and fairly specific in diagnosing COVID-19.

Some patients may be asymptomatic and may not have obvious symptoms. HRCT chest can be a valuable tool for detecting COVID-19 in the initial stage of infection when symptoms are hazy or scarce. Chest radiography and CT were the primary diagnostic tools used at the peak of SARS, in accordance with recommendations from the World Health Organization (WHO) and the Centers for Disease Control and Prevention.

Numerous research studies have examined the involvement of lungs in CT chest scans using both visual and quantitative software evaluations, and they have evaluated the relationship between the clinical profile of patients with COVID-19 illness and the HRCT chest severity ratings.[7,8] As a result, it was determined that HRCT, coupled with clinical parameter characteristics, might be useful in determining the severity and extent of the illness as well as in tracking its progression. It is noteworthy to mention that several prediction models have been given in the academic literature, with CT scan characteristics and clinical evaluation being the most commonly reported prognostic indicators.[9–11] Therefore, the current study was conducted to study the features of HRCT chest in patients with COVID-19 disease and to study its association with clinical status.

Materials and Methods

This observational study was conducted in COVID-19 block A and B of Gandhi Medical College and Associated Hamidia Hospital Bhopal, over the duration of 18 months from 1st January 2021 to 30th June 2022. All the patients diagnosed with COVID-19 and had positive results of RT-PCR/rapid antigen test (RAT) belonging to the age range of >18 years were included in our study whereas patients not giving consent for the study were excluded.

A total of 150 cases were included after obtaining ethical clearance. Patients were then subjected to detailed history including sociodemographic details, mode of presentation such as fever, cough, breathlessness, fatigue, loss of taste/smell, rhinorrhea etc., and comorbidities. SARS-CoV-2 infection from a nasopharyngeal swab was verified using the U-TOP COVID-19 detection kit, an RT-PCR test having an Emergency Use Authorization from the United States Food and Drug Administration. For RT-PCR testing, the most recent testing guidelines demanded the application of diagnostic standards from Clinical Laboratory Improvement Amendments. In all cases, two consecutive RT-PCR assays were performed.

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All the patients were then subjected to blood investigations (including complete blood counts, renal function test (RFT), lipid profile, glycated hemoglobin, inflammatory markers, CRP, IL-6, and D-dimer), Arterial blood gas analysis and radiographic tests [chest radiograph, high-resolution computed tomography (HRCT) of chest].

Patients were categorized to be suffering from varying severity of illness based upon chest CT severity score according to Radiological Society of North America criteria.[12]

Statistical Analysis

All the data were entered into the Microsoft Excel software, and the Statistical Package for the Social Sciences (SPSS) program for Windows, version 25 (SPSS, Chicago, Illinois) was used to conduct the statistical analysis. Continuous data were shown as mean standard deviation (SD), whereas categorical variables were shown as percentages and absolute values. Either Fisher's exact test or the Chi-squared test was used to analyze categorical data. One-way analysis of variance was used to come to the mean. The $p < 0.05$ was considered statistically significant.

Observations and Results

Our population included consecutive 150 COVID-19 patients.

In the present study, the majority of the patients (24%) belonged to the age-group of 41–50 years and 57.3% of patients were male. Diabetes and hypertension were present in 6.7 and 5.3% of cases, respectively. The most common clinical symptoms were fever (71%) and cough (65%). Chest CT severity score was mild in the majority of patients (46.7%), moderate in 24.7%, severe in 5.3%, and negative in 23.3% cases (Table 1).

On HRCT chest findings revealed opacity (central, peripheral, and both) in 80.7%, atelectasis (6.7%), subpleural bands (14.7%), ground glass opacity (51.3%), ground glass opacity, and consolidation (24%). More than half (>50%) of the patients had affected the right middle, upper lobe and lower lobe, left upper lobe (LUL) and left lower lobe (LLL), and both the surface of the lungs (Table 2).

The clinical symptoms of fever, cough, dyspnea, sore throat, and decreased appetite were shown to be statistically significantly correlated with the chest CT severity score ($p < 0.05$). Vital parameters respiratory rate, heart rate, systolic blood pressure and peripheral oxygen saturation, and Glasgow Coma Scale (GCS) score were significantly correlated with chest CT severity score

Table 1: Distribution based on baseline variables

Baseline variables		*Frequency (n = 150)*	*Percentage*
Age (years)	≤30	27	18.0
	31–40	17	11.3
	41–50	36	24.0
	51–60	29	19.3
	61–70	32	21.3
	>70	9	6.0
Sex	Male	64	42.7
	Female	86	57.3
Comorbidities	Hypertension	8	5.3
	Diabetes	10	6.7
Clinical features	Fever	142	71.0
	Cough	130	65.0
	Cough with expectoration	58	29.0
	Dyspnea	111	55.5
	Fatigue	108	54.0
	Sore throat	84	42.0
	Headache	99	49.5
	Nasal discharge	36	18.0
	Vomiting	53	26.5
	Body ache	107	53.5
	Decreased appetite	2	1.0
CT severity score	Normal	35	23.3
	Mild	70	46.7
	Moderate	37	24.7
	Severe	8	5.3

Table 2: Distribution of HRCT chest findings

HRCT chest findings		*Frequency*	*Percentage*
Opacity distribution	Central	2	1.3
	Peripheral	42	34.7
	Both	67	44.7
Underlying lung disease	Atelectasis	10	6.7
	Pleural effusion	0	0
	Thoracic lymphadenopathy	11	7.3
	Pulmonary nodules	2	1.3
	Cardiomegaly	3	2
	Cavitation changes	2	1.3
	Collapse	2	1.3
	Partial collapse	2	1.3
	Pleural thickening	2	1.3
	Subpleural bands	22	14.7
GGO and consolidation	GGO	77	51.3
	Consolidation	4	2.7
	Both present	36	24
	Both absent	4	2.7
Frequency of lobe affected	Right upper	101	67.3
	Right middle	77	51.3
	Right lower	115	76.7
	Left upper	91	60.7
	Left lower	101	67.3
Involved surface of lungs	Anterior	6	4
	Posterior	40	26.7
	Both	75	50

($p < 0.05$). Tachycardia and tachypnea were observed to be more evident in patients with more serious scan outcomes ($p < 0.001$) (Table 3).

The chest CT severity score substantially correlated with peripheral alone and peripheral plus central opacity ($p < 0.05$). The link between atelectasis, pleural effusion, lymphadenopathy, cardiomegaly, cavitation alterations, collapse, partial collapse, pleural thickening, and subpleural bands was found to be statistically significant ($p < 0.05$). The link between ground glass opacity and consolidation and the chest CT severity score was found to be statistically significant ($p < 0.001$). Posterior involvement alone and both surface involvement were found to be statistically significantly correlated with chest CT severity score ($p < 0.001$) (Table 4).

Discussion

In nonavailability of RT-PCR testing, results are not available right away, or there is suspicion of COVID-19 illness despite negative result of initial RT-PCR testing, the WHO advises using chest imaging as a part of the diagnostic workup for COVID-19 disease. To select the best imaging modality, radiologists, and clinicians should collaborate closely.[13] Using a CT scan, it is possible to assess each patient's disease burden.[14] A visual approach, like we used in this study or software that calculates the percentage of damaged lung volumes using deep learning algorithms can be used to assess the quantitative severity.[15]

Our goal was to assess the HRCT chest findings in 150 COVID-19 patients and determine whether they were correlated with their clinical status. In this study, the majority of patients (46.7%) had mild chest CT severity scores followed by moderate (24.7%) and severe (5.3%) scores. Similar to our study's findings, Bhandari et al.'s study discovered a relationship between the patient's clinical condition and chest CT severity score in mild cases displaying a score of 15/25 in 45.83% of patients and severe cases exhibiting a score of >15/25 in 87.50%.[16] In a few more studies, similar outcomes were noticed.[17,18]

In the present study, we found a statistically significant correlation between clinical symptoms like fever, cough, dyspnea, sore throat, and decreased appetite with the chest CT severity score ($p < 0.05$). In the study of Francone et al., fever and cough were the most frequent presenting symptoms in symptomatic patients, followed by shortness of breath, sore throat, and headache. A small number of patients also had chest pain and nonrespiratory symptoms like pain in the abdomen, fatigue, joint pain, altered sensorium, etc.[14] Clinical symptoms of the patients had a transparent correlation with the chest CT severity score in the research by Bhandari et al.[16] Similarly Saeed et al.[17] and Sharma et al.[18] reported that various clinical symptoms were significantly correlated with chest CT severity scores.

Clinical parameters respiratory rate, heart rate, systolic blood pressure, peripheral oxygen saturation, and GCS score were significantly associated with chest CT severity score in our study ($p < 0.05$). According to Yang et al., individuals with severe COVID-19 infections had a substantially higher overall chest CT severity score than those with milder infections. They added that the degree of lung involvement may be assessed using a chest CT severity score.[12] About 27 patients exhibited hypoxia by the time of admission, according to research by Alinezhad et al. These patients also had considerably higher chest CT severity scores ($p = 0.001$).[19] Patients with hypoxia had a mean chest CT severity score of 18.14 ± 7.43, while those without hypoxia had a mean chest CT severity score of 12.64 ± 7.25.[19] These findings suggest that more severe clinical problems in patients are associated with higher chest CT severity scores. We further believe that this problem may be justified by connections between higher chest CT severity ratings and hypoxia.

We found that peripheral and mixed opacity was significantly associated with chest CT severity score ($p < 0.05$) while central opacity alone was not associated with chest CT severity ($p > 0.05$). The most frequent finding in a recent investigation by Yang et al. was multifocal peripheral ground glass opacity or mixed opacity with preponderance in the lower lung. The severity of the illness was occasionally associated with the extent of

Table 3: Correlation of chest CT severity score with clinical variables

Clinical details		*Chest CT severity score*				*p-value*
		Normal	*Mild*	*Moderate*	*Severe*	
Symptoms	Fever	29 (82.9)	68 (97.1)	37 (100)	8 (100)	**0.005**
	Cough	25 (71.4)	68 (97.1)	31 (83.8)	6 (75)	**0.002**
	Cough with expectoration	10 (28.6)	24 (34.3)	20 (54.1)	4 (50)	0.102
	Dyspnea	19 (54.3)	53 (75.7)	33 (89.2)	6 (75)	**0.009**
	Fatigue	21 (60)	54 (77.1)	25 (67.6)	8 (100)	0.075
	Sore throat	21 (60)	32 (45.7)	29 (78.4)	2 (25)	**0.003**
	Headache	24 (68.6)	44 (62.9)	23 (62.2)	8 (100)	0.189
	Nasal discharge	10 (28.6)	14 (20)	10 (27)	2 (25)	0.752
	Vomiting	14 (40)	23 (32.9)	12 (32.4)	4 (50)	0.703
	Body ache	28 (80)	48 (68.6)	27 (73)	4 (50)	0.337
	Decreased appetite	0 (0)	0 (0)	0 (0)	2 (25)	**<0.001**
Clinical findings	Tachycardia	2 (4.2)	15 (31.3)	25 (52.1)	6 (12.5)	**<0.001**
	Tachypnea	0 (0)	0 (0)	29 (78.4)	8 (21.6)	**<0.001**
Vitals	RR	19.37 ± 2.26	19.71 ± 1.416	21.57 ± 2.734	22.50 ± 2.204	**<0.001**
	HR	92.40 ± 5.397	96.09 ± 6.338	100.11 ± 8.296	111.50 ± 11.25	**<0.001**
	SBP	105.2 ± 11.01	101.17 ± 10.42	105.89 ± 16.60	117.5 ± 11.65	**0.003**
	Saturation of peripheral oxygen	95.00 ± 4.59	92.59 ± 3.39	86.30 ± 7.68	73.75 ± 5.36	**<0.001**
	GCS	14.23 ± 1.629	14.63 ± 0.685	14.24 ± 0.796	11.00 ± 2.726	**<0.001**

Bold value indicates highly significant *p-value*. RR, respiratory rate; HR, heart rate; SBP, systolic blood pressure

Table 4: Correlation of CT chest findings with chest CT severity score

HRCT chest findings		*Chest CT severity score*				*p-value*
		Normal	*Mild*	*Moderate*	*Severe*	
Opacity distribution	Central	0 (0)	2 (2.9)	0 (0)	0 (0)	0.509
	Peripheral	0 (0)	36 (51.4)	16 (43.2)	0 (0)	**<0.001**
	Both	10 (28.6)	30 (42.9)	19 (51.4)	8 (100)	**0.002**
Underlying findings	Atelectasis	8 (22.9)	2 (2.9)	0 (0)	0 (0)	**<0.001**
	Pleural effusion	4 (11.4)	0 (0)	2 (5.4)	0 (0)	**0.037**
	Thoracic lymphadenopathy	9 (25.7)	2 (2.9)	0 (0)	0 (0)	**<0.001**
	Cardiomegaly	3 (8.6)	0 (0)	0 (0)	0 (0)	**0.002**
	Cavitatory changes	2 (5.7)	0 (0)	0 (0)	0 (0)	**0.004**
	Collapse	0 (0)	2 (2.9)	0 (0)	0 (0)	**0.021**
	Partial collapse	2 (5.7)	0 (0)	0 (0)	0 (0)	**0.032**
	Pleural thickening	2 (5.7)	0 (0)	0 (0)	0 (0)	**0.022**
	Subpleural bands	2 (5.7)	15 (21.4)	2 (5.4)	3 (37.5)	**0.004**
GGO and consolidation	GGO	2 (5.7)	48 (68.6)	21 (56.8)	6 (75)	**<0.001**
	Consolidation	2 (5.7)	0 (0)	0 (0)	2 (25)	**<0.001**
	Both present	0 (0)	18 (25.7)	14 (37.8)	4 (50)	**<0.001**
	Both absent	2 (5.7)	0 (0)	0 (0)	2 (25)	**<0.001**
Lobe involved	RUL	8 (22.9)	50 (71.4)	35 (94.6)	8 (100)	**<0.001**
	Right ML	4 (11.4)	40 (57.1)	25 (67.6)	8 (100)	**<0.001**
	RLL	12 (34.3)	60 (85.7)	35 (94.6)	8 (100)	**<0.001**
	LUL	6 (17.1)	44 (62.9)	33 (89.2)	8 (100)	**<0.001**
	LLL	6 (17.1)	54 (77.1)	33 (89.2)	8 (100)	**<0.001**
Surface of the lung involved	Posterior	6 (17.1)	30 (42.9)	4 (10.8)	0 (0)	**<0.001**
	Anterior	0 (0)	6 (8.6)	0 (0)	0 (0)	0.067
	Both	4 (11.4)	32 (45.7)	31 (83.8)	8 (100)	**<0.001**

Bold value indicates highly significant *p-value*

these observations.[12] According to Cheng et al., who also looked at CT scan results of patients with suspected COVID-19 illness, the patterns of imaging of multifocal, peripheral, pure ground-glass opacity (GGO), mixed GGO, or consolidation with a modest dominance in the lower lung could be viewed as observations that are very suspicious of COVID-19 infections. However, they were unable to determine whether there were any connections between these HRCT chest scan results and the clinical characteristics of the patients.[20]

Ground-glass opacity (GGO), consolidation, atelectasis, pleural effusion, lymphadenopathy, cardiomegaly, cavitation alterations, collapse, partial collapse, pleural thickening, and subpleural bands were shown to be statistically significantly correlated with the chest CT severity score in our study ($p < 0.001$). Right middle, right upper lobe (RUL) and right lower lobe (RLL), left upper and lower lobe, and both the surface of the lungs were afflicted in more than half (>50%) of the patients. A statistically significant connection between the frequency of the affected lobes and the CT severity score was discovered ($p < 0.001$). The most frequent pattern of sickness identified by Francone et al. was GGO, which affected 125 people (96.2%), and the mean of CT scores were as follows—the dimensions of the LUL are 2.2 ± 1.2, RUL is 2.2 ± 1.5, the middle lobe (ML) is 1.8 ± 1.5, RLL is 3.1 ± 1.3, and LLL is 3 ± 1.4. The relationship between the posterior alone, surface involvement, and the chest CT severity score was shown to be extremely significant ($p < 0.001$).[14]

There are several limitations in our study—first, to increase the accuracy and trustworthiness of the findings, a significant multicentric study is required. Second, the fact that the assessment of disease severity on HRCT chest scan can be subjective. This can be reduced by using two qualified readers to reach an opinion. Third, it is pivotal to include any other factors that could have an impact on the course of the disease, such as lifestyle decisions and the use of self-reporting or underreporting of comorbidities. Lastly, the study may have included unreported possible confounders, we didn't use all the pertinent information when collecting the data, and we had less evidence than prospective studies.

Conclusion

The HRCT chest severity scores in COVID-19 patients predict the severity and course of the disease and are directly corelated with the clinical symptoms and signs. It guides the management of COVID-19 pneumonia. Our study data recommends the early use of HRCT chest in COVID-19 patients to assess the severity of the disease and its management.

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ORIGINAL ARTICLE

To Estimate the Prevalence of Obesity and High Blood Pressure among Undergraduate Students at a University Medical Institution in North India

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Abstract

Background: Hypertension is a major public health issue in India. Early detection and management of high blood pressure (BP) is crucial, especially among young adults. This study aimed to estimate the prevalence of obesity and hypertension among undergraduate medical students.

Materials and methods: A cross-sectional study was conducted among 450 first year undergraduate medical students aged 18-25 years in S.M.S. Medical and Hospital Jaipur, Rajasthan after clearance from institutional ethics committee and written consent from participants. Anthropometric measurements like height, weight, BMI, waist circumference, hip circumference and blood pressure were recorded. Hypertension was defined as per JNC VIII guidelines. Data was analyzed using appropriate statistical tests.

Results: Overall, 15.56% students were hypertensive and 40.67% were prehypertensive. Hypertension was more prevalent in males (18.83%) compared to females (12.33%) ($p = 0.002$). Overweight/obesity was present in 29.33% students, more common in males (37.67%) than females (21.15%) ($p < 0.001$). Obese students had higher rates of prehypertension (47%) and hypertension (28.8%). Abnormal waist-hip ratio and waist-stature ratio were significantly associated with hypertension ($p < 0.001$).

Conclusion: Overweight/obesity and hypertension are highly prevalent among undergraduate medical students, especially males. Unhealthy lifestyles and risk factors need to be addressed to prevent long term morbidity. Routine screening and health promotion activities should be conducted for this high risk group.

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Introduction

The risk of developing cardiovascular disease, a stroke, kidney failure, and dying prematurely is increased when hypertension is present. There is a possibility that the company's bottom line and healthcare costs will be impacted. Around 9.4 million people per year lose their lives due to complications associated with hypertension. Over the course of the past several years, hypertension[1] has developed into a major concern for the medical community in India. Although, the chance of getting the condition is higher for people over the age of 40 than it is for people under the age of 30, according to.[2] Prehypertension is a precursor to hypertension and is more common in young adults in their 20s and 30s than hypertension itself. The adoption of healthy behaviors can assist in mitigating this important risk factor for developing hypertension or cardiovascular disease in later life.[3] The percentage of children and adolescents who are overweight or obese is rising at an alarming rate all across the world. Obesity is characterized by increased visceral fat, which has been linked to changes in hormone function as well as inflammatory and endothelial function. These alterations set in motion a chain reaction that acts to both keep blood pressure (BP) at persistently high levels and raise the risk of cardiovascular disease.[4] Diets that are high in salt are associated with an increased mortality risk due to factors such as obesity, smoking, inactivity, and insufficient consumption of fruits and vegetables.[5] It is absolutely necessary to bring the BP and fat levels of young people down, even just a little bit, in order to stop the spread of these noncommunicable diseases and prevent additional outbreaks. Medical school is challenging[6] all the way through because of the long hours, lack of social interaction, pressure from tests, bad eating habits, and excessive expectations. The following are some things that could be mentally draining for you. By collecting and evaluating data on the prevalence of obesity and hypertension among medical students, the goal of this study is to fill a vacuum in existing knowledge about these conditions.

Materials and Methods

In the month of July 2022, 1st-year medical students from Sawai Man Singh Medical College, Jaipur, Rajasthan, India, and affiliated facilities participated in a cross-sectional survey. The project has received the go-light from the Institution's Ethics Council. We determined that a sample size of 450 individuals would give us 80% power with an error of 0.05%, which is exactly 0.1%, due to the high rates of obesity and hypertension among college students 2.5%.

Inclusion Criteria

- Apparently, healthy subjects are free from any chronic illness.
- Those willing to participate in the study.

Exclusion Criteria

- Subjects on long-term medication for any chronic illnesses.

Methods of Evaluation

- Informed consent was obtained and participant confidentiality was assured.
- A questionnaire was used to collect details with regard to demography.
- A detailed clinical examination was performed.
- Measurements of height, weight, BP, hip circumference, and waist circumference were taken.

Measurements Taken (Table 1)

Blood Pressure (BP)

The patient's BP was taken using an auscultatory instrument that had been calibrated and tested. Each person sat in a chair with their back supported and an arm

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resting at heart level for at least 5 minutes. The ideal dimensions for a cuff's bladder are 80% forearm coverage and breadth which is about 40% of the forearm's circumference. Two measurements were collected on average. When two or more Korotkoff sounds were detected, a systolic reading was taken, and a diastolic reading was taken shortly as the sounds began to diminish.

Height

This was measured on a reliable, accurate scale. All distances were recorded to the closest centimeter.

Weight

It was measured using a validated calibrated weighing scale. Measurements were taken to the nearest kilogram.

Waist Circumference

We took a measurement that was placed precisely in the middle of the point where the lowest palpable rib and the top of the iliac crest meet. This was done in accordance with the recommendations provided by the World Health Organization. While standing with their feet together, the individual's weight was distributed equally between both feet.

Hip Circumference

We took a precise measurement across the widest area of the rear end, making sure the ruler was always perpendicular to the ground, using an inch of tape. The person's weight was divided evenly between both feet, which were planted firmly on the ground. Take your height in square meters and deduct your weight in kilograms to get your ideal weight. Obesity was also indicated by measures such as the waist-to-hip ratio (WHR), waist-to-stature ratio (WSR), and waist circumference.

Criteria for Defining Obesity Body Mass Index (BMI) (Table 2)

- Waist circumference: Men with waist circumferences over 90 cm and women over 80 can be diagnosed with central or abdominal obesity.[8]
- Waist-to-hip ratio: Truly obese people have a WHR of 0.90 or higher (for men) or 0.85 (for women).[9]

A WSR greater than 0.5 corresponds to obesity in both males and females.[10]

The BP was checked using the Joint National Committee VIII (JNC VIII) (Table 3) standards[11] for finding, checking for, and treating high BP. It was thought that systolic BP (SBP) should be <120 mm Hg and diastolic BP (DBP) should be <80 mm Hg. In the past, prehypertension meant having a systolic BP between 80 and 89 mm Hg and a diastolic BP between 120 and 139 mm Hg. People with a SBP of 140–159 mm Hg and/or a DBP of 90–99 mm Hg were said to have stage I hypertension. People with stage II hypertension have an SBP of 160 mm Hg or higher and/or a DBP of 100 mm Hg or higher.

Table 2: Southeast Asian classification[7]

S. no.	*BMI*	*Categories*
1	<18.5	Underweight
2	18.5–22.9	Normal
3	23–24.9	Overweight
4	>25	Obese

Data Analysis

The frequencies and percentages of the categorical factors were added up and then the Chi-squared test was used. To figure out how important the link was, we used the odds ratio and a 95% confidence range. The *t*-test was used to find the mean and standard deviation of continuous data. We picked a *p*-value of <0.05 as the level of statistical significance. Epi Info version 7.2.1.0 was used to figure out all the information.

Results

Out of the 450 students who took part in the study, 99.44% were women and 49.56% were men. A healthy BMI was found in 173 kids, or 37.5%. There were only 40 kids who were too thin (8.9%). Around 39% of the kids were overweight or obese (132), and 23% of the teachers were overweight (105). Out of the 132 students, 84 (63.64%) were men and 48 (36.36%) were women. The rates of obesity were very different between men and women (2 = 14.029, one degree of freedom, p = 0.001). Based on body measurements like BMI, WHR, and WSR, men were more likely to be overweight than women. The ages of overweight people and nonobese people are about the same (2 = 5.081, two degrees of freedom, and p = 0.079). All together, the kids had a BP of

Table 3: Criteria for defining high BP

Category	*Systolic*		*Diastolic*
Normal	<120	and	<80
Prehypertension	120–139	or	80–89
High BP/hypertension			
Stage I hypertension	140–159	or	90–99
Stage II hypertension	≥160	or	≥100

Table 1: Anthropometric and BP measurements of the study participants according to gender (n = 450)

Anthropometric variables	*Males (n= 223)*	*Females (n= 227)*	*Odds ratio*	χ^2	*p-value*
BMI (kg/m^2)					
<25	179 (78.85%)	139 (62.33%)	2.254 (1.483–3.424)	14.029	0.001
>25	61 (32.8%)	50 (20.2%)			
Waist circumference (cm)					
Normal	171 (77.68%)	176 (77.53%)	1.049 (0.676–1.629)	0.011	0.918
Abnormal (males >90 cm; females >80 cm)	52 (23.32%)	51 (22.47%)			
WHR					
Normal	179 (80.27%)	191 (84.14%)	1.304 (0.803–2.119)	0.904	0.342
Abnormal (males ≥0.90; females ≥0.85)	44 (19.73%)	36 (15.86%)			
BP (mm Hg)					
<120/80	74 (33.18%)	123 (54.19%)	0.41 (0.20–0.83)	6.28	0.012
>120/80	149	104			

The 95.0% confidence interval is given in parentheses

63.78/100. Around 46% of the medical students who were polled had prehypertension. Out of the children, 58 (12.89%) were found to have prehypertension and 12 (2.67%) were found to have hypertension. People between the ages of 18 and 24 did not have significantly different BP (2 = 0.432, two degrees of freedom, and $p = 0.806$). There was a big difference between men and women—42 men and 28 women had BPs of 140/90 mm Hg or higher. Obese students (those with a BMI of 30 or more and a WHR of greater than 0.5) were more likely to have high BP. People were asked about times when they had their BP checked and were told they had high BP. No one raised their hand. Students who were at risk were told what would happen if they didn't change their bad habits, and they were helped as they did so. They were also taken to the teaching hospital connected with the university for more testing and care.

Table 4: Age distribution of study subjects

Age-group (years)	*N*	*Percentage*
18–19	26	5.78
20–21	129	28.67
22–23	174	38.67
24–25	121	26.89
Total	450	100
Mean ± SD	22.26 ± 1.65 years	
Median (range)	22 (18–25 years)	

Table 5: Gender distribution of study subjects

Gender	*N*	*Percentage*
Male	227	50.44
Female	223	49.56
Total	450	100

Table 6: Distribution of study subjects according to BP status

BP status	*N*	*Percentage*
Normal	197	43.78
Prehypertension	183	40.67
Hypertension stage I	58	12.89
Hypertension stage II	12	2.67
Total	450	100

Table 7: Distribution of study subjects according to BMI

BMI	*N*	*Percentage*
Underweight	40	8.9
Normal	173	38.4
Overweight	105	23.3
Obese	132	29.3
Total	450	100

- As described in Table 4 majority of subjects were of the age-group 22–23 (38.67%).
- The mean age of our subjects was 22.26 years.
- As described in Table 5, in a study out of 450 subjects 50.44% of subjects were males and 49.56% of subjects were females.
- As described in Table 6 and Figure 1, out of 450 subjects 43.78% were normotensive, 40.67% were prehypertensive, 12.89% had stage I hypertension, and 2.67% had stage II hypertension.

In Table 7 and Figure 2 (38.4%) of total subjects were found to be falling in the category of normal BMI.

- Around 23.3% of the total subjects were overweight.
- A striking 29.3% of subjects had having BMI in the obese range.
- Lastly, 8.9% of subjects were found to be underweight.

Table 8 displays the odds ratio to illustrate the difference between the risks that men and women face in comparison to one another. Researchers classified 84 males and 48 females as being overweight out of a total of 132 participants that were overweight. There are 63.63% men and 36.36% women among those individuals.

Table 9 and Figure 3 show that out of a total sample size of 450, we were able to identify 197 individuals (123 females and 74 men) with normal BP.

Prehypertension was found in 183/450 people, with 76/183 (41.5%) women and 107/450 (58.46%) men; this is a statistically significant difference ($p = 0.001$).

Hypertension (stages I and II) was detected in 70 of 450 persons, with 28 (40%) females and 42 (60%) males. The p-value for this distinction was also very low (0.002).

Table 10 and Figure 4 show that there was no statistically significant difference in BP status between study subjects aged 18–21 and those aged 22–25.

The percentage of underweight people with normal BP is shown in Table 11 and Figure 5. Around 27.5% had prehypertension and 15% had hypertension.

Around 56.1% of people with a normal BMI had normal BP.

Normal BP was found in just 35.8% of the normal-weight population and 8.1% of the overweight population. Only 33.1% of the obese population had normal BP, whereas 45.7% of the overweight population was prehypertensive and 11.4% was hypertensive. Prehypertension (47.1% of individuals) and hypertension (28.8% of participants) were observed in the obese population.

Participants' BMI was significantly associated with their likelihood of developing hypertension. having a probability of <0.001.

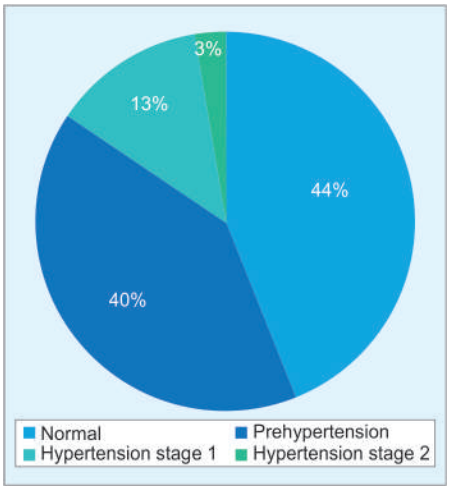

Fig. 1: Distribution of study subjects according to BP status

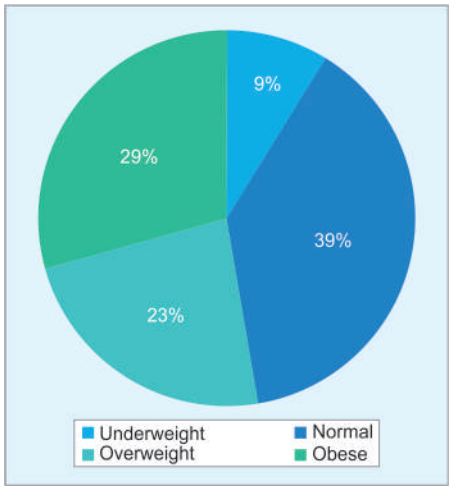

Fig. 2: Distribution of study subjects according to BMI

Table 8: BMI in relation to gender of study subjects

BMI	*Female*		*Male*		*Total*	
	N	*%*	*N*	*%*	*N*	*%*
<25 kg/m^2	179	78.85	139	62.33	318	70.67
≥25 kg/m^2	48	21.15	84	37.67	132	29.33
Total	227	100	223	100	450	100

Odds ratio = 2.254 (95% CI = 1.483–3.424); Chi-square = 14.029 with 1° of freedom; $p < 0.001$ (S)

Table 9: BP status in relation to gender of study subjects

BP status	*Female*		*Male*		*Odds ratio*	*p-value*
	N	*%*	*N*	*%*		
Normal	123	54.19	74	33.18	–	–
Prehypertension	76	33.48	107	47.98	2.340 (95% CI = 1.55–3.53)	<0.001 (S)
Hypertension	28	12.33	42	18.83	2.493 (95% CI = 1.43–4.36)	0.002 (S)
Total	227	100	223	100		

Table 10: BP status in relation to age of study subjects

BP status	*18–21 years*		*22–25 years*		*Total*	
	N	*%*	*N*	*%*	*N*	*%*
Normal	65	41.94	132	44.75	197	43.78
Prehypertension	64	41.29	119	40.34	183	40.67
Hypertension	26	16.77	44	14.92	70	15.56
Total	155	100	295	100	450	100

Chi-square = 0.432 with 2° of freedom; $p = 0.806$

Table 11: BP status in relation to BMI of study subjects

BP status	*Underweight*		*Normal*		*Overweight*		*Obese*	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Normal	23	57.5	97	56.1	45	42.9	32	24.2
Prehypertension	11	27.5	62	35.8	48	45.7	62	47.0
Hypertension	6	15	14	8.1	12	11.4	38	28.8
Total	40	100	173	100	105	100	132	100

Chi-square = 46.073 with 6° of freedom; $p < 0.001$ (S)

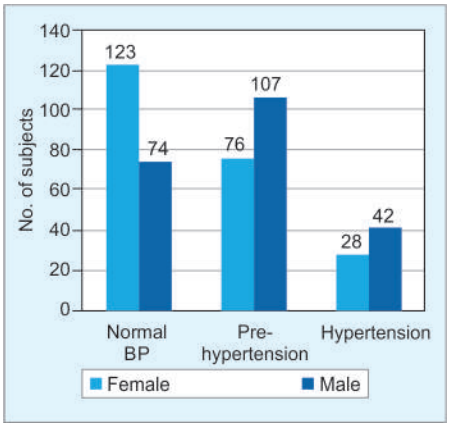

Fig. 3: BP status in relation to the gender of study subjects

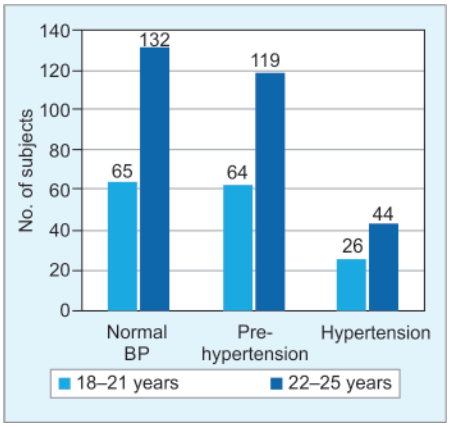

Fig. 4: BP status in relation to age of study subjects

Abnormal WHR (males 0.90, ladies 0.85).

There were 450 participants, and as shown in Table 12 and Figure 6, 180 (43.78%) had normal BP, 155 (41.89%) had prehypertension, and 35 (9.46%) had hypertension.

Out of 450 people, 80 (21.75%) had an abnormal WHR; of these, 28 (35%), were diagnosed with prehypertension, and 35 (43.75%) were diagnosed with hypertension.

The correlation between BP and WHR was statistically significant ($p = 0.001$).

Participants' BP was shown to rise in tandem with their WHR.

According to Table 13, an abnormal waist circumference in both sexes equals 0.5.

Around 399 (50.1%) of the 450 participants had normal WSR; among these, 124 (30.87%) had prehypertension, 35 (10.97%) had hypertension, and 160 (50.16%) had normal BP.

Only 37 (28.14%) of the 131 participants whose WSR was considered abnormal had a normal BP result, while 59 (45.04%) had prehypertension, and 35 (26.72%) had hypertension. The ratio of a person's waist to hips was significantly correlated with their BP (Table 14).

Discussion

Undergraduates from Sawai Man Singh Medical College, Jaipur took part in a cross-sectional study. There are 450 participants in this study. The average age of the 450 persons in the study was 22.26; they were all between the ages of 18 and 25. The results showed that 43.78% of participants had normal BP, 47% had prehypertension, 12.82% had hypertension, and 2.67% had severe hypertension. The prevalence of prehypertension is much higher in men (58.46%) than in women (41.5%) ($p = 0.001$). Around 60% of males and 40% of females had hypertension at stages I and II, respectively. Chenji, et al. others like C. R. and S. R. Among 434 medical undergraduates studying at a South Indian university, 8.1% had hypertension (stages I or II).[12] When we looked at hypertension rates for both elementary and secondary students together, we found that the combined rate was <15%. Students with high BP skewed significantly males (63%), compared to

Table 12: BP status in relation to WHR of study subjects

BP status	*Normal WHR*		*Abnormal WHR*		*Total*	
	N	*%*	*N*	*%*	*N*	*%*
Normal	180	48.65	17	21.25	197	43.78
Prehypertension	155	41.89	28	35	183	40.67
Hypertension	35	9.46	35	43.75	70	15.56
Total	370	100	80	100	450	100

Chi-square = 61.769 with 2° of freedom; $p < 0.001$ (S)

Table 13: BP status in relation to waist stature ratio of study subjects

BP status	*Normal WSR*		*Abnormal WSR*		*Total*	
	N	*%*	*N*	*%*	*N*	*%*
Normal	160	50.16	37	28.24	197	43.78
Prehypertension	124	38.87	59	45.04	183	40.67
Hypertension	35	10.97	35	26.72	70	15.56
Total	319	100	131	100	450	100

Chi-square = 25.855 with 2° of freedom; $p < 0.001$ (S)

Table 14: Comparison of various parameters among male and female

Characteristics	*Female*	*Male*	*p-value*
BMI	22.75 ± 3.99	24.19 ± 3.47	**<0.001 (S)**
WC	76.53 ± 6.62	84.58 ± 6.46	**<0.001 (S)**
WHR	0.79 ± 0.05	0.85 ± 0.05	**<0.001 (S)**
WSR	0.48 ± 0.04	0.48 ± 0.04	0.342
SBP	116.75 ± 10.15	123.72 ± 10.56	**<0.001 (S)**
DBP	76.77 ± 8.25	80.96 ± 8.79	**<0.001 (S)**

Bold letter indicates significant *p-values*

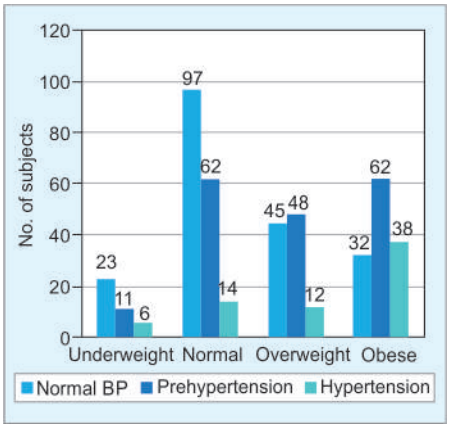

Fig. 5: BP status in relation to BMI of study subjects

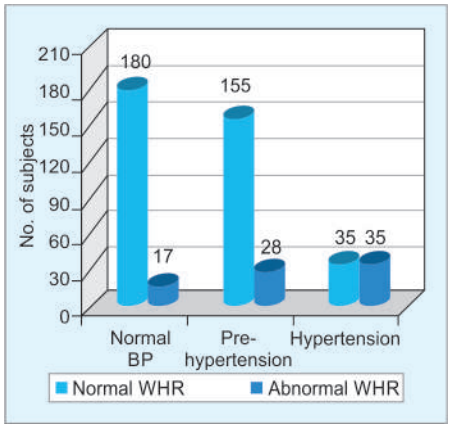

Fig. 6: BP status in relation to WHR of study subjects

females (37%). Our research showed that the incidence of hypertension was greater in men than in women. Around 1/14 of the 130 students (mean age = 22.45 1.67, range = 19–27) surveyed in the study by Alwabel et al. were found to have hypertension. Hypertension was found to be significantly more common among men (18.5%) than among women (5.3%). Alwabel et al. identified a prevalence of hypertension of 12.33% among females and 18.83% among males, which is at odds with these results. Prehypertension was found to affect 29.2% of the population. Compared to the 40. 67% who had prehypertension, this number[13] is significantly lower. Medical students in Kolkata were studied by Chattopadhyay et al. to determine the prevalence of hypertension and the factors that lead to it. The sample size for the study was 850 people. Our own anecdotal evidence is in agreement with the study's finding that 13. 88% of the pupils had hypertension and 19. 18% were prehypertensive.[6] There is a significant rate of obesity and hypertension among university students in Jeddah, Saudi Arabia, yet they are not well informed about the causes of these conditions. To the best of our knowledge, Baig et al. hypertension affected only 7.5% of the eyes in our sample.[14]

Around 25% of the people who took part in our study were considered obese by medical standards. Based on their BMI, 29. 3% of the South and Southeast Asian participants in this study were considered obese. There were significantly more men than women who were overweight (63.3%), with men having an odds ratio of 2.254 [95% confidence interval (CI) = 1.483–3.424] and women having an odds ratio of 36.6% (Chi-square = 14.029).

It was determined that 52.6% of the population is overweight or obese. Overweight women made up only 21.1 5%, while men made up 37. 67%.

According to reports, men have a higher obesity rate than women.

Khan et al. conducted a cross-sectional survey at four medical institutions in Lahore, Pakistan, between March and June of 2012. There were 244 male and female medical students in the study, with a median age of 20 (range = 18–25). Anthropometric measurements of humans were taken. Participants self-assessed their caloric intake and body composition. Approximately, 21% of medical students were overweight or obese (approximately, 30.5% of males and 16%).[15] We had anticipated a larger number, so this is disappointing. At-risk individuals are those with a BMI between 23 and 24.9, those with grade I obesity have a BMI between 25 and 29.9, and those with grade II obesity have a BMI of 30 or more. There are 101 persons who are overweight or obese (30.6% of the population) when we combine the grades I and II at-risk and obese groups. Only 11% of

boys were at a healthy weight, while 49.2% were overweight, and 39.8% were obese. Women were particularly affected, with only 23% falling within the recommended weight range, 54% being overweight, and 25% being dangerously obese. Our results demonstrate a substantially higher incidence of obesity in males than in females, and the percentage of boys who are overweight or obese is higher when compared to girls; this difference is statistically significant ($p = 0.009$). The 1st-year medical students (aged 17–24) at a private school in Chennai, Tamil Nadu, India were the subjects of a cross-sectional survey done by Rekha et al. Female students accounted for 56.7% ($n = 247$) of the overall student body, while male students made for 43.6% ($n = 186$). Around 57% of the boys ($n = 109$ in total) were considered to be overweight. Around 49% point 7% ($n = 123$) of girls were either overweight or obese. Around 53.2% ($n = 232$) of the people in this sample are overweight or obese. Previous research has shown that 22.9% of the population is overweight and 30.3% are obese.[16] Our study confirms these findings by finding that 23.3% of all respondents were overweight. Obesity affects 29.3% of the population, according to the study.

The prevalence of prehypertension was 47.0% among the obese, while the prevalence of stages I and II of hypertension was 28.8% among the obese. The figures here are comparable to those reported by Chenji et al. There was also a strong correlation between BP and WHR ($p = 0.001$), with 45.04 participants with an abnormal WHR being prehypertensive and 26.72% being hypertensive. The shockingly high rates of obesity among children and teenagers. Poor eating habits, lifestyle changes, academic stress, cultural differences, isolation from family, lack of exercise, living in dorms, and a lack of time are all factors that may put undergraduate medical students at a higher risk for obesity and hypertension. We were only able to take two readings of each participant's BP before we ran out of time and money, so we could only recommend those with persistently high readings to the medication. OPD.

Conclusion

Medical students were more likely to be overweight (29.3%) and have high BP (15.7%) than groups of people the same age that had been studied before. Keeping an eye on how weight and BP change over time could help lower the number of noncommunicable diseases that young people get today. Also, the number of diseases that could be avoided would go down a lot if medical schools taught their students how important it is to live a healthy life, eat a balanced diet, and work out daily.

Limitations

This was done twice because there wasn't enough time for one. Since the data only came from one medical school, it's not clear how well it can be used in other places. To be able to compare how well different medical schools do, data must be taken in the same way over time.

Acknowledgments

We'd like to thank everyone who took part in the study and the school for letting us do it.

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ORIGINAL ARTICLE

Efficacy of Pulse Methylprednisolone in Treatment of Acute Respiratory Distress Syndrome due to Malaria: A Randomized Controlled Clinical Trial

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Abstract

Objective: To study the efficacy of pulse methylprednisolone (MPS) therapy in patients with malaria-associated acute respiratory distress syndrome (ARDS).

Materials and methods: The study was a randomized, single-blind, placebo-controlled trial with a total sample size of 44 patients. The total random number table was used on a computer for randomization. The sample size was divided into either the study group that received pulse MPS therapy along with the standard therapy to manage acute lung injury (ALI)/ARDS or the control group that received a placebo in the form of 100 mL of normal saline with the standard therapy to manage ALI/ARDS. The primary outcome was defined as either death of the patient or discharge from the hospital. The sequential organ failure assessment (SOFA) score, the lung injury score (LIS), duration of stay in the medical intensive care unit (MICU), number of days for which mechanical ventilation was required, and the rate of secondary infections between the study and the control groups were also calculated. Statistically significant differences among continuous variables were analyzed by *t*-test, and differences between categorical variables were assessed by Chi-squared test.

Results: A total of 30 patients passed initial screening, out of which 60% were males and 40% were females. About 73.3% of the patients fell between the age groups of 36–45 years. A total of 20 patients (66.7%) were discharged from the hospital, while the remaining 10 patients succumbed to death in the intensive care unit (ICU) (33.3%). The outcome of death or discharge was found to be independent of the use of pulse MPS therapy ($p = 0.44$). No statistically significant difference was found between the partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio, SOFA score, and LIS between the two groups. Furthermore, the differences between the mean duration of stay in the MICU, the mean duration for the provision of mechanical ventilation ($p = 0.41$), and the rate of secondary infections ($p = 0.46$) remained unaffected with the use of pulse MPS therapy.

Conclusion: Pulse MPS therapy has not shown any clear-cut benefit in the management of malaria-associated ARDS, and in fact, the continuous use of this treatment in hospitals may lead to worsened outcomes. A novel, effective therapy for this grave complication needs to be developed to reduce the morbidity and mortality in such patients, which is frequently encountered. The development of a robust surveillance system is required for adequate monitoring and early diagnosis of this complication, along with larger multicentric randomized clinical trials.

Disclosures: Approval was granted by the Institutional Ethics Committee (IEC). All participants were only selected after taking their written informed consent. The authors have no conflicts of interest or acknowledgments to report.

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Introduction

Malaria is a life-threatening disease caused by an infection with *Plasmodium* protozoa, transmitted by the bite of an infective female *Anopheles* mosquito. Five *Plasmodium* species cause the disease in humans—*Plasmodium falciparum* (*P. falciparum*), *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*, the majority being caused by *P. falciparum* and *P. vivax* with *P. falciparum* being responsible for the most severe disease.[1] The outcome of the infection depends on the infecting species, the patient's age, and the level of host immunity. *P. falciparum* malaria is notorious for causing debilitating complications such as cerebral malaria, acute renal failure,[2] jaundice, acute respiratory distress syndrome (ARDS),[3] and multiple organ failure.[4]

Most of the cases of malaria-associated ARDS are reversible,[5] therefore, reversing the adverse pathological state and tiding over the crisis is expected to improve the outcomes. Ventilator strategies for ARDS have evolved, and the use of low tidal volume ventilation[6] and the appropriate use of positive end-expiratory pressure[7] have had a favorable impact on the outcome of these patients. However, there is still a continuous search for an effective pharmacological agent that would improve the outcome of patients suffering from this complication. Physicians have been prescribing pulse methylprednisolone (MPS) therapy at the dose of 1 mg/kg/day for 5–7 days for the management of ARDS in tropical infections. Neither is this the standard of care nor is there any robust existing evidence supporting this use. The above practice is still being continued in various setups, including our hospital. The literature further lacked any strong evidence in this direction. Thus, we intend to generate evidence to find out the efficacy of using pulse MPS in patients with malaria associated with ARDS. We further studied the severity of ARDS in malarial infections and the outcomes associated with this dreadful complication.

Materials and Methods

Study Design

The study was a 1:1 randomized, single-blind clinical trial. Patients were assigned to either the treatment group receiving pulse MPS at a dose of 1 mg/kg for 5–7 days along with the standard therapy for the management of ARDS or the control group receiving a placebo in the form of 100 mL of normal saline, along with the standard therapy for the management of ARDS. The duration of the study was 16 months. A total of 44 patients were screened for eligibility, out of which 30 patients were selected and randomized using the total random number table on a computer (Fig. 1). The study was conducted in the intensive care unit (ICU) of a tertiary care hospital in Mumbai, India.

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Inclusion Criteria

Patients of age >12 years who had tested positive for *P. falciparum*, *P. vivax*, or both on a peripheral smear or rapid antigen test and who had been admitted to the ICU with a diagnosis of ARDS or acute lung injury (ALI) were included in the study. ARDS/ALI was defined as the acute onset of respiratory failure, bilateral infiltrates on chest radiography sparing the costophrenic angles, partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) of <300 mm Hg, and lack of clinical evidence of left ventricular failure.[8]

Exclusion Criteria

Patients with left ventricular failure or congestive cardiac failure, undifferentiated acute febrile illness, or other tropical infections (other than malaria) complicated with ARDS and women who were pregnant were excluded from the study.

Study Procedure

The details of the patients were noted along with their history and clinical and physical examination findings. Intention to treat analysis was used in our study. The PaO_2/FiO_2 ratio was recorded at the time of admission, 48 hours after admission, and at the time of primary outcome. The primary outcome was defined as either the death of the patient or discharge from the hospital.

The sequential organ failure assessment (SOFA) score[9] and the lung injury score (LIS)[10] were calculated as clinical variables at the time of admission, 48 hours after admission, and upon reaching the primary outcome. The duration of stay in the medical intensive care unit (MICU), the number of days for which mechanical ventilation was required, and the rate of secondary infections between the study and the control groups were also calculated.

Statistical Analysis

Statistically significant differences among continuous variables were analyzed by *t*-test and differences between categorical variables were assessed by Chi-squared test. All *p*-values ≤ 0.05 were considered statistically significant with a confidence interval of 95%. All statistical analyzes were performed with the use of Statistical Package for Social Sciences (SPSS) software, IBM SPSS version 28.

Ethical Consideration

This study received ethical approval from Seth GS Medical College and KEM Hospital Institutional Ethics Committee-II (ethics committee registration number ECR/417/Inst./MH/2013). The approval number was EC/176/2016. Furthermore, this study followed the principles of the Declaration of Helsinki.

Results

Demographic Variables

About 73.3% of the patients fell in the age group between 36 and 45 years. About 60% (*n* = 18) of the patients were males, and 40% (*n* = 12) were females (Table 1).

Primary Outcomes of Death or Discharge

A total of 20 patients (66.7%) were discharged from the hospital, while the remaining 10 patients succumbed to death in the ICU (33.3%). The rate of mortality was higher in the study group (40%) compared to the control group (26.7%). The death or discharge outcome was independent of the use of pulse MPS therapy ($p = 0.44$). The findings are summarized in Table 2.

Assessment of Clinical Variables

No significant association between the study and control groups was found regarding the PaO_2/FiO_2 ratio at 0 hours, 48 hours, and at the time of outcome (death or discharge). Furthermore, no significant association was found between the two groups at the time of the outcome in regards to the LIS, the SOFA score, and the duration of stay in MICU (Table 3).

The mean duration for which mechanical ventilation was required in the study group was 5.3 ± 2.4 days, while in the control group, it was 6.5 ± 4.7 days, but this finding was not significant ($p = 0.41$). There were 12 cases of secondary infection in the study, and most of the cases belonged to the MPS group (58.3%). The use of MPS did not decrease the rate of secondary infections ($p = 0.46$) (Table 4).

Discussion and Conclusion

In our study, 60.0% of the patients were males and 40.0% were females. Thus, the male-to-female ratio was 1.5:1. Furthermore, the maximum number of patients was between 36 and 45 years old. This was similar to the studies conducted by Magazine et al.,[11] Bhadade et al.,[12] and Vigg et al.[13] in India. This demonstrates that it might be a male-preponderant disease with more prevalence in middle-aged adults.

The studies conducted by Weigelt et al.[14] and Schein et al. conducted in the United

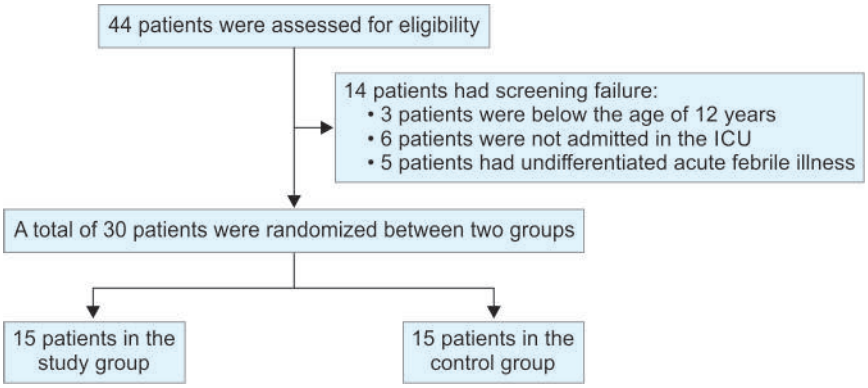

Fig. 1: Participant flowchart. All patients were screened and those who passed were randomized into the study group and control groups

Table 1: Distribution of demographic variables

Variable		*Study group (n = 15)*	*Control group (n = 15)*
Age (in years)	12–25	2 (13.3)	0 (0)
	26–35	1 (6.7)	0 (0)
	36–45	9 (60.0)	13 (86.7)
	46–55	2 (13.3)	2 (13.3)
	56–65	1 (6.7)	0 (0)
Gender	Male	10 (66.7)	8 (53.3)
	Female	5 (33.3)	7 (46.7)

Number in parenthesis signifies percentage unless otherwise specified

Table 2: Primary outcome of the patients in the study and control groups

Primary outcome	*Study group (n = 15)*	*Control group (n = 15)*
Discharge	9 (60)	11 (73.3)
All-cause mortality	6 (40)	4 (26.7)

Number in parenthesis signifies percentage unless otherwise specified

Table 3: Assessment of clinical variables

Outcome variables		*Patients with discharge as the outcome (n = 20)*			*Patients with death as the outcome (n = 10)*		
PaO_2/FiO_2 ratio	Follow-up time	Study group (*n* = 9)	Control group (*n* = 11)	*p*-value	Study group (*n* = 6)	Control group (*n* = 4)	*p*-value
	At 0 hours	214.11 ± 62.55	240 ± 43	0.29	159.58 ± 75.47	195.75 ± 71.47	0.47
	At 48 hours	317.22 ± 78.63	298.64 ± 64.78	0.57	186.17 ± 55.69	138 ± 52.69	0.21
	At the time of death or discharge	473.78 ± 13.84	462.09 ± 22.58	0.19	186.17 ± 55.69	92 ± 21.85	0.21
LIS score		0.53 ± 0.51	0.52 ± 0.51	0.98	2.79 ± 0.56	2.94 ± 0.43	0.67
SOFA score		0.67 ± 1	0.27 ± 0.65	0.30	7.83 ± 2.93	8.5 ± 2.08	0.71
Duration in MICU (in days)		8.11 ± 1.96	9.64 ± 6.23	0.49	4.33 ± 3.01	4.5 ± 3.7	0.94

The value following ± indicates the standard deviation

Table 4: Duration for the requirement of mechanical ventilation and rate of secondary infections

Variables		*Study group (n = 15)*	*Control group (n = 15)*
Requirement of mechanical ventilation (in days)	1	1 (6.7)	1 (6.7)
	2	0 (0)	1 (6.7)
	3	4 (26.7)	2 (13.3)
	4	1 (6.7)	2 (13.3)
	5	2 (13.3)	00
	6	1 (6.7)	5 (33.3)
	7	2 (13.3)	0 (0)
	8	3 (20)	0 (0)
	9	1 (6.7)	1 (6.7)
	10	0 (0)	1 (6.7)
	11	0 (0)	1 (6.7)
	12	0 (0)	1 (6.7)
Prevalence of secondary infections	Yes	7 (46.7)	5 (33.3)
	No	8 (53.3)	10 (66.7)

Number in parenthesis signifies percentage unless otherwise specified

States[15] demonstrated that MPS therapy failed to improve PaO_2/FiO_2 ratio and pulmonary function in patients with ARDS. Also, in a study conducted by Bernard et al.,[16] no difference was observed in the LIS upon using MPS in patients with malaria-associated ARDS, consistent with our findings. However, in a study conducted by Meduri et al.,[17] it was reported that MPS-induced downregulation of systemic inflammation was associated with significant improvement in pulmonary function and reduction in the duration of stay in MICU and duration for which mechanical ventilation was provided. On the contrary, a study by Takaki et al.[18] showed that these variables were prolonged with the use of MPS. We found no such associations.

In the study conducted by Weigelt et al.,[14] MPS therapy was associated with an increased risk of secondary infections. In the study conducted by Bernard et al.,[16] infectious complications were similar in both groups. In our study, the type of treatment did not have a statistically significant impact on the risk of secondary infections. The studies conducted by Bone et al. in Michigan, the United States of America[19] and Bernard et al.[16] did not show any reduction in mortality with the use of MPS, which was also demonstrated in our study. A study by Weigelt et al.[14] demonstrated an increased rate of mortality among patients taking MPS.

An increase in the respiratory rate in the absence of metabolic acidosis and anemia is usually the first clinical manifestation pointing toward the presence of pulmonary edema. Bilateral basal crepitation, raised jugular venous pressure and chest radiograph demonstrating hilar congestion, bilateral diffuse infiltrations, and reduced arterial pO_2 are other obvious manifestations of ARDS.[20] The development of a robust surveillance system is required for adequate monitoring and early diagnosis of this complication. ARDS can even develop after successful treatment and parasite clearance. In these cases, ARDS may reflect the presence of inflammatory cytokines in the absence of infected erythrocytes. Emerging evidence suggests that even after treatment, free parasite antigens may persist, which could be stimuli for inflammation.[21] Currently, the only effective therapeutic measure to decrease mortality in severe ARDS is low tidal volume and prone mechanical ventilation.[21]

There were a few limitations in our study. Firstly, along with a smaller sample size, the sampling was done from a single center, which may limit the generalizability of the study. Secondly, it is nearly impossible to standardize all the parameters in critically ill patients. Lastly, the respiratory mechanics and ventilator strategies used to treat different patients may result in significant heterogeneity in the all-cause mortality of patients.

In conclusion, pulse MPS therapy has not shown any concrete benefit in the management of malaria-associated ARDS, and in fact, the continuous use of this treatment in hospitals may lead to worsening outcomes. A novel, effective therapy for this grave complication needs to be developed to reduce the morbidity and mortality in such patients, which is frequently encountered.[22]

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ORIGINAL ARTICLE

Analysis of Hematological Indices in Patients of Systemic Lupus Erythematous and Its Correlation with SLEDAI-2K

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Abstract

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease which is characterized by chronic multisystem inflammation and end-organ damage. In recent times there has been a need for new hematological markers to assess disease activity in SLE patients specifically, which can be easily available like eosinophil, basophil, neutrophil, monocytes, and platelet to lymphocyte ratios (ELR, BLR, NLR, MLR, and PLR, respectively).

Materials and methods: The present investigation determines the use of a different peripheral hematological marker to assess SLE activity in 106 patients attended for medical care at Sawai Mansingh (SMS) Medical College and attached hospital, Jaipur. SLE disease activity index 2000 (SLEDAI-2K) was used to assess the disease activity in all patients. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were assessed in each subject. The ratio of various hematological indices, like NLR, BLR, ELR, MLR, PLR, etc., analyzed and correlated with CRP, ESR, and SLEDAI-2K.

Results: The present study revealed that the SLEDAI-2K score showed a significant positive correlation with ELR and MLR ($p < 0.005$). CRP showed a significant positive correlation with PLR ($p < 0.005$). ESR showed a significant positive correlation with ELR, MLR, PLR, and NLR ($p < 0.005$).

Conclusion: The final results demonstrate that in SLE patients, the ratio of hematological indices like ELR, MLR, and PLR can be employed as disease activity markers.

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Introduction

Systemic lupus erythematosus (SLE), often known as SLE, is a chronic autoimmune illness that affects the body's connective tissue and is distinguished by a predisposition for flares.[1] The condition can vary in its severity and its duration. According to the many pieces of epidemiologic evidence available, the frequency of SLE ranges from 6.5 to 178.0 per 100,000 people worldwide. The prevalence of SLE in India is estimated to be 3.2 cases per 100,000 people. Patients with SLE in North and West India were more likely to have a malar rash, arthritis, renal, and hematological features than discoid lesions, serositis, and neurological signs.[2] Malar rash, arthritis, renal, and hematological manifestations were recorded in larger proportions. Neutrophils have been identified as key effector cells in the pathogenesis of SLE. In more recent studies, it was shown that neutrophils, specifically the formation of neutrophil extracellular traps, have a role in the development of SLE. This is a process in which nuclear and cytosolic debris is extruded from neutrophils that are in the process of dying.[3] It is absolutely necessary for persons who have SLE and also have renal impairment to have frequent follow-up appointments. For the purpose of determining the severity of SLE disease activity, composite measures such as the SLE Disease Activity Index 2000 (SLEDAI-2K) are utilized. However, relying on these indices on a consistent basis is not something that is especially feasible. The search for uncomplicated laboratory indicators that can be acquired at almost any healthcare facility in order to evaluate disease activity and renal affection in SLE patients is an important challenge that needs to be addressed.[3] This is a problem that has to be addressed since it is an essential issue. Indirect evidence of subclinical inflammation has been shown to be present in platelet-to-lymphocyte ratios (PLR), neutrophil-to-lymphocyte ratios (NLR), basophil-to-lymphocyte ratios (BLR), and eosinophil-to-lymphocyte ratios (ELR).[4] In recent years, there has been a growing interest in the function that complete blood count (CBC) parameters play in evaluating disease activity in a variety of autoimmune disorders. This interest stems from the fact that CBC parameters may be found in blood tests. Several different things have contributed to the rise in interest. Various components of the CBC, such as NLR, monocyte-to-lymphocyte ratio (MLR), and PLR, have been used as efficient markers of inflammation in inflammatory and autoimmune disorders in recent reports.[5] The PLR has recently come to be recognized as an informative measure that can identify changes in platelet and lymphocyte counts that are caused by acute inflammatory and prothrombotic states.[6] Their levels shift depending on whether the overall inflammatory response in the body is becoming worse or getting better. A comprehensive blood count, which can be carried out at a wide range of medical facilities, may be used to estimate the total number of cells that are circulating in the body.[7] On the other hand, there is a paucity of information addressing the use of NLR, MLR, and PLR in SLE patients. Within the context of this investigation, we investigated the possibility of a connection between NLR, MLR, ELR, BLR, and PLR levels and disease activity in SLE patients.

Materials and Methods

A hospital-based retrospective study was conducted on 106 SLE patients at the internal medicine wards of Sawai Mansingh (SMS) Hospital, Jaipur. Patients diagnosed on the basis of Systemic Lupus International Collaborating Clinic (SLICC) and European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) criteria are included in this study. Participants were given a thorough explanation of the study, and after receiving their informed consent, the research was carried out. A routine blood investigation was done, and patients were evaluated for disease activity as per the SLEDAI-2K scale. The hematological markers (NLR, BLR, ELR, MLR, and PLR) were evaluated and subsequently correlated with disease activity parameters C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and SLEDAI-2K score. Inclusion of patients with a diagnosis of illness according to SLICC classification criteria from 2012 or the criteria from the 2019 EULAR/ACR. Patients who had a current infection, overlap syndrome,

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pregnancy, known cases of chronic liver disease (CLD), end-stage renal illness, or active cancer were excluded from this study.

Statistical Analysis

The presentation of the qualitative data included percentages and proportions. The mean and standard deviation (SD) were used to show the quantitative data. Analyzing nonparametric or categorical data was accomplished with the use of the Chi-squared test. The student's *t*-test was applied to the examination of the data collected using ordinal scales. In order to examine the degree of connection between the variables, the Karl–Pearson correlation coefficient was computed. The level of significance was determined to be $p = 0.05$.

Results

In our study, 106 patients with SLE were recruited. There is a female preponderance, with 103 (97.17%) females and three (2.83%) males. The mean age of cases is 26.74 years (26.74 ± 6.74), and the mean SLEDAI-2K is 9.71 (9.71 ± 5.82). Most of the patients in the study are 16–35 years of age. In cases, mean NLR, BLR, ELR, MLR, and PLR are 5.35 (5.35 ± 3.52), 0.00 (0.00 ± 0.01), 0.15 (0.15 ± 0.15), 0.21 (0.21 ± 0.20), and 219.42 (219.42 ± 147.91), respectively. The mean ESR is 47.62 mm/hour. We found that in our patients SLEDAI-2K score is high because most of the patients that are included in the study are indoor patients with high disease activity and relapse (Table 1).

This study showed that ELR and MLR were found to be increased in patients with high SLEDAI-2K scores, and MLR, ELR, NLR, and PLR are increased in patients with high ESR. Here, our study revealed that ELR (p-value = 0.0002) and MLR (p-value = 0.0088) show a significant positive correlation with the SLEDAI-2K score. CRP also show a significant positive correlation with PLR (p-value = 0.0192). ESR also show a significant positive correlation with ELR (p-value = 0.01), MLR (p-value = 0.001), PLR (p-value = 0.035), and NLR (p-value = 0.02). The mean hemoglobin of cases is 9.88 gm/dL, the mean total leukocyte count is 7365.42/cumm, the mean mean corpuscular volume (MCV) is 87.9 fL, the mean platelet count is 2.24 lakh/cumm, and the mean ESR is 47.62 mm/hour (Table 2).

It was observed that NLR ($r = 0.219$; p-value 0.0241), ELR ($r = 0.247$; p-value 0.0106), MLR ($r = 0.425$; p-value 0.001), and PLR ($r = 0.204$; p-value 0.0359) with ESR and PLR ($r = 0.227$; p-value 0.0192) with CRP show a statically significant correlation in SLE cases. Here, we find that ELR ($r = 0.395$; p-value 0.0002) and MLR ($r = 0.253$; p-value 0.0088) show a statistically significant correlation with SLEDAI-2K in SLE cases.

Table 1: Hematological profile of the study population

Hematological indices	*Mean*	*SD*
Hemoglobin (gm/dL)	9.88	2.12
Total leukocyte count (cells per cumm)	7365.42	2837.74
MCV (fL)	87.91	10.08
PLT (Lakhs/cumm)	2.24	1.04
ESR (mm/hour)	47.62	24.92
NLR	5.35	3.52
ELR	0.15	0.15
MLR	0.21	0.20
BLR	0.00	0.01
PLR	219.42	147.91

Discussion

Here, in our study, NLR shows statistically significant correlation with ESR ($r = 0.219$; p-value = 0.0241), ELR shows statistically significant correlation with ESR ($r = 0.247$; p-value = 0.0106), and SLEDAI-2K ($r = 0.395$; p-value = 0.0002), MLR shows statistically significant correlation with ESR ($r = 0.425$; p-value = 0.001) and SLEDAI-2K ($r = 0.253$; p-value = 0.0088) and PLR shows statistically significant correlation with ESR ($r = 0.204$; p-value = 0.0359), and with CRP ($r = 0.227$; p-value = 0.0192).

In their research, Abdulrahman et al. discovered that NLR and PLR levels, when correlated with other illness features in patients with lupus nephritis, exhibited a strong association with ESR.[8] Our research also demonstrates a link between ESR and NLR and PLR, in addition to demonstrating a correlation between ESR and ELR and PLR. According to the findings of research conducted by Wu et al., the levels of NLR and PLR were significantly greater in SLE patients than in the healthy control group. The SLEDAI-2K was strongly linked with both ratios.[9] However, the results of our research do not demonstrate such a link with SLEDAI-2K.

In their study of patients with SLE, Farouk et al. discovered a substantial positive connection between NLR, PLR, and ESR.[10] Under conditions of systemic inflammation, it is usual for circulating white blood cells to undergo relative alterations. These changes are often reflected by lymphopenia and neutrophilia, and they might explain elevated NLR in SLE patients, particularly when activity is present. In addition, lupus activity often results in a reduction in platelet counts; however, lymphocyte numbers drop more rapidly than platelet counts, which may help to explain why elevated PLR is associated with SLE activity.[11] According to the results of our research, ELR and MLR are both high in patients who have a high SLEDAI-2K score; however, NLR and PLR do not exhibit any link with the SLEDAI-2K score. The CRP has a statistically significant connection with both the PLR. A substantial link may be shown between ESR and MLR, ELR, PLR, and NLR. There is a correlation between having a high SLEDAI-2K and ESR and having a high ELR and MLR. PLR has been found to have a substantial connection with both CRP and ESR. The research conducted by Suszek et al.[5] demonstrates that there is a substantial link between SLEDAI-2K, ELR, and MLR. On the other hand, there is no relationship between NLR and PLR and the disease activity indicators. The NLR/MLR/PLR markers and the ESR/CRP readings all showed a substantial positive connection with one another.

According to Qin et al.,[12] both NLR and PLR had a positive correlation with SLEDAI-2K ($r = 0.471$, $p = 0.01$) and ($r = 0.44$, $p = 0.01$), respectively. The PLR was shown to have a somewhat favorable association with the MEX-SLEDAI score, with a value of $r = +0.366$ and a p-value that was <0.001. This was discovered by Fikri et al.[13] According to the results of our research, these hematological ratios are easily measurable and have the potential to serve as a useful marker for determining the progression of a disease and its subsequent treatment.

Conclusion

Other inflammatory indicators are more complicated, but hematological ratios may be easily determined from routine CBC tests performed in health clinics. Additionally, they are less expensive. In addition, these ratios are rather stable despite the fact that

Table 2: Pearson correlation between ESR, CRP, and different hematological ratios

Ratio of blood cells	*ESR*	*CRP*	*SLEDAI-2K*
	r (p-value)	*r (p-value)*	*r (p-value)*
NLR	+0.219 (0.0241)	+0.141 (0.1493)	+0.170 (0.0814)
ELR	+0.247 (0.0106)	+0.119 (0.2243)	+0.395 (0.0002)
MLR	+0.425 (0.001)	+0.112 (0.253)	+0.253 (0.0088)
PLR	+0.204 (0.0359)	+0.227 (0.0192)	+0.133 (0.1741)

each leukocyte count may be altered by dehydration, rehydration, and dilution of blood samples; hence, these hematological indicators may be utilized to evaluate the disease activity of SLE patients.

Ethical Approval

This study was approved by the ethical and research committee of SMS Medical College and Hospital, Jaipur, India.

Author Contributions

Prakash, Meena, and Chejara formulated the research questions, designed the study, developed the preliminary search strategy, and drafted the manuscript. Prakash, Singh, Nawal, and Rankawat collected and analyzed data for the study and wrote the manuscript. Nawal and Chejara conducted the quality assessment. All authors critically reviewed the manuscript for relevant intellectual content. All authors have read and approved the final version of the manuscript.

Availability of Data and Materials

Available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

The Indian REgistry on Current Patient PrOfiles and TReatment TrenDs in Hypertension (RECORD): Final Outcomes of the Real-world Observational Study

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Abstract

Objectives: The Indian Registry on Current Patient Profiles and Treatment Trends in Hypertension (Record) evaluated the current trends and outcomes related to hypertension (HTN) management at 3, 6, 12, and 24 months in India. This study highlights and evaluates the outcomes and trends noted at 24 months.

Materials and methods: The detailed study methodology is provided in the earlier publication (interim analysis at 12 months). Aspects such as changes in the quality of life (QOL), percentage of patients reaching target blood pressure (BP), treatment pattern among patients with comorbid conditions, and difference in treatment patterns between public and private healthcare settings, at 24 months, were evaluated in the current study.

Results: The study population included 2,000 patients (55.7% males) with a mean age of 54.45 years. Telmisartan (43.7%) and amlodipine + telmisartan (16.4%) were the most prescribed monotherapy and combination therapy among patients with newly diagnosed HTN. A significant decrease in both systolic BP (SBP) and diastolic BP (DBP) was noted in the overall patient population at 24 months ($p < 0.001$). The mean change in SBP and DBP was slightly higher at 24 months compared to 12 months. This was more evident among patients on combination therapy. A significant improvement in QOL was noted at 24 months.

Conclusion: Treatment strategies in HTN management are changing and are associated with effective HTN control and improvements in QOL. However, there is a further need for improved awareness regarding the optimal usage of combination therapy for better management of uncontrolled HTN.

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Introduction

Globally, about 1.28 billion adults have hypertension (HTN) with two-thirds of these living in low- and middle-income countries. Among these, HTN is controlled in only about 21% of adults.[1] HTN prevalence in India is quite high and is one of the most important risk factors for the increasing burden of chronic diseases in India. According to recent estimates, 13.8% of men and 8.8% of women in India are diagnosed with HTN.[2] As described in the publication on interim 1-year analysis, there is a lack of prospective cohort study that has evaluated the trends in HTN management among newly diagnosed and preexisting HTN patients in India.[3]

The Indian Registry On Current Patient Profiles And Treatment Trends in Hypertension (Record) was a long-term registry planned to observe the current trends in clinical practice for HTN management in India and the outcomes noted with these approaches at 3, 6, 12, and 24 months.

The interim analysis published previously has reported the outcomes noted at 3, 6, and 12 months. A total of 2,000 patients with preexisting or newly diagnosed HTN were enrolled in this study. The study outcomes revealed several facets related to the real-world scenario in HTN management across India.

The mean age of the participants was 54 years, and the average duration of preexisting HTN was 68 months. The most commonly used antihypertensive medications were telmisartan, amlodipine, and their combinations. The proportion of patients with controlled HTN (<140/90 mm Hg) increased by >30% in 1 year. Further, physical and emotional role functioning, social functioning, and health had all improved considerably at the end of 1 year.

The present study highlights the outcomes noted at 24 months and attempts to provide a comparison with the earlier outcomes and evaluate the trends noted.

Materials and Methods

The study methodology, HTN definition, patient inclusion and exclusion criteria, and other relevant information are explained in detail in the earlier publication. Patients were enrolled in the study after approval from the study site's Institutional Review Board or Ethics Committee. Consent was obtained from all patients before enrolment into the study.

The primary and secondary endpoints are as follows:

- Primary endpoints: The pattern of pharmacotherapy prescribed by physicians in India to treat patients with newly diagnosed essential HTN or patients with existing essential HTN and on the same treatment for the past 3 months.
- Changes in the QOL before and after these antihypertensive treatment regimens.
- Secondary endpoints: Percentage of patients reaching target blood pressure (BP) according to the American College of Cardiology/American Heart Association 2017 or European Society of Cardiology/European Society of Hypertension 2018 criteria, as per the prevailing clinical practices, at follow-up with various antihypertensive treatment regimens.

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- Percentage of patients with a change in the antihypertensive treatment regimen (up-titration/down-titration of dose or addition/removal of antihypertensive drugs) during follow-up, and reasons for the same at 24 months.
- Treatment patterns in hypertensive patients with comorbid conditions.
- The difference in treatment patterns between public and private healthcare settings.

Statistical Methods

No formal sample size calculations were performed for this study as it was an observational, single-arm study. Categorical variables were presented as numbers and percentages, and continuous data as mean and standard deviation (SD). Categorical variables were tested for differences between groups using the Fisher exact test, while continuous variables were tested using the Mann–Whitney *U* test. A *p*-value of <0.05 was considered to be statistically significant for all analyzes.

Results

Baseline Characteristics

Patient demographics and baseline characteristics (including treatment patterns and prescribed medications) have been elaborated in detail in the earlier publication (interim analysis at 1 year) and are available as supplementary material.[3]

A total of 2,000 patients were enrolled (55.7% males). The mean age (±SD) of the participants was 54.45 (±11.93) years. The study population comprised nearly similar proportions of preexisting (52.15%) and newly diagnosed (47.85%) hypertensive patients. At baseline, the mean systolic BP (SBP) and diastolic BP (DBP) (±SD) among patients with newly diagnosed HTN were 150.70 (±14.52) mm Hg and 86.72 (±10.73) mm Hg, respectively, while it was 146.79 (±21.32) mm Hg and 85.70 (±11.68) mm Hg, respectively, among patients with preexisting HTN. The most common comorbidity observed was diabetes mellitus (DM) (*n* = 632, 31.6%) followed by dyslipidemia (*n* = 316, 15.80%) in the overall population.

At baseline (diagnosis), >50% of the population was prescribed combination therapy among patients with newly diagnosed HTN, while usage of monotherapy was higher in patients with preexisting HTN. An increasing trend was noted with the usage of combination therapy at initial diagnosis (Fig. 1). Telmisartan (43.7%) was the most frequently prescribed medication among patients with newly diagnosed HTN, followed by amlodipine (25.8%) for monotherapy, and amlodipine + telmisartan (16.4%) for combination therapy (Fig. 2).

Treatment Patterns in Patients with Stage II HTN

Among the 957 newly diagnosed patients with stage II HTN (≥140 or ≥90 mm Hg), 511 patients were initiated on combination therapy. Amlodipine + telmisartan was the most commonly prescribed combination therapy in the newly diagnosed (42.9%) patients with stage II HTN (Table 1).

Changes in BP in the Overall Population

In the overall population of patients with HTN (*N* = 1,986), there was a significant decrease in both SBP and DBP from baseline to 24 months ($p < 0.001$).

Changes in BP Based on the Type of Diagnosis and Therapy

In the subgroup analysis based on diagnosis, a significant decrease in SBP and DBP was observed at 24 months in the preexisting and newly diagnosed group of patients. The mean change in SBP and DBP in the preexisting and

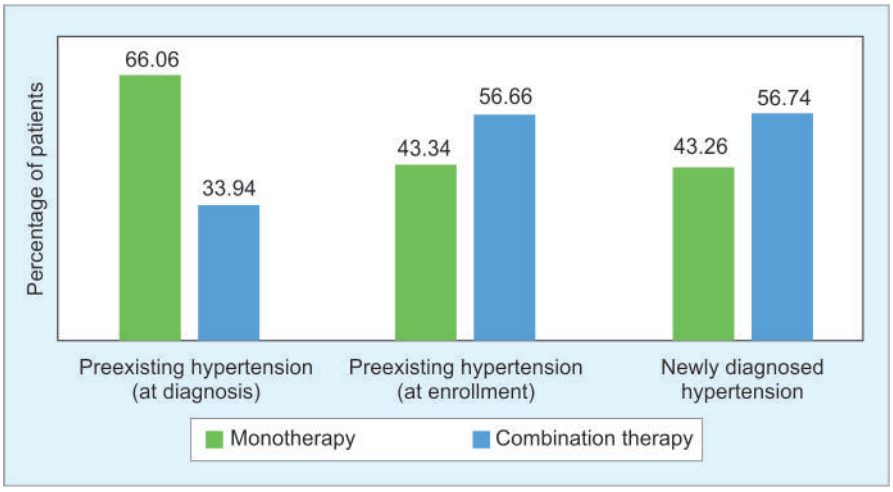

Fig. 1: Distribution of monotherapy and combination therapy in patients with preexisting and newly diagnosed hypertension

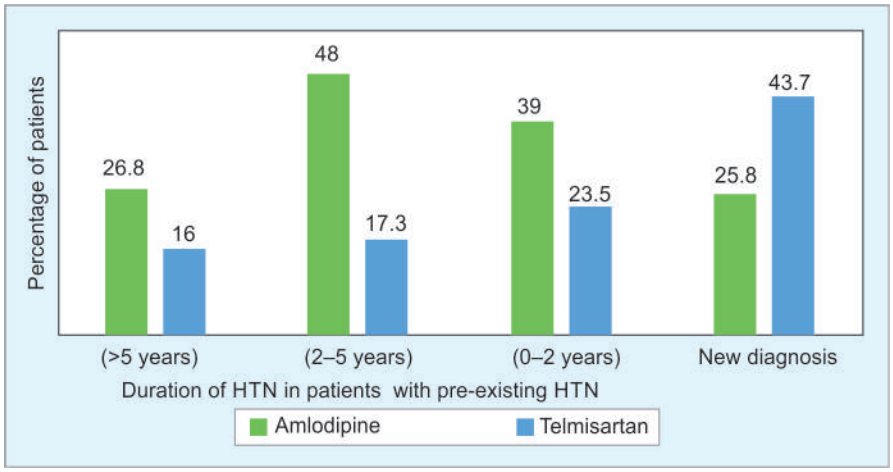

Fig. 2: Distribution of commonly prescribed monotherapies at diagnosis

Table 1: Top 10 most frequently used combination therapy at baseline in patients with newly diagnosed stage II hypertension (*n* = 511)

Combination therapy	*Count*	*Percentage*
Amlodipine + telmisartan	219	42.9
Metoprolol + telmisartan	42	8.21
Amlodipine + hydrochlorothiazide	38	7.43
Hydrochlorothiazide + telmisartan	35	6.85
Amlodipine + atenolol	18	3.52
Cilnidipine + telmisartan	15	2.93
Telmisartan + torsemide	12	2.34
Amlodipine + telmisartan + torsemide	11	2.15
Chlorthalidone + telmisartan	11	2.15
Amlodipine + metoprolol	10	1.95

newly diagnosed groups was slightly higher at 24 months compared to 12 months (Table 2).

When the BP changes were analyzed in subgroups based on the type of therapy, it was observed that in comparison to monotherapy, combination therapy showed significant reductions in mean SBP, and mean DBP at all time points studied (Table 3). This decrease was slightly higher at 24 months compared to 12 months in both monotherapy and combination therapy groups.

Comparison of Most Commonly Observed Treatment Strategies

Changes in BP with Telmisartan or Amlodipine Monotherapy

In newly diagnosed hypertensive patients (N = 957), a significant decrease from baseline in mean SBP and DBP was observed for both the medications at time points of 12 and 24 months ($p < 0.001$ for each medication at both time points; Table 4). As compared to telmisartan, the decrease in mean DBP from baseline was significantly higher with amlodipine at 12 months ($p = 0.010$) and 24 months ($p = 0.009$) of follow-up.

Changes in BP with Amlodipine + Telmisartan and Amlodipine + Atenolol Combination Therapy

In the newly diagnosed hypertensive patients, a significant reduction in mean SBP and DBP from baseline was noted for both amlodipine +

Table 2: Subgroup analysis for change in blood pressure based on the type of diagnosis

BP parameter	*Type of hypertension*	*Visit*	*Mean ± SD*	*Mean change (from baseline)*	*p-value*
SBP	Preexisting (*n* = 1031)	Baseline	134.14 ± 19.31	–	–
		12 months	123.94 ± 12.63	−10.54	<0.001
		24 months	122.9 ± 9.89	−11.24	<0.001
	Newly diagnosed (*n* = 955)	Baseline	150.63 ± 14.62	–	–
		12 months	133.44 ± 8.74	−17.19	<0.001
		24 months	127.9 ± 6.22	−22.72	<0.001
DBP	Preexisting (*n* = 1031)	Baseline	80.85 ± 10.79	–	–
		12 months	77.87 ± 23.25	−2.27	0.0045
		24 months	77.4 ± 6.74	−3.4	<0.001
	Newly diagnosed (*n* = 955)	Baseline	86.69 ± 10.77	–	–
		12 months	74.5 ± 6.87	−12.19	<0.001
		24 months	70.2 ± 8.63	−16.5	<0.001

Table 3: Subgroup analysis for change in blood pressure based on the type of therapy

BP parameter	*Type of hypertension*	*Visit*	*Mean ± SD*	*Mean change (from baseline)*	*p-value*
SBP	Monotherapy (*n* = 856)	Baseline	138.00 ± 17.61	–	–
		12 months	126.73 ± 11.52	−11.56	<0.001
		24 months	124.3 ± 8.53	−13.68	<0.001
	Combination therapy (*n* = 1130)	Baseline	145.15 ± 19.58	–	–
		12 months	129.76 ± 12.14	−15.39	<0.001
		24 months	126.1 ± 8.75	−19.1	<0.001
DBP	Monotherapy (*n* = 856)	Baseline	82.99 ± 10.19	–	–
		12 months	76.77 ± 6.36	−6.18	<0.001
		24 months	74.6 ± 8.25	−8.4	<0.001
	Combination therapy (*n* = 1130)	Baseline	84.17 ± 11.83	–	–
		12 months	76.49 ± 22.59	−7.68	<0.001
		24 months	73.4 ± 8.69	−10.7	<0.001

Table 4: Subgroup analysis for change in blood pressure based on telmisartan and amlodipine monotherapy

Parameter	*SBP*		*DBP*	
	Telmisartan	*Amlodipine*	*Telmisartan*	*Amlodipine*
N	180	106	180	106
Baseline (mean ± SD)	146.74 ± 13.11	146.83 ± 13.02	85.62 ± 9.66	87.17 ± 7.57
12-month BP	131.92 ± 7.10	132.30 ± 10.39	75.56 ± 6.61	73.64 ± 7.65
Change	−14.83	−14.53	−10.06	−3.53
p-value (intragroup)	<0.001	<0.001	<0.001	<0.001
p-value (intergroup)	0.864		0.01	
24-month BP	128.7 ± 4.42	125.2 ± 7.62	71.2 ± 7.59	69.1 ± 9.79
Change	−18	−21.6	−14.4	−8.1
p-value (intragroup)	<0.001	<0.001	<0.001	<0.001
p-value (intergroup)	0.0254		0.009	

telmisartan and amlodipine + atenolol at all time points ($p < 0.001$ for both combinations at most time points) (Table 5). The mean SBP and DBP reduction was significantly higher at 24 months compared to 12 months ($p < 0.001$) for both combinations.

In patients with preexisting HTN, a significant reduction in mean SBP from baseline was noted for both amlodipine + telmisartan and amlodipine + atenolol at 12- and 24-month follow-up ($p < 0.001$ for both combinations at 12 and 24 months). A significant reduction in mean DBP from baseline at 12 and 24 months was noted with amlodipine + telmisartan, whereas a similar outcome was noted only at 24 months with amlodipine + atenolol. The change in mean SBP and DBP values was higher at 24 months compared to 12 months in both groups.

Patterns of Treatment Modification

Treatment modifications were infrequent and were more common at 6 months (63 patients) and 2 years (73 patients) in the overall population (Table 6). In the newly diagnosed population, 43 patients required treatment modification at 2 years (Table 6). Among these, medications were removed in 23 patients, added in four, substituted in five, and the dose of medication decreased in 11 patients.

Control Rates of HTN

In patients newly diagnosed with HTN and taking monotherapy, 95.39% had controlled HTN (<140/90) at 24 months, while in patients receiving combination therapy, 97.79% had controlled HTN. In patients with preexisting HTN, 91.22 and 89.10% on monotherapy and combination therapy, respectively, had controlled HTN (Table 7A). The percentage of patients with controlled HTN (both newly diagnosed and preexisting) when the cutoff was considered at 130/90 was much lesser compared to the 140/90 cutoff. However, an increasing trend in the control rates was noted at each time point, reaching the highest at 24 months (Table 7A).

In the newly diagnosed patients, the percentage of patients with controlled HTN (<140/90) was higher with telmisartan compared to amlodipine at 1 year. However, at 2 years, the percentage of patients with controlled HTN was similar. A similar trend was noted when the cutoff of <130/80 was considered (Table 7B). The control rates were similar with amlodipine + telmisartan and amlodipine + atenolol combination therapies.

Subgroup Analysis for Hypertensive Patients with DM

The number of patients with coexisting DM was 632. In the overall population, a higher proportion of patients with diabetes were on combination therapy than monotherapy (55.4 vs 44.6%). In the preexisting hypertensive patients with diabetes, the majority of the patients were receiving amlodipine monotherapy (35.4%) and amlodipine + indapamide + perindopril (19.8%) combination therapy at the time of diagnosis. However, telmisartan (47.6%) and amlodipine + telmisartan (34.1%) were the most preferred monotherapy and dual therapy agents, respectively, in the newly diagnosed patients.

Subanalysis for change in BP in the overall population of hypertensive patients with DM based on the type of diagnosis revealed that in patients with preexisting HTN, the reduction in mean SBP and DBP from baseline was significant at 12 and 24 months. For patients newly diagnosed with HTN, the reductions in mean SBP and DBP from baseline were significant at all time points studied (3, 6, 12,

Table 5: Subgroup analysis for change in blood pressure based on amlodipine + telmisartan and amlodipine + atenolol combination therapy in newly diagnosed patients

Parameter	*SBP*		*DBP*	
	Amlodipine + telmisartan	*Amlodipine + atenolol*	*Amlodipine + telmisartan*	*Amlodipine + atenolol*
N	232	18	232	18
Baseline (mean ± SD)	154.06 ± 14.88	154.78 ± 11.23	87.61 ± 11.52	83.33 ± 9.96
12-month BP	135.03 ± 7.94	137.00 ± 7.36	74.14 ± 6.03	71.11 ± 7.2
Change	−19.03	−17.78	−13.47	−12.22
p-value (intragroup)	<0.001	<0.001	<0.001	<0.001
p-value (intergroup)	0.6457		0.5587	
24-month BP	128.5 ± 4.93	126.6 ± 5.50	70.0 ± 8.16	65.2 ± 6.76
Change	−25.6	−8.2	−17.6	−18.1
p-value (intragroup)	<0.001	<0.001	<0.001	<0.001
p-value (intergroup)	0.3528		0.8238	

Table 6: Treatment modification in newly diagnosed population

	Medications substituted	*Medications added*	*Medications removed*	*Dose increased*	*Dose decreased*	*Total*
Overall population						
3 months	37	8	2	1		48
6 months	31	4	25	2	1	63
1 year	3	3	2			8
2 years	17	14	30		12	73
Newly diagnosed population						
3 months	1	1	2	0	0	4
6 months	5	0	17	0	1	23
1 year	2	0	1	0	0	3
2 years	5	4	23	0	11	43

Table 7A: Control rates of hypertension (<140/90 and <130/80) with monotherapy and combination therapy in the newly diagnosed and preexisting hypertensive patients

Category	*Overall patient population (n = 1986)*							
	Newly diagnosed HTN (n = 955)				*Preexisting HTN (n = 1031)*			
	Monotherapy (n = 412)		*Combination therapy (n = 543)*		*Monotherapy (n = 444)*		*Combination therapy (n = 587)*	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*
<140/90								
1 year	314	76.2	349	64.3	403	90.8	478	81.43
2 years	393	95.4	531	97.8	405	91.2	523	89.1
<130/80								
1 year	67	16.3	62	11.4	78	17.6	103	17.55
2 years	195	47.3	246	45.3	107	24.1	146	24.87

Table 7B: Control rates of hypertension (<140/90 and <130/80) with telmisartan or amlodipine monotherapy and amlodipine + telmisartan or amlodipine + atenolol combination therapy in the newly diagnosed hypertensive patients

Category	*Telmisartan*		*Amlodipine*		*Amlodipine + telmisartan*		*Amlodipine + atenolol*	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*
<140/90								
1 year	148	82.22	69	65.09	154	66.38	9	50.00
2 years	175	97.22	101	95.28	227	97.84	18	100.00
<130/80								
1 year	36	20.00	3	2.83	26	11.21	0	0
2 years	89	49.44	40	37.74	107	46.12	11	61.11

and 24 months; $p < 0.001$). Similar outcomes were noted with amlodipine + telmisartan combination therapy at all time points ($p < 0.001$).

Public vs Private Hospital

The proportion of patients with preexisting HTN was higher in private hospitals compared to public hospitals (90.0 vs 10.0%). However, public hospitals had a greater proportion of patients with newly diagnosed HTN compared to private hospitals (63.7 vs 36.3%). In the overall population, patients in both public and private hospitals received a larger proportion of combination therapy than monotherapy (56.1 vs 43.9%; 58.1 vs 41.9%, respectively). The most commonly prescribed medications for monotherapy were amlodipine (41.4 and 19.8%) followed by telmisartan (36.8 and 18.6%) in both private and public hospitals, respectively. The most commonly prescribed combination therapy was amlodipine + telmisartan (38.2%) in public and amlodipine + indapamide + perindopril (30.6%) in private hospitals. For patients newly diagnosed with HTN, the most commonly prescribed medication was amlodipine + indapamide + perindopril (13.6%) in private hospitals and amlodipine + telmisartan (25.7%) in public hospitals. For the patient population with preexisting HTN, the most commonly prescribed therapy was amlodipine + indapamide + perindopril (18.3%) combination in private hospitals and amlodipine (30.3%) monotherapy in public hospitals.

Adverse Events

Adverse events (AEs) were reported by 144 patients (7.2%). The total number of events reported was 156 among which 140 events (89.74% of total events) were AEs and 16 events (9.6% of total events) were serious AEs (SAEs). The major events were fever (16.0%), headache (11.5%), cough (6.4%), and weakness (6.4%).

In the groups based on diagnosis, 71 events (45.5%) were observed in patients with preexisting HTN, and 85 events (54.5%) in newly diagnosed patients. Patients who received monotherapy had 65 events (41.7%), whereas those who received combination therapy had 91 events (58.3%).

The majority of the total events were of mild intensity (74.4%), followed by moderate (16.0%), and severe (9.6%). Death accounted for 7.1% of the SAEs, followed by inpatient hospitalization/prolongation of an existing hospitalization (3.2%). Among 156 total events reported, 154 events (98.7%) had no relationship with the drug and only two events (1.3%) had a possible relationship with the drug. The medication was discontinued only in six patients (3.8%) due to AEs. Death was reported in 11 (7.1%) patients, of which 73% (8/11) were on monotherapy with amlodipine and bisoprolol in most patients, and 82% (9/11) of patients had preexisting HTN.

Quality of Life (QOL)

Significant improvement was noted in all the parameters included in the 36-item short form survey (SF-36) health survey questionnaire in the overall population at 24-month follow-up from the baseline ($p < 0.0001$ for all parameters). A similar trend was observed in patients with newly diagnosed as well as preexisting HTN. In patients with newly diagnosed HTN and taking telmisartan, significant improvements were noted in all parameters from baseline to 24 months ($p < 0.0001$ for all parameters) (Table 8). However, in patients taking amlodipine, marked improvements were noted in all parameters except emotional role functioning, emotional well-being, and pain from baseline to 24 months. For patients receiving amlodipine + telmisartan combination therapy, significant improvements were noted in all parameters from baseline to 24 months ($p < 0.0001$ for most parameters), whereas for amlodipine + atenolol combination therapy, marked improvements were observed in physical and social functioning, general health, and health change. For patients receiving monotherapy and combination therapy, all parameters were markedly improved at 24-month follow-up from baseline ($p < 0.0001$ for all parameters).

Discussion

The benefits noted in the 12-month interim analysis were extended in the 24-month

Table 8: Mean changes in parameters included in the SF-36 health survey questionnaire in patients with newly diagnosed hypertension at 24 months

Scale	*Telmisartan*			*Amlodipine*			*Amlodipine + telmisartan*			*Amlodipine + atenolol*		
	24 months	*Mean change**	*p-value*	*24 months*	*Mean change**	*p-value*	*24 months*	*Mean change**	*p-value*	*24 months*	*Mean change**	*p-value*
Physical functioning	70.5	23.4	<0.0001	69.3	13.3	0.0006	70.8	24.1	<0.0001	69.4	24.4	0.0305
Role functioning/ physical	82.5	55.1	<0.0001	73.6	20.5	0.0001	72.6	41.8	<0.0001	63.9	13.9	0.1631
Role functioning/ emotional	68.5	44.6	<0.0001	58.5	6.6	0.2602	66.4	38.6	<0.0001	48.1	1.9	0.8972
Energy/fatigue	59.4	11.7	<0.0001	54	5.8	0.0008	56.3	7.7	<0.0001	50.3	4.7	0.1545
Emotional well-being	67.8	14.8	<0.0001	58.5	2.2	0.2767	63.1	8	0	55.8	4	0.42
Social functioning	72.2	19	<0.0001	71	10.7	0.0001	72.8	15.9	<0.0001	72.2	18.1	0.0024
Pain	76	15.4	<0.0001	58.9	−3.4	0.1674	68.4	8	0	45.6	−3.6	0.5007
General health	51.2	12.6	<0.0001	42.9	5	0.0039	46.9	9.6	<0.0001	34.4	10.3	0.0076
Health change	67.5	33.2	<0.0001	55.2	22.6	<0.0001	61.7	28.3	<0.0001	52.8	29.2	0

*from baseline

analysis with a significant decrease noted in both SBP and DBP from baseline to 24 months in the overall population. Notably, the reduction in mean SBP and DBP (with both monotherapy and combination therapy) was slightly higher at 24 months compared to that noted in the interim analysis at 12 months. The successful treatment of chronic disorders such as hypertension is primarily influenced by patient compliance and the use of an appropriate antihypertensive agent.[4,5] Factors commonly influencing patient compliance include knowledge/education level, support from healthcare professionals, family members and friends, self-motivation and confidence, gender, age, and duration of HTN. Higher education, better support, self-confidence, female gender, younger age, and lower duration of HTN were all associated with better compliance.[6] Better compliance in the current study may be attributed to the type of population which mainly comprised of urban population. Similar results were noted in another study from Korea, which reported better adherence among patients being managed in metropolitan cities, aged ≥65 years, taking medication for comorbidities such as diabetes, and being managed with ≥2 classes of medications.[7]

The World Health Organization recommends the usage of any of the following three classes of medications for the initial management of HTN—(1) angiotensin-converting enzyme inhibitors/ angiotensin receptor blockers, (2) thiazide and thiazide-like agents, and (3) long-acting dihydropyridine calcium channel blockers. Combination therapy, preferably in the form of a single pill, involving the same class of drugs is also recommended, if considered necessary.[8] According to a systematic review that evaluated the prescription patterns for chronic diseases in India, most of the practitioners followed international guidelines for the management of HTN. Combination drugs are used considerably in India with a positive impact on BP control.[9]

Calcium channel and angiotensin receptor blockers are the commonly prescribed antihypertensive medications in India, either alone or in combination. According to available data, monotherapy was prescribed in 50.76% of patients followed by dual drug therapy (42.13%) and triple drug therapy (6.09%).[10] In the present study, monotherapy was used in 43.3% of patients, while combination therapy was preferred in 56.7% of patients; among patients on combination therapy, the fixed-dose combination was preferred in 70.55% of patients. Telmisartan, amlodipine, and their combinations were the most preferred antihypertensive medications in the present study. Compared to 1-year results, the percentage of patients with controlled HTN (<140/90 mm Hg) was considerably higher at 2 years for patients with newly diagnosed HTN (both monotherapy and combination therapy) in the present study. In the newly diagnosed patients, the mean SBP and DBP reduction was significantly higher at 24 months compared to 12 months for both amlodipine + telmisartan and amlodipine + atenolol combinations. This may be attributed to the improvement noted with consistent treatment.

The fixed-dose combination has been recommended across several guidelines to achieve optimal BP control and reduce the risk of cardiovascular complications. The usage of telmisartan/amlodipine fixed-dose combination was associated with a significant decrease in 24-hour BP and was independent of administration time among patients with uncontrolled HTN following administration of amlodipine alone.[11] Significantly greater reductions in BP were also noted with telmisartan/amlodipine fixed-dose combination at 8 weeks when compared to telmisartan or amlodipine alone.[12] Low-dose combination of amlodipine 5 mg and telmisartan 40 mg was associated with a statistically significant reduction in SBP as compared to either drug as monotherapy. Low-dose combination therapy has been suggested as a better approach instead of high-dose monotherapy.[13]

In a similar observational but retrospective study from India that evaluated the clinical effectiveness and safety of telmisartan as monotherapy or combination among Indian adults with HTN, a significant reduction was noted in the mean SBP and DBP following treatment with telmisartan (both as monotherapy and as a combination). The efficacy and tolerability of telmisartan was rated as "good to excellent" by the patients.[14]

Treatment modification (including medication substitution, addition, removal, or dose modification) was required in 4.5% of the patients with newly diagnosed HTN. Some of the reasons for modifications included the need for better BP control, improvement in BP, and the nonavailability of certain combinations. Reduction in the dosage of antihypertensive medications can be carried out if the BP is maintained for 6–12 months.[4] Outcomes from the OPTIMISE trial indicated that medication reduction in older patients was not associated with significant changes in SBP control.[15] Notably, in the present study, the proportion of patients with newly diagnosed HTN who were prescribed monotherapy increased by 3.7% from baseline at 24 months, while the percentage of patients receiving combination therapy decreased by 3.7% at 24 months. A similar trend was noted in patients with

preexisting HTN. Better HTN control over 24 months may have resulted in a reduction in the number and dosage of antihypertensive medications, along with patient or healthcare practitioner preference (Table 6).

The subgroup analysis of patients with DM revealed trends similar to that noted in the overall population. The usage of combination therapy at initial diagnosis was found to be increasing among patients with DM. However, as the duration of the study increased, there was a shift of trend from amlodipine to telmisartan monotherapy at diagnosis. The most common combination therapy prescribed at diagnosis in newly diagnosed and preexisting hypertensive patients with diabetes was amlodipine + telmisartan, with the preference for this combination increasing over the years. Telmisartan has been suggested as the first choice in hypertensive patients with DM owing to its agonist action at peroxisome proliferator-activated receptor γ. Telmisartan therapy among hypertensive patients with DM was associated with increased high-molecular-weight adiponectin levels and improved insulin resistance, apart from a significant reduction in BP.[16] Telmisartan prescribed as monotherapy or combination therapy was associated with a significant reduction in BP among Indian patients with mild-to-moderate HTN with comorbidities such as diabetes or dyslipidemia.[17]

The occurrence of AEs is a common concern with the long-term administration of antihypertensive drugs. This is especially true among patients with comorbidities wherein the risks and benefits need to be carefully assessed before the administration of any medications. According to a recent systematic review and meta-analysis, antihypertensive therapy was associated with a decreased risk of stroke, all-cause mortality, and cardiovascular death; however, it was also associated with an increased risk of adverse effects such as acute kidney injury, hypotension, and hyperkalemia.[18] Nevertheless, the majority (98.7%) of the AEs noted in the overall population did not have a causal relation with the drugs being administered in the present study.

Adherence to pharmacologic treatment for the management of HTN has a positive impact on the physical and mental domains along with overall improvement in the QOL.[19] In the present study, significant improvements were noted in all parameters of the SF-36 survey at 24 months among patients with newly diagnosed HTN taking telmisartan, whereas among patients on amlodipine therapy, significant improvements were lacking in terms of emotional role functioning, emotional well-being, and pain from baseline to 24 months (Table 8). In terms of combination therapy, patients receiving amlodipine + telmisartan combination therapy reported significant improvements in all parameters from baseline to 24 months ($p < 0.0001$ for most parameters), whereas patients on amlodipine + atenolol combination therapy reported marked improvements only with physical and social functioning, general health, and health change.

Conclusion

This study shows effective HTN control rates with significant BP reductions and improvements in QOL at all following visits in both old as well as newly diagnosed patients. Over due course (from old to newly diagnosed patients) the usage of combination therapy at initial diagnosis was found to be increasing. The most preferred dual combination was telmisartan + amlodipine with a consistent increase in its usage. There was a shift from amlodipine to telmisartan in the preference for monotherapy prescribed at diagnosis. There is inadequate usage of dual combination therapy in stage II HTN at initial diagnosis. Overall, it is observed that the treatment strategies are changing and becoming more aligned with the guideline recommendations; however, still there is scope for improved awareness and usage of initial combination therapy to reduce the burden of uncontrolled HTN, to improve QOL, and thereby to prevent complications.

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ORIGINAL ARTICLE

Role of miRNA Let-7 in Plasma of Rheumatoid Arthritis

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Abstract

Objectives: Micro ribonucleic acids (miRNAs) are noncoding RNAs, recently implicated as potential biomarkers or therapeutic targets for autoimmune diseases such as rheumatoid arthritis (RA). The aim of this study is to assess the role of miRNA Let-7 in the plasma of RA.

Materials and methods: Trained medical staff already enrolled for the study collected blood samples from healthy controls ($N = 42$) and RA patients ($N = 44$). In the laboratory, these samples were centrifuged at 2000 rpm for 8 minutes to separate serum from the sample, which was then transferred to a plain vial. Until transport to the genetic lab, samples were stored at −20°C Deoxyribonucleic acid (DNA) was isolated using a standard protocol cohort of controls and patient blood samples. Quantification of DNA was conducted using ultraviolet (UV) spectroscopy, and DNA quality was assessed on 0.8% agarose gel. A comparison of genotype frequencies in the different study groups was performed using the Chi-squared test, while a comparison of allelic frequencies was conducted using Fisher's exact test.

Results: Variations of alleles, such as 16539423 (G>T), 16539433 (T>G), and 16539629 (A>T) were found only in RA patients. On the other hand, 16539617 (T>A), 16539622 (G>T), and 16539624 (T>C) were found only in control cases. Five sequences (three RA variants and two control variants) with minimal alignment were compared to the wild-type sequence. We found that the sequence modification of pre-miRNA 16539623 del G was significantly higher and had a risk allele in the study group [odds ratio (OR) = 3.29].

Conclusion: Rheumatoid arthritis (RA) is an autoimmune disorder that presents with a variety of clinical manifestations. Genetic factors possibly account for about 60% of disease susceptibility and expression, thus playing a very important role in disease pathogenesis.

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Introduction

Rheumatoid arthritis (RA) is a chronic, multisystem inflammatory disorder of autoimmune origin that involves multiple body parts. However, it primarily affects the articular system, resulting in inflammation and synovitis, often progressing to damage of articular cartilage and eventually stiffness and fixation of the joints.[1] The incidence of RA is three cases per 10,000 populations per year. The incidence of RA increases with age until the age of 80, while its onset is uncommon before the age of 15 years. The prevalence of RA is 1%. Females are three to five times more commonly involved compared to men[1] and four times more common among smokers compared to nonsmokers.

Insights gained from multiple clinical research over the past two decades have miraculously improved the paradigms for the diagnostic approach and management of RA. Serum antibodies to cyclic citrullinated peptides (anti-CCPs) are now identified as a significant serum biomarker for diagnostic and prognostic purposes. Advances in imaging techniques like ultrasound and MRI have facilitated early detection of joint inflammation and subsequent destruction in RA. The science behind the pathogenesis of RA has taken a great step forward with an understanding of new disease-related genes and their molecular pathways of disease pathogenesis. The relative importance of these different mechanisms is evidenced by the adequate benefits of the new class of highly targeted biologic therapies. However, partial descry of the initiating disease-related pathways of RA still remains a significant hurdle.

Rheumatoid arthritis (RA) is strongly associated with human leukocyte antigens (HLA)—DR4 (most specifically DR0401 and 0404), making family history one of the important risk factors.[2] HLA-DRB1 and protein tyrosine phosphatase non-receptor type 22 1858T gene variants are important risk factors for the progression of joint destruction in RA. Presently, many immunopathogenetic study models of other genes are undergoing discussion. The outcome of a few of the gene polymorphisms related to RA and pharmacogenetic concepts are being applied to different classes of available medical therapeutics, such as classical disease-modifying antirheumatic drugs and newer biological agents.[3]

The lethal-7 (Let-7) gene was first found among nematodes, where it acts as the main developmental regulator and now has become one of the first two known micro ribonucleic acids (miRNAs) (the other one is lin-4).[4] It plays an important role in posttranscriptional regulation of innate immune responses to pathogenic agents. Numerous reports have depicted a lower frequency of expression levels of Let-7, whereas chromosomal clusters of Let-7 are often found to be deleted in many cancers.[5] Let-7 is also a potential therapeutic agent to prevent tumour growth and angiogenesis, specifically the cancers with under-expression of Let-742. Intranasal use of Let-7 was found to be highly useful in controlling tumorigenesis in a transgenic mouse model of lung cancer.[6] Similar restoration of Let-7 has been observed to be beneficial in controlling tumor growth in other cancers (like breast, colon cancers and uterine leiomyoma).[7] The aim of this study is to define the role of miRNA Let-7 in RA patients.

Material and Methods

This is a case-control study that involved all patients visiting our hospital and volunteers for blood investigations. Blood samples of patients were drawn at Medicine and Rheumatology, Outpatient Department of Sardar Patel Medical College, Bikaner, Rajasthan, India after properly performed informed consent from the patients or from patient guardians.

Inclusion Criteria

All patients aged 16 years or older, who were diagnosed with RA using the latest RA classification criteria from 2010, were included as cases.

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Exclusion Criteria

All patients of RA who meet the following criteria are included:

- Those who are critically ill and have associated illnesses such as malignancy, renal failure, or liver failure.
- Those who have overlap syndrome (other connective tissue disorders like scleroderma, SLE, polio, etc.).

Diagnostic Criteria

The 2010 American College of Rheumatology and the European League Against Rheumatism classification criteria for RA.[8]

Blood Sampling Procedure

Blood samples of patients or volunteers were taken by already enrolled skilled medical staff, prior to discharge, under aseptic precautions and after immediately transferring to already labelled blood vials containing ethylenediaminetetraacetic acid as anticoagulant were carried in a cold chain from the sample collection site to the lab.

In the laboratory, these blood samples were centrifuged at 2000 rpm for 8 minutes to separate serum, which was then collected to a plain vial and kept at –20°C till shifted to the genomic lab.

Analytical Method

Clinical and Biochemistry Evaluations

Clinical data and lab investigations, including a complete blood count, random blood sugar, serum electrolytes such as sodium, calcium, potassium, blood urea, creatinine, AST/ALT ratio, serum ALP, and total proteins were conducted and were recorded from the patient's IPD sheets.

Isolation of Deoxyribonucleic Acid (DNA)

Deoxyribonucleic acid (DNA) from blood samples of both the case and control groups was isolated as per standard protocol. DNA quantification was done using ultraviolet (UV) spectroscopy, and the quality was assessed on a 0.8% agarose gel.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) reactions were carried out with genomic DNA, using forward and reverse primers (as mentioned below), 1X PCR buffer (sigma), 200 µM of deoxynucleotide triphosphates (dNTPs) and 1 unit of Taq polymerase (sigma). The annealing temperature was standardized for the above primer pair to obtain a 245 bp amplicon. Primers used for Let-7c PCR were: 5′-GGTTTGGACCAGGATCTGAA-3′ (forward) and 5′-TTGGGTTTCCAGCATAGGTC-3′ (reverse) as described by Kim et al.[9]

Polymerase chain reaction (PCR) settings used were as per below:

- 94°C for 5 minutes for initial denaturation.
- 94°C for 15 seconds.
- 53°C for 25 seconds.
- 72°C for 15 seconds; 39 cycles.
- 72°C for 10 minutes for final elongation.

Agarose Gel Electrophoresis

Polymerase chain reaction (PCR) products were evaluated by electrophoresis on 1.5% agarose gels to find out their quality and amplification. Agarose gel (1.5% w/v agarose) was made by dissolving agarose in the required quantity of 0.5X TBE electrophoresis buffer by heating in a microwave oven, followed by addition of 0.5 µg/mL of ethidium bromide. This agarose solution was then spread over the gel tray with a comb, allowed to cool and solidify and then placed in an electrophoresis tank and submerged in 0.5X TBE buffer.

The PCR reaction mix was mixed with DNA loading buffer (6X; 0.25% bromophenol blue, 0.25% xylene cyanol, 40% w/v sucrose). DNA samples were loaded into the wells as per size standard. Horizontal electrophoresis was performed at approximately 75V for 1.5 hours. DNA fragments are identified after staining them with ethidium bromide visualized on a UV transillumination, and the gel picture was captured.

Purification of PCR Products

Polymerase chain reaction (PCR) products were purified to remove primer dimers and excess dNTPs and nonspecific products prior to sequencing by using a GenElute Gel Extraction Kit (sigma).

Precipitation of PCR Products

The amplified products were precipitated using the polyethylene glycol (PEG) method.

- Transferred the PCR product into 1.5 mL microfuge tubes.
- Added 0.5 V of 22% PEG to each tube (final concentration is 11%).
- Added 0.1 M $MgCl_2$ in each tube (0.01 M final concentration).
- Mixed the sample thoroughly and kept it on ice for 20 minutes.
- Centrifuged the samples at 4°C, 14000 rpm for 15 minutes.
- Removed the supernatant carefully and again added 70% ethanol in each tube and centrifuged at 14000 rpm for 20 minutes twice.
- Decanted the ethanol carefully and air-dried the pellets.
- Pelleted PCR products were sequenced.

Statistical Analysis

Genotype distributions were determined by using the Chi-square-test for significant deviation from Hardy–Weinberg equilibrium, and a trend test was used to find out any rise in risk with the rise in the number of risk alleles. The risk associated with C17T and A118G polymorphism was calculated using odds ratios (ORs) and 95% confidence intervals, for genotypes in both control and case groups. In study groups, comparisons of genotype frequencies were done using the Chi-squared test, while comparisons of allelic frequencies were done using Fisher's exact test.

Result and Discussion

The PCR product obtained was purified and sent for DNA sequencing. The DNA sequences were subjected to multiple sequence alignment using NC_000021.9 as a reference sequence for the region under study. The Basic Local Alignment Search Tool was used to analyze the differences in the sequences. Various insertions, deletions, and variations were observed in the 245 bp amplicon (Table1).[10]

Several variations were found both in RA patients and controls, namely, 16539425 (A>C), 16539427 (A>G), and 16539594 (T>C) were found in both RA and control cases. 16539423 (G>T), 16539433 (T>G), and 16539629 (A>T) were found only in RA patients. 16539617

Table 1: Deletions present in RA and control cases

Sequence modifications	*Cases*	*Controls*	*Odds ratio*	*Remarks*
16539609 del T	7	12	0.17	Protective
16539611 del G	10	10	0.37	Protective
15639615 del G	1	3	0.15	Protective
16539617 del T	13	9	0.62	Protective
16539621 del T	5	4	0.58	Protective
16539622 del G	2	3	0.30	Protective
16539623 del G	6	1	3.29	Risk allele

(T>A), 16539622 (G>T), and 16539624 (T>C) were found only in control cases.

Longer primary transcripts are referred to as pre-miRNAs. These act as the initial substrates to generate the miRNA precursor forms by the nuclear Drosha RNase. Albeit sequences outside of the pre-miRNA hairpin are important for RNA processing or stability but actual composition of pre-miRNAs is still a mystery. Sequences were aligned using Clustal W and based on the minimal alignment, the variants were chosen for MFold analysis. Five sequences (three RA variants and two control variants) with minimal alignment were compared to the wild-type sequence. It is obvious from the folding of the messenger ribonucleic acid (mRNA) structures that the mutations (deletions and insertions) cause a variation in the secondary structure of the mRNA as a consequence of which the processing of pre-miRNA to miRNA could be affected leading to long-ranging effects on gene expression in the prechondrocytes and chondrocytes. Specific changes occurring due to these mutations in the upstream region of the Let-7 gene cluster need to be further characterized in a larger cohort and the resultant changes in expression/regulation of expression of genes involved in the etiology and/or progression of the immunological events underlying the development of RA.[11]

In multiple studies, aberrant expression of miRNAs has been documented in RA patients. The majority of them focused on T cell differentiation (Th17), which results in the formation of inflammatory cytokines, and B cell activation. These inflammatory markers and miRNAs that control their expression might be used as genetic biomarkers in RA.[12]

Murata et al. in a study concluded that the involvement of miR-16, miR-146a, miR-155, and miR-223 in the pathogenesis of RA.[13]

The expression of miR-155 and miR-146a is enhanced in synovial fibroblasts of RA patients after stimulation by pro-inflammatory mediators. This enhanced expression of miR-155 in RA patients' synovial fluid suppresses the production of MMP-3 and counters MMPs-1 and -3 induction by pro-inflammatory cytokines and toll-like receptor ligands.[14]

Eight miRNA (miR-126 - 3p, Let-7d-5p, miR-431-3p, miR-221-3p, miR-24-3p, miR-130a-3p, miR-339-5p, Let-7i-5p) were significantly elevated in RA serum compared to HC (all $p < 0.01$) and one miRNA (miR-17-5p) was significantly lower in RA ($p < 0.01$).

Conclusion

Rheumatoid arthritis (RA) is a systemic inflammatory disease with autoimmunity with multiple clinicophysical presentations. It affects approximately 70% of women. Genetic factors, accounting for about 60% of disease susceptibility, play a crucial role in pathogenesis. Several studies performed in past to evaluate the specific genes and chromosomal markers attributing to RA in European and American populations, but no such study has been conducted on Indian populations by any rheumatologist or molecular biologist so far. We found that sequence modification of pre-miRNA 16539623 del G was significantly higher and had a risk allele in the study group (OR = 3.29).

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REVIEW ARTICLE

Role of Bilastine in Allergic Rhinitis: A Narrative Review

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Abstract

Allergic rhinitis (AR) is considered a trivial disease and is often self-treated with over-the-counter drugs and home remedies. However, AR is a contributing risk factor for asthma associated with complications, including chronic cough, eosinophilic esophagitis, and otitis media with effusion. In AR, inflammation is primarily mediated by histamines. Guidelines advise using second-generation oral H1 antihistamines as the primary treatment for AR. Second-generation H1 antihistamines strongly prefer the H1 receptor, limiting their ability to enter the central nervous system. Thus, they have minimal adverse effects. Among these H1 antihistamines, bilastine is highly specific for H1 receptors with a slight affinity for other receptors. It has a rapid and prolonged action, which reduces the need for frequent dosing and has better compliance. In the long term, bilastine is well-tolerated with minimal adverse effects. It is not associated with drug interactions, so dosage adjustment is unnecessary. Bilastine does not penetrate the brain and is nonsedating at 80 mg once daily. The low possibility of drug–drug interactions and pharmacokinetics of bilastine makes it suitable for elderly patients, even with compromised hepatic and renal function, without dose adjustment. This review comprehensively discusses the guidelines and the role of bilastine in treating AR.

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Introduction

Allergic rhinitis (AR) occurs due to inflammation of the upper airways triggered by allergens; it is usually a chronic disease with episodes of acute exacerbation.[1] In the acute stage, it commonly presents as a blocked nose, sneezing, rhinorrhoea, and itching.[2] The overall prevalence of AR in India is 17.9–24.7%; however, the prevalence varies based on different geographical locations. It might be due to air pollution levels, heterogeneity in weather, diet, religious and cultural factors, socioeconomic levels, and literacy.[3] Historically, AR was considered an ailment of the nasal airway alone. Today, it is recognized as a systemic allergic reaction that may be connected to other illnesses, including asthma and atopic dermatitis.[4] Nevertheless, AR is often considered a trivial disease, and many people choose self-treatment with over-the-counter drugs and home remedies. Most patients are unaware of AR and the risks of associated complications.[1] Certain patients may exhibit a nonproductive cough accompanied by allergic conjunctivitis, persistent sinus inflammation, and eustachian tube dysfunction.[5] If nasal congestion or discharge symptoms last longer than 3 months, acute AR can progress to chronic rhinosinusitis. Chronic rhinosinusitis can cause nasal polyps due to chronic inflammation of the paranasal sinus mucosa.[4] Further, sensitization to allergens can result in adenoid hypertrophy.[6] Additionally, research indicates that AR can be a predisposing factor for asthma, mainly when diagnosed in infancy. Other complications linked to AR encompass chronic cough, eosinophilic esophagitis, and otitis media with effusion.[7]

Why is it Essential to Treat AR?

Allergic rhinitis (AR) results in impaired work productivity, absenteeism, and reduced performance at work.[8] According to a study, people with rhinitis (36%) were likelier to have worse self-rated work performance than those with asthma (19%).[9] Quality of life (QoL) is another important and perhaps undervalued aspect of AR. Almost 90% of patients with AR complain of nasal congestion and associated sleep problems. Furthermore, nasal congestion, rhinorrhea, and sneezing are most intense early in the morning, further exacerbating their sleep effects. Thus, people with AR are more likely to experience daytime fatigue due to disrupted sleep during the night. Sleep impairment can lead to depression, irritability, memory deficits, difficulty concentrating, and a decreased QoL.[10] Although clinicians perceive AR as a chronic but nonserious medical condition with limited symptoms, patients perceive it as limiting and disabling. This disconnect may lead to suboptimal treatment.[11] Hence, it is important not to trivialize AR and treat it according to guideline recommendations.

Guidelines for AR Management

The principal objective of AR treatment is the relief of symptoms. The first-line treatment involves avoiding relevant triggers, for example, molds, house dust mites, pollens, pets, tobacco smoke, etc. However, it might not always be possible. Leukotriene receptor antagonists, combined intranasal corticosteroid (CS)/antihistamine sprays, intranasal CSs, allergen immunotherapy, and oral antihistamines are pharmacological alternatives for symptomatic alleviation. Decongestants and oral CSs are other treatment options that may benefit some people.[12]

The Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines for treating AR are accepted globally (Fig. 1).[13]

Based on systematic research, the guidelines provide explicit, clear, and transparent clinical recommendations for treating AR. According to the ARIA guidelines, the selection of pharmacotherapy for patients with AR is aimed at effectively managing and controlling the disease. Factors affecting include (1) patient preferences, empowerment, and age; (2) major symptoms, disease severity,

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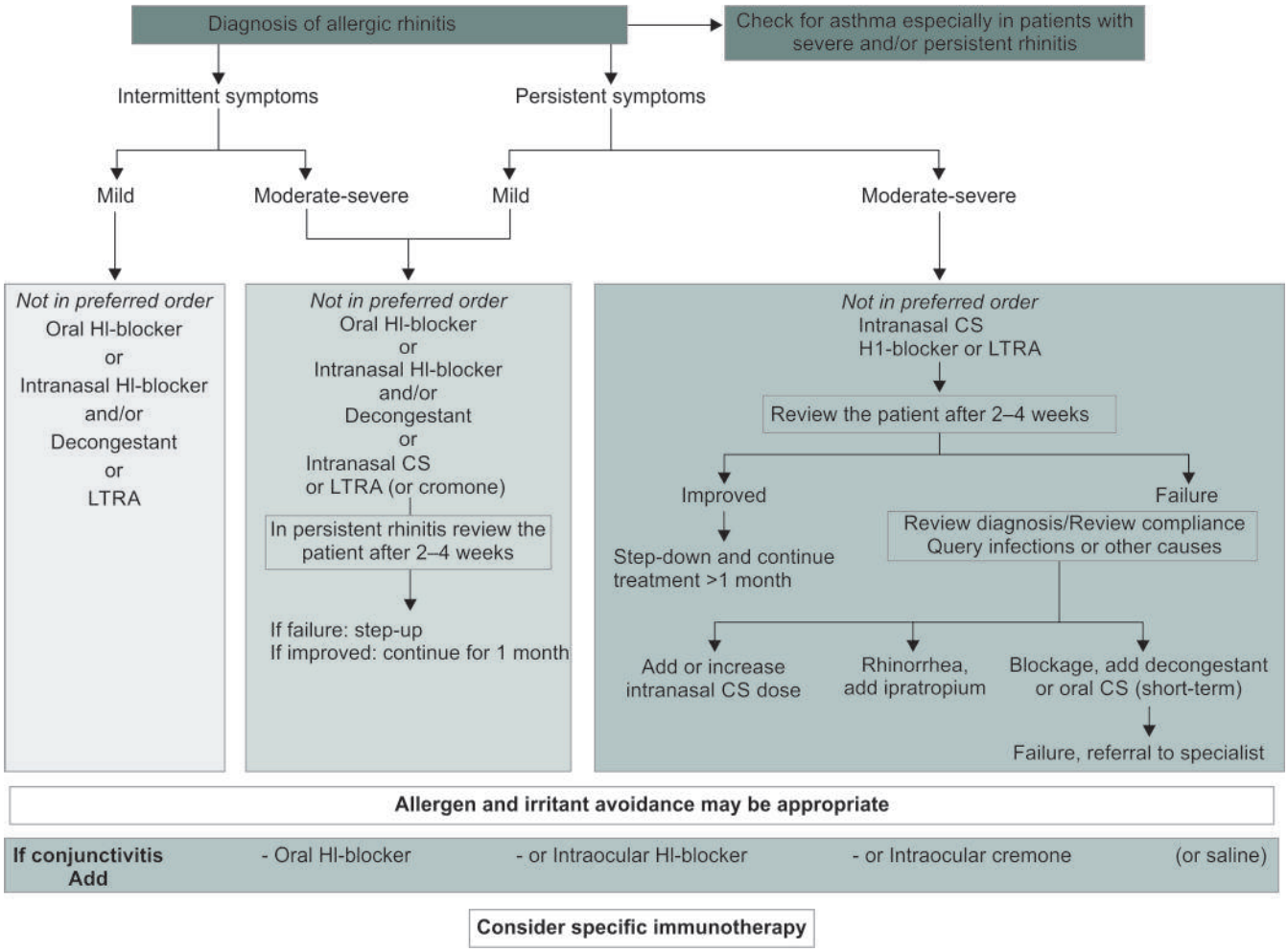

Fig. 1: Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines; CS, corticosteroids; LTRA, leukotriene receptor antagonist

and multimorbidity; (3) safety and efficacy of treatment; (4) speed of treatment onset action; (5) ongoing treatment; (6) past response to treatment; (7) effect on work productivity and sleep; (8) self-management strategies; (9) resource use. Among other guidelines, the Association of Otolaryngologists of India (AOI) released practice-oriented guidelines for integrated care of AR patients in India in 2022 (Fig. 2).[14]

Similarly, the Indian Academy of Pediatrics (IAP) has recommended a practical approach to managing AR in the pediatric population (Fig. 3). In AR, inflammation is primarily mediated by histamines; hence, antihistamine therapy plays an essential role in managing AR, and all guidelines recommend second-generation (new-generation) oral H1 antihistamines as the primary treatment.

Antihistamines

Oral H1 antihistamines inhibit histamine's proinflammatory effects by binding to the H1 receptor. Antihistamines are generally classified according to their generation as first- or second-generation agents. The first-generation H1 antihistamines (diphenhydramine, chlorpheniramine, and triprolidine) readily cross the blood–brain barrier. However, they may lead to side effects like drowsiness, sedation, fatigue, anti-muscarinic effects, impaired concentration and memory, and psychomotor performance.[15] Majority of first-generation H1 antihistamines inhibit cytochrome P450 (isoenzyme CYP2D6) and can interfere with other drugs metabolized via this pathway, such as β-blockers, tricyclic antidepressants, tramadol, and antiarrhythmic drugs.[16] In contrast, second-generation H1 antihistamines (desloratadine, loratadine, bilastine, fexofenadine, cetirizine, and levocetirizine) have limited central nervous system infiltration and are very selective for H1 receptor. Thus, they have minimal adverse effects. They also do not interact with cytochrome P450 enzymes. Hence, they are advised to treat AR.[17] Although second-generation H1 antihistamines have similar effectiveness, different medications have different pharmacokinetic characteristics and possibilities for food– and drug–drug interactions.[18] The choice of drug is often determined by a combination of factors, including dosing, onset time, drug interactions, and adverse effects. Moreover, bilastine has shown distinct advantages over other agents in these aspects.

Pharmacological Properties of Bilastine

Bilastine has a low affinity for other receptors and a high selectivity for H1-receptors.[19] Its activity has a quick onset and a longer time of action.[20] It has an extended residence period at the H1- receptor, leading to 60–70% antagonism visible 24 hours after dosage.[21] After oral administration, it is quickly absorbed, reaching maximum plasma concentrations within 1–1.5 hours and having a mean elimination half-life of around 12–14.5 hours.[22] According to a double-blind cross-over research comparing bilastine, cetirizine, and fexofenadine in AR, within an hour of taking the medication, bilastine decreased sneezing and eye symptoms (itchy eyes, watery eyes, and red eyes).[23] Thus, bilastine has a rapid action that lasts for a prolonged

period, reducing the need for frequent dosing and leading to better compliance.

Efficacy of Bilastine in AR

Numerous studies have compared the efficacy and safety of bilastine with a placebo and other second-generation antihistamines. Bilastine showed significant improvement in total symptom score (TSS) comprising four nasal symptoms (rhinorrhoea, itching, congestion, and sneezing) and six nonnasal symptoms (burning, tearing, ocular itching, redness, and itching of ears or palate, and a feeling of a foreign body in the eye) against placebo. It has also shown comparable efficacy to cetirizine, fexofenadine, and desloratadine in clinical trials on AR.[17,24–26]

Belastine demonstrated a superior adverse event profile compared to cetirizine. It was significantly associated with less fatigue (0.4 vs 4.8; $p = 0.02$) and somnolence (1.8 vs 7.5%; $p < 0.001$) than cetirizine.[26] It was also reported to have an extended action time than fexofenadine.[27] In another study, 20 mg bilastine had a quicker onset of action than 60 mg fexofenadine.[23] Bilastine successfully managed the ocular symptoms and nasal obstruction associated with allergic rhinoconjunctivitis, according to analyses from seven clinical trials.[28,29] Once daily, bilastine 20 mg effectively reduced TSS and improved QoL in 64 patients with perennial AR for up to 52 weeks. This effect was sustained for up to 1 year.[30] In a multicenter, placebo-controlled, randomized, parallel-group, double-blind research including 513 Caucasian patients, bilastine 20 mg once a day was well-tolerated and safe throughout a 1-year treatment period.[26]

The Safety Profile of Bilastine

In the long term, bilastine showed minimal side effects and is well-tolerated. The side effects included dizziness, headache, somnolence, and fatigue.[26] *In vitro*, bilastine did not induce or inhibit CYP3A isoenzyme activity, and its metabolism in humans is insignificant, thus having a minimal chance for metabolic drug–drug interactions resulting in no dosage adjustments.[13,31] Liver impairment does not affect bilastine since it is not metabolized in the liver; thus, dose adjustment is not needed in patients with liver impairment.[32] Bilastine, like placebo, has minimal H1-receptor occupancy (H1RO) in the central nervous system. Among first- and second-generation H1 antihistamines, bilastine shows the lowest H1RO; hence, it is classified as a nonbrain penetrating antihistamine.[33] Even at an 80 mg once-day dosage, bilastine is still nonsedating.[34,35] Driving ability was unaffected by 20 mg bilastine or 40 mg in a trial with healthy participants.[36] Even when given up to four times the recommended amount, bilastine has no discernible impact on the QT corrected for heart rate interval and shows no signs of cardiotoxicity.[19]

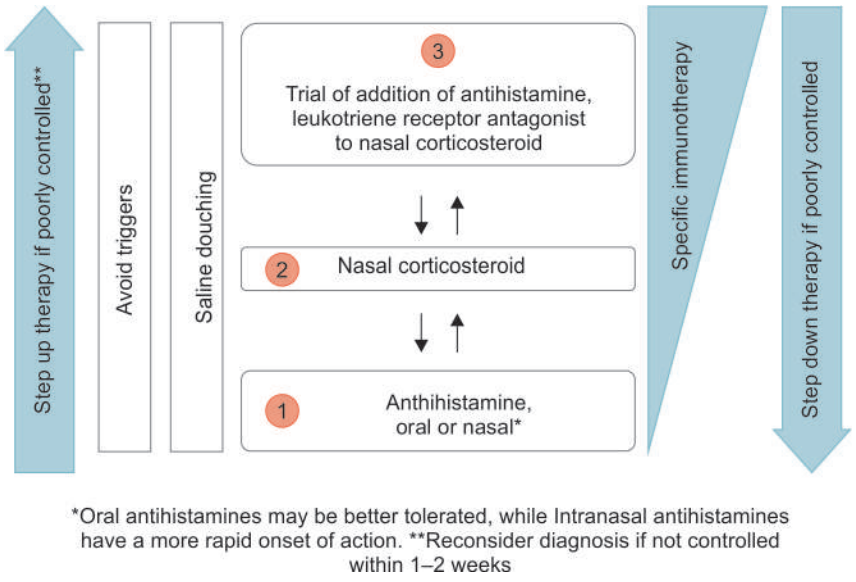

Fig. 2: Guidelines of AOI, 2022

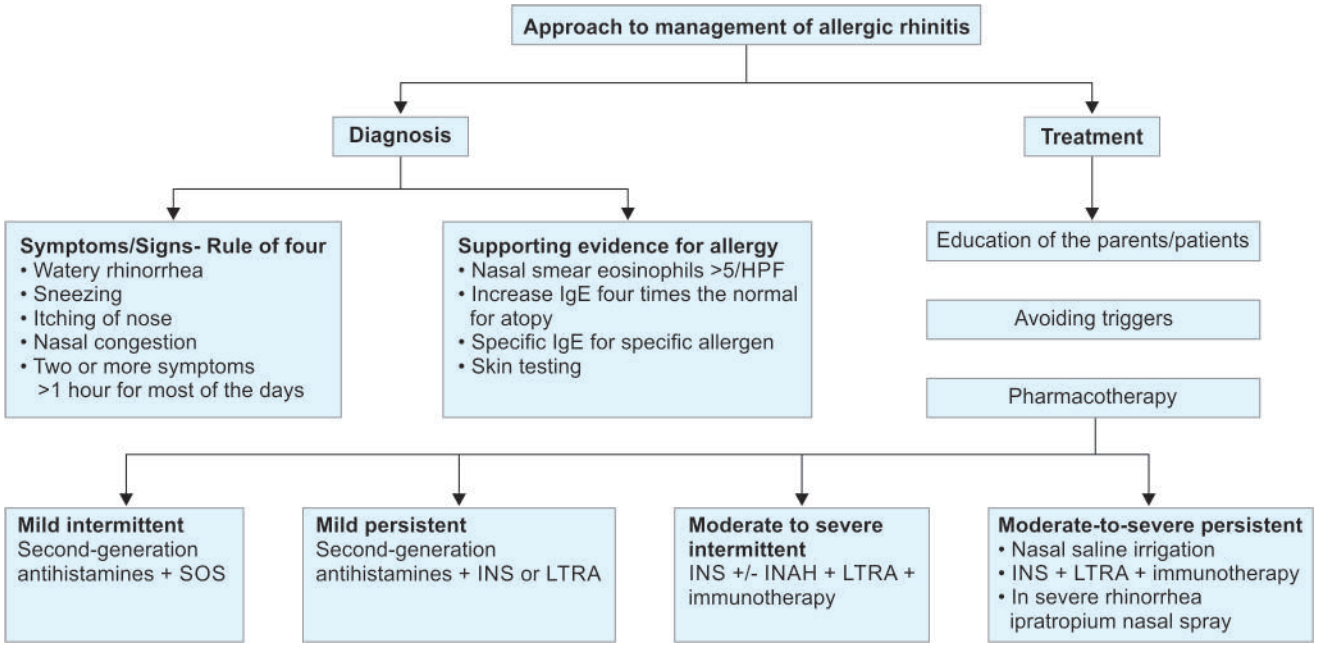

Fig. 3: Indian Academy of Pediatrics (IAP) guidelines; HPF, high power field; IgE, immunoglobulin E; INAH, intranasal antihistamine; INS, inhaled nasal steroids; LTRA, leukotriene receptor antagonist

Bilastine in Special Populations

Older adults frequently have comorbid conditions and take numerous medications, increasing the risk of drug interactions and adverse drug responses. Elderly individuals might also have reduced cognitive performance. Moreover, they might have age-related compromised hepatic and renal function, which can impact drug clearance.[37] The low possibility of drug–drug interactions and pharmacokinetics of bilastine makes it suitable for elderly patients without needing dose adjustment, even with compromised hepatic and renal function. Bilastine showed a favorable safety profile in an observational study in patients 65 years and older, with a very low rate of serious adverse effects.[38] Bilastine is also safe for 2-year-old kids and is permitted in Europe for kids between the ages of 6 and 12.[39] There is insufficient or no data on using bilastine in pregnant or lactating women. Humans have not been examined for bilastine excretion in milk.

In summary, bilastine has good effectiveness with a quick onset and longer duration of action. It is well tolerated, has a little sedative effect, and exhibits fewer drug interactions. Bilastine can be used as a first-line pharmaceutical agent for treating patients with AR in all age groups, from school children to older people, either as a monotherapy or a combination therapy component.

Author Contributions

All authors contributed and approved the final manuscript. All authors met ICMJE criteria, and those who fulfilled those criteria were enlisted as authors. All authors had access to the study data and made the final decision regarding where to publish these data and approved submission to this journal.

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REVIEW ARTICLE

Antibiotic-associated Gut Dysbiosis

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Abstract

The human gut microbiota plays a crucial role in maintaining overall health. However, the widespread use of antibiotics has raised concerns about its impact on the microbial ecosystem. This review explores the multifaceted relationship between antibiotics and gut dysbiosis, highlighting the mechanisms underlying these interactions and their implications for human health. Antibiotics, while invaluable in treating infections, disrupt the gut microbiota by indiscriminately targeting both harmful and beneficial microorganisms. This disturbance leads to a reduction in microbial diversity, altered metabolite production, and compromised immune responses, resulting in a state referred to as dysbiosis. Broad-spectrum antibiotics tend to induce more severe dysbiosis compared to narrow-spectrum agents. Antibiotic-induced dysbiosis has been linked to the onset and progression of these disorders, emphasizing the far-reaching consequences of microbial imbalance. The review highlights various strategies to mitigate the adverse effects of antibiotics on gut health, like probiotics, fecal microbiota transplantation (FMT), and phage therapy, as promising approaches to restore and maintain a balanced gut microbiota.

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An Overview of Human Gut Microbiota

The human gut microbiota plays a critical role in supporting the integrity and functionality of the gastrointestinal (GI) tract and immunological and metabolic systems.[1] The 10^{14} naturally occurring bacterial cells normally present in the human intestine and colon belong to around 500–1,000 species that have a mutually beneficial relationship, and they colonize the human gut.[2] Over 90% of the total bacterial population that lives in the gut belongs to five major bacterial phyla: Verrucomicrobia, Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes. *Fusobacteria* and *Fibrobacteres* are less abundant bacteria.[3] This dynamic ecosystem upholds a two-way connection with the host, a relationship crucial for both the normal physiological and pathophysiological state.[4] Microbiota helps in processing nutrients, producing essential compounds, shaping the immune system, and maturing the GI system. Microbiota is constantly altered by several factors like genetics, sex, diet, lifestyle, age, and medicine.[2] The microbial denizens of the gut have many interactions; some are cooperative, such as sharing of nutrients, and others inhibitory, such as bacteriocins. In a healthy individual, a balance is maintained between beneficial and aggressive microbial communities, a state that is referred to as eubiosis. An imbalance between possibly detrimental and helpful bacteria leads to a state called alteration of the normal composition of gut microbes, causing intestinal dysbiosis, which may, in turn, lead to pathological abnormalities and inflammation. This intestinal dysbiosis has been linked to various diseases, both intestinal and extraintestinal.[5,6] This review examines the role of antibiotics in inducing prolonged changes in the gut microbiome and the potential association of such changes with disease states.

Antibiotic-Associated Changes in the Gut Microbiota

One of the foremost achievements in medicine of the 20th century was the discovery of antibiotics, which completely changed how infectious diseases were treated.[5,7] Nevertheless, their impact extends beyond the targeted pathogens, as they indiscriminately stop the proliferation of helpful microorganisms, including those inhabiting the gut.[3,8] This reduction in microbiota diversity caused by antibiotics undermines interactions between the host and microbes, disrupts the equilibrium of the immune system, and weakens the body's ability to resist the colonization of harmful bacteria.[8] The influence of antibiotics on gut dysbiosis and their potential function in disease, even after brief usage, may cause long-term dysbiosis, which is marked by loss of important taxa, change of diversity, and metabolic changes. These changes may lead to impaired colonization resistance against intestinal pathogens, most clearly demonstrated in the emergence of *Clostridiodes difficile* (*C. difficile)*-associated diseases, even after short-term antibiotic administration in susceptible individuals. Numerous medical conditions have been investigated and shown to cause dysbiosis of the gut microbiota. The interaction between gut health and microbiota is thought to have a role in the pathogenesis and seriousness of metabolic conditions, autoimmune disorders, infections, and cancer malignancies and is expected to play a role in the pathogenesis and severity of these diseases. Despite this, a 65% increase in the consumption of antibiotics worldwide was observed between 2000 and 2015.[5,9] It has been estimated that nearly 50% of the time antibiotics are prescribed unnecessarily.[10,11] In 2010, India stood as the foremost consumer of antibiotics for human health.[12] Sometimes, inappropriate and irrational use of antibiotics against infectious diseases is found to be the highest in India[13] and is considered a major driver of resistance.[12] In a survey by Basu et al., the doctors participating in the study identified infants and children under 5 years as the most susceptible group to potential negative effects of antibiotics stemming from disruptions in the gut microbiome.[14] Another age group that is susceptible to developing dysbiosis is the geriatric population. In the elderly, the makeup of the gut microbiota changes, leading to a mild inflammatory state. This transformation can be further intensified by various internal and external factors, including the consumption of antibiotics and dietary choices.[15] The excessive utilization of broad-spectrum antibiotics for conditions that could be effectively treated with narrow-spectrum agents has been steadily rising.[10,16] This irrational, extended, and relentless use contributes to the emergence of dysbiosis, which subsequently gives rise to pathological complications in the host.[17] This disturbance in the equilibrium of gut microbiota is accompanied by the loss of some essential taxa that can cause metabolic shifts, a rise in

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the vulnerability of the gut to colonization, and the development of bacterial antibiotic resistance.[5] Antibiotic treatment is the most immediate and drastic exposure that causes dysbiosis.

In this review, we have summarized the most recent research on the gut microbiome and its alterations in relation to antibiotics, along with the impact of antibiotic-associated dysbiosis in specific conditions and strategies to reduce this dysbiosis. A literature search of published articles in the English language was conducted using search engines like PubMed and Google Scholar from 2010 to 2021. A total of 150 abstracts were identified using the relevant search terms such as antibiotic-associated dysbiosis, the impact of antibiotics on gut microbiota, gut dysbiosis, classes of antibiotic, antibiotic-associated diarrhea (AAD), *C. difficile*-associated diarrhea, inflammatory bowel disease (IBD), atopic disorders, allergy, coronavirus disease 2019 (COVID-19), and treatment for antibiotic-associated dysbiosis. The manual reference and review article searches were conducted in addition to the computerized search. Full-text articles and those describing the results on human subjects of all age groups were considered. Research related to various methodologies investigating alterations in the microbiome linked to specific antibiotic usage, diseases connected with antibiotic-associated dysbiosis, and strategies like fecal microbiota transplantation (FMT), probiotics, and phage therapy were included in the search. Animal studies were a part of the exclusion criteria. After applying the predetermined criteria for inclusion and exclusion, a total of 62 pertinent articles were ultimately selected for use.

The Direct and Indirect Effects of Antibiotics on Gut Microbiota

Antibiotics exert their effect on gut microbiota through either direct or indirect mechanisms. Direct action involves reducing pathogenic bacteria; however, due to their wide-ranging effects, antibiotics can also eliminate or hinder certain groups of beneficial microbes that inhabit the gut. Broad-spectrum antibiotics have been reported to affect 30% of gut bacteria, leading to a prompt and significant decrease in evenness, diversity, and taxonomic richness.[18–20]

The indirect effect of antibiotics on the gut microbiome is discussed below:

- Effects on bacterial metabolism: Different subsets of gut microbiota universally exhibit symbiosis and codependency. A homeostatic state is maintained by the microbiota under normal physiological conditions. The secondary compounds produced by certain microbiota species might offer essential nutritional value to other colonizing organisms. If not, some metabolites may accumulate in the gut and exhibit toxicity toward other microorganisms. The microbial biotransformation of these toxic metabolites is species-specific. Hence, disturbance in the microbiota can lead to changes in metabolites and the gut's microenvironment, subsequently impacting the growth of other constituents within the gut microbiota.[3] Antibiotic treatment also causes the loss of Firmicutes, Bacteroidetes, and Proteobacteria, diminishes the healthy combination of phyla, which in turn causes metabolic shifts, makes the gut more prone to colonization, and stimulates the development of bacterial antibiotic resistance.[5,17] Metabonomic analysis[3] suggested that lipids, bile acids, amino acids, and amino acid-related materials are affected greatly by antibiotics in the gut. An alteration in the short-chain fatty acids (SCFA) production is the primary route through which microbiota dysbiosis induces their effects on immunity and metabolism.
- Impact on host immune system: Molecules derived from luminal gut microbes trigger the immune response. For example, microbial-associated molecular patterns are sensed by innate immune receptors on epithelial cells and dendritic cells. These toll-like receptors (TLRs) influence cells in the lamina propria to elaborate cytokines and chemokines. They also influence the maturation of naïve T-cells in the intestinal mucosa and direct their differentiation to different classes of T-helper (Th) cells (such as Th1, Th2, Th 17, etc.) or T-regulatory cells. In a healthy state, it is believed that a preponderance of the T-regulatory cells maintains immune homeostasis and prevents unbridled inflammation within the intestine and further systemic circulation. The microbial imbalance caused by antibiotic use leads to dysregulation of immune response, which can potentially manifest as a minor systemic inflammatory state or as an inflammation in a variety of end-organs. SCFA results in a lower frequency of Th and T_{reg} cells, which are considered critical for immune homeostasis. Deregulation of gut microbiota disturbs this balance, which impairs the immune responses, hence leading to a variety of disease outcomes.[18] Antibiotics affect the immunity of the host by changing the metabolites of bacteria and specifically inhibiting bacterial colonies. Intestinal epithelial cells identify the signals communicated from the gut microbiota to the host, and intestinal immunity may skew the immune response to cause inflammation or allergic reactions.[3] The hygiene hypothesis specifies that, as hygiene improves, autoinflammatory and allergic diseases become more prevalent in society, and a large component of this is mediated by changes in gut microbiota. Recent research has shown that the reduction of resident microbiota affects systemic immunity, innate immunity, and adaptive immunity.[3,21] Antibiotic-induced dysbiosis promotes sustained T-cell-mediated dysfunction, thereby increasing the susceptibility to inflammation and infections by meddling with regulation dependent on the microbiota of intestinal innate immunity.[1] Antibiotics also have a profound impact on group 3 innate lymphoid cell recruitment and development, which in turn reduces interleukin 22 (IL-22) production and makes the host more susceptible to invading pathogens.[22] The effect of antibiotics/dysbiosis on immune cells in humans is explored by relatively fewer studies.[23] In a specific subset of full-term infants, Oosterloo et al. investigated how neonatal antibiotic treatment influenced the expression of 84 distinct markers on circulating immune cells. The findings revealed a notable correlation between the use of broad-spectrum antibiotics and the presence of immune-inflammatory markers [such as heat shock protein 70, soluble form of vascular endothelial growth factor receptor 1, IL–1RII, soluble cluster of differentiation 19 (sCD19), sCD14, and soluble vascular cell adhesion molecule-1] at the age of 1 year.[23] The host's adaptive immunity against hepatitis B virus (HBV) infection was also depleted due to dysbiosis. Antibiotics-treated patients showed a decline in interferon-γ production and impaired clearance of HBV.[24] Figure 1[25] describes the mechanism of immunomodulation caused by antibiotics.
- Increased susceptibility to infection: An excess of inflammatory vs anti-inflammatory microbial species is also related to low microbial richness, which in turn causes inflammation of the intestine and disruption in the function of the mucosal barrier.[5,26] Antibiotic-associated dysbiosis leads to a plethora of imbalances in the immune system like (1) impairment of pulmonary defense against pathogens,

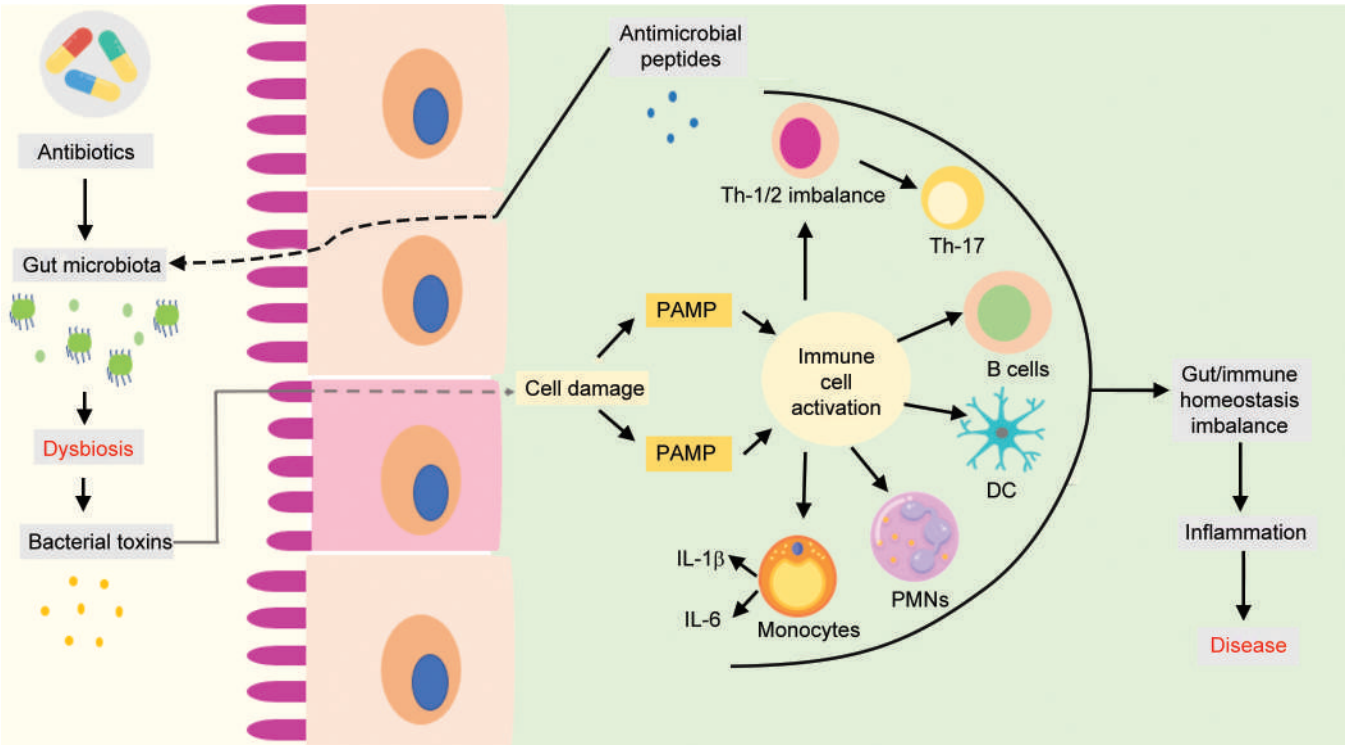

Fig. 1: Pathophysiology of dysbiosis associated changes. DC, dendritic cells; DAMP, damage-associated molecular patterns; PMNs, polymorphonuclear leukocytes; PAMP, pathogen-associated molecular patterns; Th, T helper cells

(2) alteration of metabolism within alveolar macrophages, consequently leading to a deterioration in the ability to phagocytose *S. pneumoniae,* (3) impaired reaction to stimulation from lipopolysaccharides to lipoteichoic acid, and (4) diminished expression of a proliferation-inducing ligand and decreased pulmonary immunoglobulin A production in critically ill patients due to deregulation of TLRs signaling.[27] These changes could potentially play a role in the development of various noncommunicable diseases and the progression of these conditions to a chronic state.[5,28,29]

Impact of Different Antibiotic Classes on Gut Microbiota

The usage of antibiotics is one of many elements that may disturb the balance between human hosts and related microorganisms.[30] The effect of antibiotics on intestine microbiota varies among different classes of these drugs. The extent to which antibiotics can alter the microbiota depends on factors like (1) the spectrum of the agent, (2) the duration and dosage of treatment, (3) the pharmacodynamic and pharmacokinetic properties of the agent, and (4) the route of administration.[31] To increase the spectra of antibacterial activity, usually a combination of antibiotics is used, but this produces severe dysbiosis compared to treatment with a single antibiotic. Treatment with cefazolin, ampicillin, and vancomycin in humans has been shown to decrease the population of Firmicutes [*Clostridium* cluster IV and XIVa (*Eubacterium* and *Subdoligranulum*), *Anaerobutyricum hallii* and *Faecalibacterium prausnitzii*] with a simultaneous rise in the abundance of Proteobacteria.[17,32] The importance of this observation lies in the fact that *Clostridium* clusters IV and XIV, *Eubacterium*, *Roseburia*, and *Faecalibacterium prausnitzii* are important for producing SCFA, particularly butyrate, while phylum Proteobacteria contains potentially pathogenic microbiota including *Escherichia coli*, *Vibrio cholerae*, and *Shigella* species. Clindamycin, a broad-spectrum antibiotic that is excreted in bile and mainly concentrated in the feces, is known to target anaerobic bacteria. Clindamycin has an adverse impact on the gut microbiota by diminishing its ability to resist colonization by harmful pathogens and promoting the proliferation of *C. difficile*. Additionally, it also causes inflammation of the lining of the stomach and diarrhea. These disruptions in regular bowel function may cause bloating and discomfort in the abdomen.[30,31]

On the other hand, β-lactams are known to double the average microbial burden. The patients undergoing moxifloxacin treatment experienced a decrease in the abundance of *Bacteroides* and *Faecalibacterium* genera. However, in the early stages of treatment, there was an active presence of other bacteria that produce butyrate, such as *Roseburia* and *Lachnospiraceae incertaesedis*, as well as H2-consuming bacteria like *Blautia*, *Collinsella*, and *Bifidobacterium*. These bacteria provide energy to the host's epithelial cells lining the colon. When a combination of clindamycin and penicillin G was used, a marked decrease in *Blautia* and *Bacteroides* genera was observed in the active microbiota.[30,31] These were some common examples of the perturbations caused by antibiotics. Table 1 lists more such examples.

Impact of Antibiotic-Associated Dysbiosis in the Following Conditions

Dysbiosis induced by antibiotics has been linked to the genesis of several disorders, including obesity, AAD, *C. difficile*-associated diarrhea, IBD, atopic disorders, allergy, and COVID-19. The aforementioned conditions are discussed in detail below:

- Antibiotic-associated diarrhea (AAD): The prevalence among patients receiving antibiotics is about 5–35%.[5,34] While certain antibiotics may induce diarrhea through effects on motility, modification in the variety of the gut microbiota is supposedly

Table 1: Impact of antibiotics on gut microbiota and immunity[31,33,43]

Antibiotics	*Class*	*Resistance mechanism*	*Effect on gut microbiota*	*Effect on immunity*
Amoxicillin	β-lactam	Altered target, β-lactamase	Reduction of *Enterobacteria*	NA
Ampicillin	β-lactam	Altered target, β-lactamase	Greater prevalence of *Enterobacter* spp. and decreased bacterial diversity	Reduced immune cell
Cefotaxime	β-lactam (third generation cephalosporin)	Altered target	Reduction in the number of bacterial cells; decline in the abundance of anaerobic and *Enterobacterial* species	NA
Ceftriaxone	β-lactam (third generation cephalosporin)	NA	Increase in gram-positive bacteria, reduction in *Enterobacteria*	Decrease in sIgA
Ciprofloxacin	Fluoroquinolone	Altered target, efflux	Decreased abundance of *Enterobacteria*; lower bacterial diversity, decrease in short-chain fatty acid (SCFA) producers	Decrease in antimicrobial peptide
Clindamycin	Lincosamide	Altered target	Initially decreased abundance of *Enterococci*, *Streptococci*, and anaerobic bacteria, subsequent recovery of abundance of *Streptococci* and anaerobic bacteria; reduced diversity of *Bacteroides* spp.; decrease in an abundance of bacteria producing short-chain fatty acids	Not reported
Vancomycin	Glycopeptide	Altered peptidoglycan target	Decreased bacterial diversity	Decrease in antimicrobial peptide, immune cells, and intestinal lymphoid follicles
Amoxicillin/ clavulanic acid	Penicillins with β-lactamase inhibitors	NA	Increase in aerobic gram-positive cocci and *Enterobacteria*	Decrease in antimicrobial peptide and antigen-presenting capacity
Clarithromycin/ metronidazole	Macrolide (clarithromycin) and nitroimidazole (metronidazole)	Altered target/drug inactivation (clarithromycin) and efflux (metronidazole)	Reduction in an abundance of Actinobacteria, partial recovery of pretreatment state; increase in *enterobacteria*	Decrease in antimicrobial peptide and intestinal innate immune cells

an underlying factor of AAD. One of the mechanisms by which AAD can occur is due to the decrease of advantageous metabolic activities of intestinal microbes. By altering global colonic metabolism, alterations in the composition and amount of the intestinal microbiota (even in the absence of excess growth of pathogenic microorganisms) may lead to AAD.[5,35] Since the colon cannot absorb carbohydrates, anaerobic colonic bacteria metabolize them as an important source of energy, creating lactic acid and SCFAs that the colon can easily absorb. Due to antibiotic treatment, such bacteria are lost, which leads to an increase in the amounts of carbohydrates in the lumen, further causing osmotic diarrhea.[36] A meta-analysis by Szajewska, Kolodziej et al. revealed that certain strains of probiotics, such as those composed of *Lactobacillus rhamnosus* GG or *Saccharomyces boulardii* might be useful for the prevention of AAD.[5,37,38]

- *Clostridium difficile*(*C. difficile)*—associated diarrhea: *C. difficile*, an anaerobic, gram-positive bacillus, possesses resilient spores resistant to extreme temperatures, chemicals, and desiccation. It generates toxins A and B, capable of damaging the gut mucosa.[5,39,40] Antibiotics such as clindamycin, cefoperazone, and vancomycin produce an environment that is favorable for the development of *C. difficile* by increasing the levels of carbohydrates like mannitol and sorbitol, reducing SCFAs and glucose, creating an increase in tauro-conjugated primary BAs and levels of glycine in the intestinal lumen.[18]
- Obesity: Obesity and obesity-related metabolic disorders are linked to dysbiosis of the gut microbiota. Diets, in addition to genetics, both have a significant impact on obesity. Over the past 3 decades, there has been a rise in the number of cases of obesity, which has been linked to changes in the composition of the intestinal microbiota.[5] Obesity is related to changed microbial profile, including diminished diversity,[41,42] increased abundance of pro-inflammatory bacteria, and decreased abundance of anti-inflammatory bacteria. In comparison to their lean counterparts, obese people have fewer Bacteroidetes, Christensenellaceae, and Akkermansia and a higher concentration of Firmicutes. Numerous studies have consistently linked antibiotic-induced alterations in the gut microbiota to either children being overweight or adiposity. Evidence indicates that early antibiotic exposure can trigger long-term and substantial changes in an infant's gut microbiota.[42] It has been noted that antibiotic use in infants under 6 months of age is linked to higher body mass index in later life.[10] Changes in metabolic function, an increase in adipose tissue in the body, and childhood obesity have all been linked to altered gut microbiota. Since the published results are not causal, the mechanism that underlies the linking of early childhood antibiotic use, being obese, and gut microbiota is unknown.[42]
- Inflammatory bowel disease (IBD): A close connection between altered gut microbiota and IBD can be made from several clinical observations.[43] A

population-based study by Kronman et al. stated that infants receiving anti-anaerobic antibiotics in the first year of life had higher chances of being diagnosed with IBD than those who were not treated with antibiotics.[5,44] A Danish (Hviid et al.) study observed that the probability of IBD was highest in the initial 3 months after usage of antibiotics and in children who had received at least seven courses of antibiotics.[5,45] Antibiotic-induced dysbiosis may contribute to the onset and progression of IBD. The widespread presence of supposedly immune-protective bacteria from the phylum Firmicutes, especially *F. prausnitzii* and *Akkermansia muciniphila*, is decreasing, whereas adherent, invasive Escherichia coli is increasing. *F. prausnitzii* secretes anti-inflammatory metabolites that block nuclear factor-β activation and IL-8 development by epithelial cells, resulting in anti-inflammatory effects in cellular and animal models of colitis. *F. prausnitzii* and *A. muciniphila* contain the SCFAs butyrate and propionate, which maintain the integrity of the mucosal barrier in addition to their direct anti-inflammatory effects. In line with the idea that antibiotic exposure will lead to a decrease in abundance and the extinction of certain taxa.[43] The overall decrease in Firmicutes and *F. prausnitzii* is linked to an increased risk of recrudescence of CD, ulcerative colitis (UC), and IBD.[46]

- Allergy and atopic disorders: Allergies are a very common chronic disease where there is hyperactivation of Th2 of the adaptive immune response. Important components of the gut microbiota are necessary for the development of the immune system's regulatory components as well as for maintaining homeostasis at the gut epithelium. It is assumed that dysbiosis due to antibiotics can affect the response of Th2, making it more vulnerable to allergies.[47] Multiple studies have revealed that the first 6 months of life are the most important for the development of the immune system, suggesting host-microbiome interactions play an important factor.[10] In a study by Kummeling et al., an investigation into the connection between infants being exposed to antibiotics within their first 6 months of life and the occurrence of eczema, wheezing, and allergies at the age of 2 was conducted. The study revealed that antibiotics were associated with an elevated likelihood of recurrent and extended wheezing, although no significant links were observed with allergic sensitization or eczema.[47,48] According to Stensballe et al., children born to asthmatic mothers having a higher risk of asthma were linked to maternal antibiotics used during the third trimester of pregnancy.[47,49] The widespread use of antibiotics may be one of the factors leading to the dramatic rise in autoimmunity and allergies over the last few decades.
- Coronavirus disease 2019 (COVID-19): Prescribing antibiotics to prevent/treat superinfections in COVID-19 patients might have a significant effect on the patient's gut microbiota.[50] COVID-19 patients have demonstrated a more heterogeneous microbiome configuration, and there is a shift of gut microbiota toward an unhealthier spectrum. A study conducted by Zuo et al. concluded that even in patients with COVID-19 naïve to antibiotic therapy, depletion of beneficial commensals was a peculiar characteristic.[51] Azithromycin showed a higher potential to rapidly worsen the already weak microbiota of elderly and COVID patients with comorbid conditions because of its ability to rapidly reduce bacterial richness (23%) and Shannon diversity (13%). However, further research is needed to figure out the effect of antibiotic-associated dysbiosis on COVID patients.[50]

Strategies to Reduce Antibiotic-induced Gut Microbial Dysbiosis and Immune Disorders

Overuse of antibiotics must be moderated due to the numerous adverse effects that come with the consumption of antibiotics. The use of antibiotics cannot be abandoned, especially when patients have severe infections. Hence, multiple approaches have been proposed to lessen immunological diseases and dysbiosis caused due to antibiotics.[3] Some of these strategies are mentioned below:

- Probiotics: Live probiotics are generally administered to restore the balance of microbiota in the gut and to promote anti-inflammatory responses. *Lactobacillus*, *Saccharomyces*, *Bacillus*, *Bifidobacterium*, and *Enterococcus* are the most frequently used probiotics. Goldenberg et al. revealed that probiotics may be useful to prevent *C. difficile* infection in patients who are being administered antibiotics.[3,52] Multiple studies have observed that oral administration of a combination of probiotics, such as *Bifidobacterium*, *L. acidophilus*, *L. casei*, and *L. rhbamnosus,* can decrease the risk of AAD, probably due to regulation of the gut microbiota, modulation of immune responses, and increase in the gut barrier function. Li et al. encapsulated probiotics in alginate, coadministered them with antibiotics, and observed that the antibiotic did not affect the metabolic activity of the probiotics. These could also exert a synergistic role against two methicillin-resistant pathogens and, hence, can be coadministered to treat complex infections and the prevalent antimicrobial resistance. Because of the strain-specific effects of probiotics, it is essential to emphasize on development of new personalized probiotic supplementation approaches rather than the general ones.[53] Yeast probiotics, especially *S. bourlardii*, are now gaining a lot of popularity for the treatment of dysbiosis. A recent review confirmed that treatment with *S. boulardii* CNCM I-745 in dysbiosis results in a faster reestablishment of healthy microbiota. *S. boulardii,* being yeast, is resistant to antibiotics, and the administration of *S. boulardii* CNCM I-745 after antibiotic therapy has been reported to accelerate the recovery of the intestinal microbiota at the initial level.[54] Therefore, the use of yeast probiotics can be further explored for the treatment of antibiotic-associated dysbiosis.
- Fecal microbiota transplantation (FMT): FMT is considered to be a novel method to control multidrug-resistant pathogens and to avoid potential or future severe infections, and is generally used to treat patients whose gut is colonized with such pathogens as *C. difficile* or for patients who are at greater risk of infection after treatment with antibiotics.[53] FMT controls intestinal inflammation and maintains the balance of gut microbiota through several mechanisms, such as increasing the production of IL-10 by APCs, iNKT cells, and $CD4^+$ T-cells, restoration of secondary bile acid metabolism, provision of signals for epithelial regeneration, and stimulation of secretion of antimicrobial peptide. Selective transfer of *Clostridium scindens* has demonstrated considerable advantages in addressing recurrent or recalcitrant *C. difficile* infections, yielding a notable cure rate ranging from, approximately, 87 to 90%. This beneficial outcome arises from the augmentation of host resistance against the infection, as *C. scindens* possesses the capability to enzymatically transform primary bile salts into secondary bile salts, which exhibit potent inhibitory properties against the colonization of *C. difficile*.[3] Lastly, autologous FMT also increases the

diversity of microbiota and restores the gut microbiota in healthy adults, patients, and mice ingested antibiotics.[53]

- Phage therapy: Along with bacterial diversity, the human gut is also a host to a fascinating viral community that exerts its effect on microbiota as well as the host.[33] Phage therapy was the choice of treatment before the advent of antibiotics and is now gaining popularity either for restoring microbial equilibrium in chronic health conditions or for compassionate intervention in acute cases.[33,55] Phage therapy capitalizes on the intrinsic capacity of bacteriophages to selectively target specific bacteria, harnessing both lytic and temperate phages as agents for microbiome intervention to engineer and manipulate the microbiota within a specific ecological niche to attain a state of well-balanced and health-promoting microflora.[55,56] Phages are now recommended because of (1) their high specificity to the target bacteria, which helps in reducing the off-target impacts on broader microbiota and (2) their inherent self-replicative nature, thereby contributing to the cost-effective production of phage-centered therapeutics.[33] Genetically engineered phages show an improved function in modulating the gut ecosystem.[57] One such example is the incorporation of a biofilm-degrading enzyme into the genetic makeup of T7 phages. It allowed for the reduction of the biofilm and lysis of the bacteria simultaneously in a positive feedback manner.[58] Many active phages like those against *E. faecalis*,[59] *Bacillus cereus*,[60] and *P. aeruginosa*[61] have been discovered. A study by Yosef et al. identified the use of phage therapy to treat antibiotic resistance.[62] On the basis of these reports, phage therapy is recommended to be used for diseases in which (1) the bacterial cause is well defined, (2) refractory to antibiotics, and (3) phages are accessible.[62] However, more research needs to be done on resistance to both phages and engineered nucleases. Nonetheless, natural and engineered phages are promising future tools in the battle against pathogens and the state of the dysbiotic community.[62]

Conclusion and Future Directions

The gut, housing a vast community of trillions of microbes known as the gut microbiota, plays a pivotal role in maintaining equilibrium within the body. It accomplishes this by safeguarding against diseases modulating both the immune response and energy utilization. Despite being one of the most often prescribed medications, the excess dependence on them can be a serious problem as they can disrupt the equilibrium of the gut microorganisms and further lead to serious disorders. Antibiotics have extreme effects on the gut microbiota as they diminish the plethora of useful commensals and increase the abundance of harmful pathogens or commensals. These adverse effects of antibiotics are particularly a major concern to infants and should be carefully considered while it is prescribed without affecting the clinical practice. Disturbances in microbiota equilibrium triggered by antibiotics can have adverse health implications. These perturbations may amplify vulnerability to infections and disrupt immune homeostasis, potentially leading to heightened allergies, IBD, colorectal cancer, obesity, and asthma. However, recent advances bring a new road map for combating disease-causing bacteria while minimizing harm to the microbiota. The forthcoming emergence of targeted antivirulence agents, alongside a revitalized exploration of probiotics, phage therapy, and FMTs, promises to revolutionize treatment approaches for dysbiosis. Furthermore, advancements in our understanding of antibiotics can give us a better insight to fight pathogenic bacteria.

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REVIEW ARTICLE

COVID-19 Therapeutics Why Not Angiotensin Receptor Blockers (ARBs)?

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Abstract

Overactivity of the renin–angiotensin–aldosterone system (RAAS) is a consistent feature of COVID-19 as indicated by high concentrations of angiotensin II (Ang II) in lungs and other tissues. Virus-induced downregulation of angiotensin-converting enzyme-2 (ACE2) explains the raised Ang II levels. Available evidence points to the crucial role of Ang II in the pathogenesis of coronavirus disease. The proinflammatory, immune stimulant, and procoagulant effects exhibited by the peptide at high tissue levels explain lung injury, a characteristic feature of severe COVID-19.

Angiotensin II (Ang II) inhibitors [both the angiotensin-converting enzyme inhibitors (ACEIs) and the angiotensin receptor blockers (ARBs)] constitute the logical therapy for established COVID-19 infection. While ACEIs help to lower Ang II levels in the tissues, ARBs antagonize the effects of the peptide on the target tissues. Of the two, ARBs offer a better choice because of the minimal adverse effects of dry cough and angioedema. The effectiveness of Ang II inhibitors in COVID-19 is well supported by their protective effect against lung injury in animals induced by the virus spike protein as well as the clinical improvement of shortened hospital stay and reduced mortality in observational studies in humans.

A unique feature of these agents is that mutations of the coronavirus 2 (CoV-2) would have little impact on their effectiveness since they do not interfere with the host cell entry of the virus or its replication. Expectedly, the agents might retain their usefulness against variant strains, including "o" and its subvariants.

The overall safety of Ang II inhibitors has been well established beyond doubt since they have been in use for years in the management of cardiovascular (CV) diseases, diabetes mellitus, and chronic kidney disease (CKD).

Regular use of ARBs in all patients who are COVID-19 positive and symptomatic (mild, moderate, or severe) offers a good option worth serious consideration.

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Introduction

The COVID-19 outbreak reported in Wuhan, China, toward the end of December 2019,[1] was declared a pandemic by the World Health Organization (WHO) 3 months later. As of 15th July 2022, confirmed cases of COVID-19 globally have risen to 55.80 crores, including 63.59 lakhs deaths, while the figures for India read 4.37 crores and 5.26 lakhs, respectively (as reported by WHO).

Renin–Angiotensin–Aldosterone System (RAAS) Activation, a Feature in COVID-19

Several studies have demonstrated overactivity of the renin–angiotensin–aldosterone system (RAAS) in COVID-19 as indicated by high levels of angiotensin II (Ang II) in lungs and other tissues in both animals and human beings infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[2,3] Virus-induced down-regulation of angiotensin-converting enzyme-2 (ACE2) undisputedly explains raised Ang II levels.

Angiotensin II Plays a Pathological Role

Besides the conventional effects of vasoconstriction and aldosterone release, Ang II is known to exert proinflammatory and procoagulant effects at high levels found in disease states. The peptide also causes immune stimulation, endothelial dysfunction, and increased oxidative stress.[4–6] Increasing evidence points to a crucial role of Ang II in the pathogenesis of coronavirus disease. Damage to the lungs leading to pulmonary edema and acute respiratory distress syndrome (ARDS) are characteristic features of severe infection (see below).

Angiotensin-converting Enzyme-2 (ACE2) is the Crucial Link

The ACE2 constitutes the crucial link between the SARS-Cov-2 virus and the host RAAS. The molecule appears to play a dual role. While it is a negative regulator of the RAAS in the host by degrading Ang II, it facilitates virus entry into the host cells by serving as the receptor for the virus spike protein.[7–9]

Regulation of Ang II levels—in normal conditions, tissue Ang II levels are regulated by two enzymes, ACE and ACE2, the former catalyzing its formation and the latter its degradation.

Mechanism of Action of ANG II

The active moiety of the RAAS exerts all its effects, both physiological and pathological, through the activation of angiotensin II type 1 (AT-1) receptors.

Angiotensin II (ANG II) Inhibitors, the Logical Therapy

Inhibitors of Ang II [both the angiotensin-converting enzyme inhibitors (ACEIs) and the angiotensin receptor blockers (ARBs)] constitute the logical and the most specific therapy for established COVID-19 infection. While ACEIs help to lower Ang II levels (Fig. 1) in the tissues, ARBs antagonize the effects

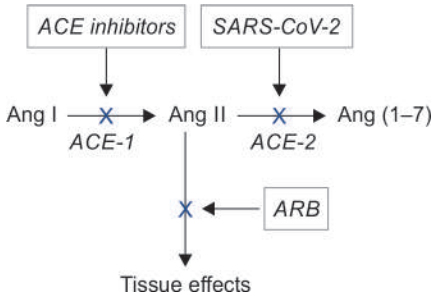

Fig. 1: Effects of ACE1 and ARB

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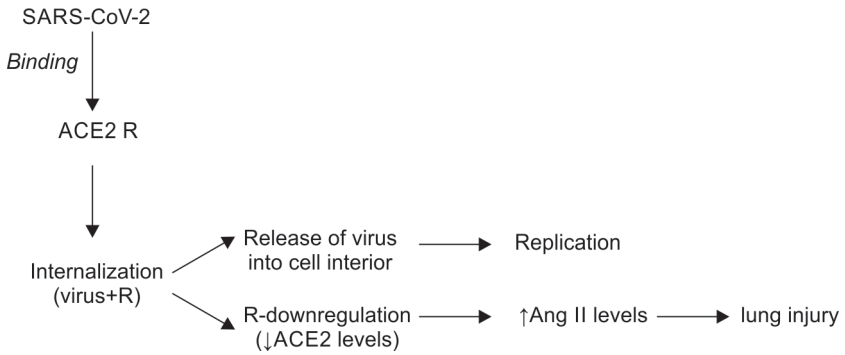

Fig. 2: Virus entry into host cells and related events

of the peptide at the tissue level. Of the two, ARBs offer a better choice because of the minimal adverse effects of dry cough and angioedema.

Sun et al.,[10] Gurwitz,[11] and Hanff et al.[12] had earlier expressed support for the use of RAAS inhibitors in COVID-19-associated pneumonia.

COVID-19-Infectivity and Pathogenesis

Infectivity

Virus Entry into Host Cells

The entry process involves the following steps:

Binding of spike protein-S of the virus to the ectodomain of the ACE2 receptor (ACE2-R) removal of the ectodomain and its release into the circulation (ACE2 shedding)[13] fusion of viral envelope with the host cell membrane and internalization of the virus-receptor complex[14] release of viral RNA into the host cell cytosol and degradation of ACE2-R (Fig. 2).

Down-regulation of ACE2 and Enhanced Ang II Levels

Internalization with subsequent degradation results in a decrease in the number of receptors on the cell surface. The host cell thus loses one receptor molecule with the entry of each virus particle. This is the most crucial aspect of the coronavirus infection and has been corroborated by all the studies.

The ACE2 down-regulation leads to an increase in tissue levels of Ang II since ACE2 is also the enzyme for Ang II degradation.

Pathogenesis of Lung Injury

High ACE2 expression in Lungs

Lungs constitute the prime target for coronavirus. ACE2-R is expressed in abundance in the lungs, especially in the epithelial cells of alveoli (pneumocytes type II) and endothelial cells of alveolar capillaries.[15,16] Direct exposure to the external environment and the large surface area of these cells explain the high vulnerability of lung tissue. This also indicates the main route of virus entry.

Angiotensin II Plays a Crucial Role

The Ang II levels are significantly raised in the lung and other tissues in patients infected with COVID-19.[2,3] Studies using various animal models of lung injury have demonstrated severe inflammatory lesions in the lungs, which appeared to be mediated by Ang II.[17–19] In a landmark study, Kuba et al.,[20] demonstrated that the isolated spike protein of the coronavirus caused down-regulation of ACE2 with a concomitant increase in Ang II in the lungs and precipitation of severe inflammatory lesions and that Ang II inhibitors attenuated these lesions.

Progressive Diffuse Alveolar Damage[21–23]

Elevated levels of Ang II in COVID-19 cause all-round depletion of pneumocytes, together with damage to capillary endothelium. Thickening of the alveolar-capillary membrane decreases surfactant production, and the formation of fibrin exudates causes a decrease in the elasticity of the lungs and impaired gas exchange. The resultant alveolar damage manifests as pneumonia, ARDS, and respiratory failure.

Beneficial Effects of ARBs in COVID-19

Attenuation of Lung Injury

Studies using animal models and cell lines on Vero cells of monkeys indicate that Ang II inhibitors exert a protective effect on lungs from severe forms of injury induced by CoV-2. Retrospective/observational studies in humans corroborate this point.

Animal Studies

Kuba et al.[20] showed that blockade of AT-1 receptors by losartan attenuates pulmonary edema and lung damage in mice induced by injection of Spike-Fc protein of SARS-CoV. Interestingly, ARBs exhibited the same protective effects in animals against lung injury induced by exposure to other stimuli, including oleic acid and lipopolysaccharide.[17,18] Moreover, the severity of lung injury induced by influenza H7N9 virus[19] was markedly reduced by the blockade of angiotensin receptors, indicating that activation of the RAAS is crucial in all forms of lung injury.

Human studies (Observational Studies in COVID-19-infected Patients)

In this regard, four studies[24–27] have revealed significant improvement in clinical outcomes with the use of Ang II inhibitors. The study subjects (716 in all) were patients with hypertension already on Ang II inhibitor therapy and hospitalized for COVID-19. The most recent ongoing clinical trial, in its interim report, shows that telmisartan, in addition, shortened hospital stays.[28]

Improvement in the clinical outcomes has been shown by either a decrease in the severity of clinical manifestations, a reduction in lymphocyte count and inflammatory markers, or a reduction in 28-day all-cause mortality.

Beneficial Effects on Comorbidities

Hypertension, cardiovascular (CV) conditions, diabetes mellitus, and chronic kidney disease (CKD) constitute important comorbidities for COVID-19 and are well known to contribute to the severity of the latter.

The ARBs and ACEIs have established benefits in protecting the myocardium and kidney; they reduce mortality in CV disease while ensuring system stability and also delay disease progression in CKD and diabetic nephropathy. Abrupt withdrawal of these drugs in these patients is certain to adversely affect the preexisting comorbidities and enhance the risk of clinical decompensation. As Vaduganathan et al. have stressed, RAAS inhibitors should be continued in patients in otherwise stable conditions when they get infected with COVID-19.[29]

Special Advantages over Antiviral Agents

As it stands today, Ang II inhibitors (ARBs and ACEIs) offer the most specific form of therapy in the clinical management of the disease in COVID-19-infected patients since they act to interfere with disease progression by lowering tissue levels of Ang II, the peptide responsible for organ damage. Moreover, mutations of the CoV-2 would have little impact on the effectiveness of these drugs since they do

not interfere with viral replication. This factor could be applicable to the latest variants, "δ" and "o."

Safety Profile of ARBs

Overall Safety: Excellent

Ang II inhibitors have been in use for years in the management of CV diseases, diabetes mellitus, and CKD, and their overall safety has been well established beyond doubt. No serious adverse effects have been reported so far. Common problems include dry cough, angioedema, hypotension (with the ACEI group), and hyperkalemia.

Safety in COVID-19 Patients: Concern does Exist, but Misplaced

Many healthcare providers appear to harbor serious concern about the potential for enhanced severity of the disease and mortality following the use of Ang II inhibitors.

Position Statement of Scientific Societies

Settling the uncertainty in this regard, scientific societies have come out with statements allaying such apprehensions. In a joint statement released in March 2020, the American College of Cardiology, American Heart Association, and the Heart Failure Society of America[30] advised clinicians against discontinuing the use of Ang II inhibitors in COVID-19 patients. The position also got the support of other academic bodies, including the International Society of Hypertension, European Society of Hypertension, European Society of Cardiology, Canadian CV Society, Canadian Heart Failure Society, and International Society of Hypertension. This has certainly helped to provide some reassurance to healthcare providers. Most clinicians, however, remain confused since the opinions expressed by the societies are quite guarded and with a certain degree of caution, the only exception being that of the Council on Hypertension of the European Society of Cardiology.

The Scientific Brief Issued by WHO[31]

The note, released in May 2020, summarizes the most recent evidence on the impact of ACEIs or ARBs on severe acute respiratory illness due to SARS-CoV-2. A rapid review of 14 studies was carried out using Ovid Medline and the Cochrane Database as well as the WHO database of COVID-19 publications. The review concluded that ACEI or ARB use was not found to be associated with increased severity of COVID-19 illness.

Studies in Human Subjects on Safety

Published Studies Have Provided Evidence for the Safety of ARBs/ACEIs in COVID-19 Patients: Retrospective Studies[32–34]

The subjects were mostly patients with hypertension (a handful having cardiac problems) who were hospitalized for COVID-19, with a majority of them on RAAS inhibitors or other antihypertensives on admission. The study involved a total of 362 patients, 163 on Ang II inhibitors therapy.

Population-based studies[35–38] covered a very large number of COVID-19 patients drawn from different geographical areas, including Denmark, Italy, Spain, South Korea, and the city of New York. Each study worked with the official Administration Registries of the population concerned. Taken together, the five studies pertain to 5,686 patients drawn from five different communities. These patients were already on ACEI/ARB therapy at the time of hospitalization with COVID-19.

The results of these studies provide convincing evidence that ACEI/ARB therapy does not enhance susceptibility to or increase the severity of COVID-19. The 30-day mortality rate also did not differ significantly from those not receiving ACEI/ARB therapy.

Concluding Remarks

As Curfman[39] observes in his editorial in JAMA, these studies on such large patient populations should lay to rest concerns about the use of these drugs in patients with or at risk of COVID-19.

Current Status: ANG II Inhibitors Do Not Find a Place in COVID-19 Therapy: Why?

It is quite surprising that neither ARBs nor ACEIs have been officially approved for use in COVID-19 management, irrespective of all the plus points, including effectiveness, safety, and a sound basis for their use. Uncertainty also continues to exist as to whether individuals on these drugs could continue with them or should stop their use once they become infected with the SARS-CoV-2.

The answer to the question appears to be the presumed risk of worsening the disease condition and enhancing mortality. The argument runs on the following lines.

The ACE2 molecule constitutes the gateway for the host cell entry of coronavirus. Ang II inhibitors could arguably promote virus entry through overexpression (upregulation) of the ACE2 and increase the viral load in tissues. The enhanced viral load could increase the severity of the disease and mortality. This is more so that many patients with comorbid conditions would be on therapy with Ang II inhibitors. Generally, these assumptions are sufficient to raise serious apprehension about their use in patients infected with COVID-19. However, a detailed analysis of available facts disputes such an argument.

Angiotensin-converting Enzyme-2 Receptor (ACE2-R) Overexpression (Upregulation)

Overexpression of receptors as such is a well-established state of an enhanced number of the receptors and is intended to maintain routine tissue function in the face of an adverse situation. This usually occurs in the absence of an endogenous ligand or with the use of a receptor antagonist.

The points to be considered in this regard are:

- Does ACE2 overexpression occur with the use of Ang II inhibitors?
- Could this phenomenon enhance virus entry?

Angiotensin-converting Enzyme-2 (ACE2) Overexpression and ARB/ACE Use

The idea of upregulation of ACE2 arose from a few studies in mouse/rat[40–42] and had caught undue attention from scientists and clinicians. However, neither is this concept logical nor is it supported by concrete evidence. It remains mere speculation. Many scientists concur that "ACE2 upregulation is a possibility, but not the real fact," evading a clear-cut position. According to Danser et al.,[43] current data are not sufficient to conclude that RAAS inhibitors facilitate virus entry into cells by ACE2 overexpression. Whereas Perrotta et al.[44] consider as highly hypothetical the issue of increased receptor availability in the lungs from exposure to RAAS inhibitors.

A Critical Analysis of Available Data on ACE2 Expression

A point that is beyond dispute is that the binding of the virus molecule leads to a decrease in the receptor number downregulation. The receptor molecule gets lost from the cell surface due to internalization and lysosomal degradation[13,14] (see viral entry). The fear of enhanced ACE2 expression by Ang II inhibitors is thus unconvincing.

Studies on Humans

The study by Milne et al.[45] involved the estimation of levels of both ACE2 and ACE genes from lung tissue. More than 1,000 samples were obtained from the Human Lung Tissue Expression Quantitative Trait Loci Study. Whereas ACEI use decreased the expression of both ACE2 and ACE genes, ARBs were devoid of any effect on the expression of any of them. Increased risk of COVID-19, if any, is thus not related to upregulation of ACE2. This study is exceptional in that it recorded ACE2 levels in tissues (lungs). A few other studies[46–53] performed on patients with comorbidities such as CV conditions, CKD, or diabetes mellitus monitored soluble ACE2 levels in plasma or urine samples. Here again, there is no indication of any overexpression of ACE2 protein by ARBs or ACEIs. It is to be noted, however, that levels of soluble ACE2 are not true markers of RAAS activity.

Studies on Animals

Data are confusing, even though a majority of studies in animals rule out overexpression of ACE2 following administration of ARBs. Four studies[20,54–56] relate to ACE2 expression in lung tissue in response to different stimuli, including SARS-CoV Spike protein, whereas a few other studies involved cardiac and renal tissues.[57–61] In these studies on animals, basal levels of ACE2 were lower than normal due to exposure to the applied stimuli or others, and these levels had been restored to normal by ACEIs/ARBs. This obviously could mean only an apparent overexpression since ACE2 levels in control animals remained largely unchanged. Of course, as mentioned earlier, three studies by Ferrario et al.,[40] Ishiyama et al.,[41] and Karram et al.[42] did demonstrate upregulation with the use of ACEIs/ARBs, which had triggered confusion and apprehension concerning their safety.

Data from animal studies may not corroborate fully with the effects of Ang II inhibitors in humans. All the studies on animals mentioned above were conducted in rodents (rats/mice). Significant variations in ACE2 molecules have been reported among human and animal species and in animals themselves. ACE2 of human/rhesus monkeys exhibited the highest, while that of the rat/mouse showed the lowest receptor activity.[62]

Overexpression and Enhanced Virus Entry

Even if increased expression of ACE2 does occur, this need not enhance virus entry into the host cell cytoplasm. Cellular access requires additional downstream steps, such as virus S-protein priming, internalization, etc., that are unlikely to be affected by ARBs or ACEIs.

Conclusion

Therapeutic options are very limited in patients infected with COVID-19. Currently, antivirals such as remdesivir, systemic dexamethasone, and therapeutic antibodies, including tocilizumab and bebtelovimab are the drugs approved for the treatment of COVID-19-infected persons.

Remdesivir acts by interfering with viral replication inside the host cell. WHO recommends the use of remdesivir in patients in the early stage of the disease and who require minimal supplemental oxygen; the organization advises against initiating monotherapy with the drug in those who require mechanical ventilation. In fact, evidence for its effectiveness in COVID-19 is still unconvincing. Corticosteroids, as well as antibodies, act by suppressing the inflammatory damage to the lung tissue. Dexamethasone is recommended specifically for use in hospitalized persons on mechanical ventilation. Tocilizumab is an add-on agent in patients not responding satisfactorily to dexamethasone.

The action of ARBs resembles that of steroids and tocilizumab in blocking the proinflammatory and immune-stimulant effects of cytokines in coronavirus disease, though the molecular mechanism might be different.

A detailed perusal of the literature and in-depth analysis of studies reveal that Ang II inhibitors, both receptor blockers and ACEIs, constitute useful therapeutic agents in COVID-19. These agents are the most specific for COVID-19 management since they target the Ang II peptide involved in tissue damage. Their effectiveness is well supported by clinical improvement, shortened hospital stay in human studies, and protection effect against lung injury in animals induced by virus spike protein. The safe use of these medicines in COVID-19-infected individuals has been established in many retrospective and epidemiological studies. ARBs are being used on a regular basis in hypertension, heart failure, diabetes mellitus type II, and CKD, and this reflects their excellent record of safety. Since these disease states also constitute important comorbidities associated with COVID-19, ARBs can be continued without hesitation in COVID-19-infected patients. ARBs are easily available, cheap, and orally effective. In contrast, remdesivir and therapeutic antibodies are immensely costly and need to be injected. Thus, there are compelling reasons for advocating routine use of ARBs in all patients who are COVID-19 positive and symptomatic (mild, moderate, or severe).

Angiotensin receptor blockers (ARBs) score over ACEIs as first-line agents considering the minimal frequency of common adverse effects of dry cough, angioedema, and hypotension.

The author contends that Emergency Use Authorization could very well be granted for the use of ARBs in the management of COVID-19, a situation of global emergency.

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SHORT REVIEW

Role of Magnetic Resonance Mammography in the Evaluation of Indeterminate Breast Lesions

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Abstract

Background: Malignancy of the breast is one of the most common cancers among females worldwide. Magnetic resonance mammography (MRM) is a valuable complement to conventional methods for the early diagnosis of disease, thereby providing patients with a better prognosis. The number of unnecessary biopsies and repeated excisions in cases of indeterminate breast lesions detected on conventional imaging is high.

Aims: The purpose of this study was to evaluate the role of MRM in the evaluation of indeterminate breast lesions [Breast Imaging Reporting and Data System (BIRADS) 3/4] found in conventional mammography and ultrasonography (USG), taking the histopathological examination (HPE) as the gold standard.

Materials and methods: A total of 38 patients with conventional radiological imaging diagnosis of indeterminate breast lesions (BIRADS 3/4) were included in this study and evaluated using contrast-enhanced MRM according to the MR-BIRADS lexicon (5th edition). Morphological characteristics of lesions were evaluated to determine the probability of malignancy. Histopathology was kept as the gold standard for comparing all the statistical parameters.

Results: There were a total of 40 lesions, 35 masses, and five nonmass enhancement (NME) available for evaluation out of the 38 patients. The sensitivity of margins to detect malignancy approached 100%; however, it had a slightly lower specificity of 66.67%. Magnetic resonance imaging (MRI) showed good diagnostic performance with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of 85, 90, 89.47, 85.71, and 87.50%, respectively.

Conclusion: The MRI has been shown to be useful as a problem-solving tool in breast cancer screening, clarifying indeterminate findings and avoiding unnecessary short follow-ups and percutaneous biopsies.

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Introduction

Malignancy of the breast is one of the most common cancers among females worldwide. As per the Globocan data 2020, in India, breast malignancies accounted for 13.5% (178,361) of all cancer cases and 10.6% (90,408) of all deaths with a cumulative risk of 2.81.[1] The global burden is projected to cross 2 million by the year 2030.[2]

Early detection and adequate characterization of the lesion are important for planning future management.[3] Therefore, an investigation that is both sensitive and specific is required in such cases.

Radiological investigations such as conventional mammography, ultrasonography (USG), and magnetic resonance imaging (MRI) are used to diagnose and characterize various breast lesions. The latest updated version of the Breast Imaging Reporting and Data System (BIRADS) is 5th edition (2013).[4]

Conventional mammography and B-mode USG are the established gold standards for breast imaging; however, significant false positives and negatives still occur. False positive results evoke invasive diagnostic procedures, which are otherwise avoidable in these patients.[5]

The MRM has the capability of providing three-dimensional spatial information and better visual differentiation of breast lesions from normal breast tissue based on differences in vascularity and permeability of the lesions.[6] Irregular shape, noncircumscribed margins, diffusion restriction, and heterogeneous/rim enhancement with washout kinetic curve are suggestive of malignant neoplastic etiology.

Evidence in the literature shows that using cutoff apparent diffusion coefficient (ADC) ranges of $1.3–1.5 \times 10^{-3}$ mm^2/second for benign lesions and $0.85–1.1 \times 10^{-3}$ mm^2/second for malignant lesions allows differentiation of benign from malignant lesions with high sensitivity and specificity.[7–9]

As stated in the BIRADS lexicon, lesions scoring BIRADS 4A/B/C (suspicion for malignancy 2–9, 10–49, and 50–94%, respectively) are advised histopathological confirmation to know the malignant potential of the lesion.[4] However, the rate of negative biopsy is high.

Keeping in view the lack of sufficient data available for the North Indian population, this study was conducted with the main objective of evaluating the role of MRM in adequately characterizing breast masses that are found suspicious on clinical examination, routine mammography, and ultrasonography (i.e., BIRADS 3/4 lesions) taking histopathology as a gold standard to reduce the need of unnecessary biopsies and short-term follow-ups.

Materials and Methods

Patients clinically found to have indeterminate breast lesions were referred from the department of surgery to the department of radiodiagnosis for further evaluation. The relevant patient history was taken by the principal investigator, and a physical examination of the patient was carried out by the surgeon. These patients underwent conventional mammography and USG. The lesion was characterized by the BIRADS lexicon. Patients with indeterminate/suspicious (BIRADS 3/4) breast lesions detected on conventional mammography and USG, who met the criteria of inclusion and did not have any exclusion criteria, were enrolled for this study post taking written informed consent.

Inclusion Criteria

- Patients with clinically suspicious/indeterminate palpable lump in the breast, which is BIRADS 3 on conventional mammography and USG.
- Patients with clinically suspicious/indeterminate palpable lump in the breast, which is BIRADS 4 on conventional mammography and USG.
- Patients with suspicious (BIRADS 3) breast masses were seen incidentally on screening mammography and USG.

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- Patients with suspicious (BIRADS 4) breast masses were seen incidentally on screening mammography and USG.
- Patients who presented with other complaints like nipple discharge, mastalgia, or redness showed suspicious (BIRADS 3/4) lesions on conventional mammography and USG.

Exclusion Criteria

The following categories of patients were excluded from the study:

- Operative history of the breast in the past.
- History of radiotherapy (RT) or chemotherapy to the breast in the past.
- Breast lesions that were definitely benign, whether clinically or on conventional imaging, like simple cysts on mammography and sonomammography.
- Biopsy-proven cases of malignancy.
- Deranged renal function tests.
- Patients with claustrophobia
- Any known contraindication to MRI, namely cochlear implants and pacemakers, metallic implants, dental fillings, and clips in the body noncompatible with MRI.
- Patients who did not give consent to participate in the study.

All these patients were asked to get their renal function tests, and those who had normal renal function tests underwent contrast-enhanced MRM during the mid-menstrual cycle, that is, week 2 (7–14 days) in premenopausal women and at any time in the postmenopausal women.

For acquiring these MRI sequences, the patient was placed in the prone position with the breasts hanging in a double breast coil at the isocentre of the magnet.

The following MRM sequences were acquired on the Phillips ACHIEVA 1.5 Tesla MRI scanner.

- Short tau inversion recovery (STIR) axial.
- T2 weighted image (T2W)-turbo-spin-echo (TSE) axial.
- T1W-TSE axial.
- T1W-TSE coronal.
- T2W-TSE coronal.
- Diffusion-weighted imaging (DWI)/ADC axial.
- T2W-TSE RT saggital.
- T2W-TSE LT saggital.
- Dynamic-enhanced T1 high-resolution isotropic volume excitation (dyn-eTHRIVE).
- T1W-TSE-PGAD coronal.

Dynamic contrast-enhanced images were obtained after administration of 0.1 mmoL/kg body weight of gadobenate dimeglumine contrast injected intravenously with the help of an automatic injector at the rate of 2 mL/second. This sequence was repeated sequentially before and five times after a bolus of contrast over a period of 5 minutes so that the rate and duration of enhancement could be assessed.

Image Analysis

On basic MRI sequences, the breast lesions were analyzed and characterized with emphasis on the number, size, and morphology of the lesion. On DWI, diffusion restriction, if any, with ADC mapping was recorded. On dynamic contrast-enhanced T1 weighted sequences, region of interest (ROI) was drawn in the most rapidly enhancing part(s) of the lesion, and time-signal intensity curve(s) (TIC) were obtained. Background parenchymal enhancement was categorized as symmetrical or asymmetrical and was also classified as minimal, mild, moderate, or marked.

The shape of the lesion was classified as oval, round, or irregular.

The margins of the lesions were classified as circumscribed or noncircumscribed. Circumscribed margins were taken as smooth, and noncircumscribed were further categorized as irregular or spiculated margins.

Internal enhancement characteristics of the lesions included homogeneous, heterogeneous rim enhancement or dark internal septations.

We evaluated the TICs according to the BIRADS 5th edition guidelines; that is, the initial enhancement phase and the delayed enhancement phase are represented by the three primary shapes of TICs. The initial-phase enhancement pattern depicts enhancement within the first 2 minutes after injection or until peak enhancement is reached. The delayed-phase enhancement pattern occurs after 2 minutes or after the peak enhancement is attained and is typically used to characterize the curve shape. In general, for the delayed phase, persistent is often 10% more than the original enhancement, plateau is equal to the initial enhancement (within 10%), and washout is typically 10% less than the initial enhancement. Based on the time signal intensity, the TIC curves are classified as an initial and delayed phase.

The initial phase is further subclassified into the following:

- Slow (intensity increase <50%).
- Medium (intensity increase 50–100%).
- Fast (intensity increase >100%).

The delayed phase is further subclassified into the following:

- Type I/persistent (sustained enhancement).
- Type II/plateau (stable enhancement).
- Type III/washout (rapid initial enhancement and decreasing late enhancement).

Nonmass enhancement (NME) is defined as those that are neither a focus nor a mass. Depending on the way the enhancement is distributed, NME is categorized as a focal area, linear, segmental, regional, multiple regions, or diffuse. The internal enhancement of NME can be further categorized as homogenous, heterogeneous, clumped, or clustered.

Associated features like nipple retraction or inversion, skin thickening and invasion, axillary lymphadenopathy, pectoralis muscle involvement, chest wall invasion, or any other associated architectural distortion were also recorded.

Keeping in view all the above parameters, the lesions were categorized as benign or malignant and were assigned the BIRADS category. Final BIRADS was assigned to the breast as per the highest category BIRADS lesion present in one or both breasts.

- BIRADS 1—negative.
- BIRADS 2—benign.
- BIRADS 3—probably benign.
- BIRADS 4—suspicious.
- BIRADS 5—highly suggestive of malignancy.
- BIRADS 6—known biopsy-proven malignancy.

The final diagnosis of the lesions was determined by subsequent histopathological examination (HPE) (fine needle aspiration cytology/biopsy/mastectomy specimen).

In the end, the MRI findings were correlated with the HPE and the results were statistically analyzed.

Statistical Analysis

Imaging parameters concerning MRM findings for different cases were described by using frequency distribution for qualitative data and by using mean along with standard deviation (SD) for qualitative parameters. Diagnostic values of MRI were evaluated, taking histopathology as the gold standard. These values were expressed in terms of sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy. The Chi-squared test was used for testing the significance between MRM findings and histopathology findings, and the κ coefficient of agreement was calculated. Data analysis was carried out by using International Business Machines Corporation Statistical Package for the Social Sciences (25.0 version) software.

Results

A total of 38 patients referred from the department of surgery with clinical or conventional radiological imaging diagnoses of indeterminate breast lesions (BIRADS 3/4) were included in this study. A total of 40 lesions, 35 masses, and five NMEs were evaluated using contrast-enhanced MRM according to the MR BIRADS lexicon (5th edition).

Morphology

Lesion Size

Lesions were measured in three dimensions and the maximum dimension was considered for comparison analysis. The mean size of benign lesions was 24.20 mm (±25.899) mean (± SD) and of malignant lesions was 35.05 mm (±33.622) mean (SD). There was no statistically significant difference between the mean size of benign and malignant lesions (*p*-value of 0.307).

Margins

The margins of the lesions were described as circumscribed or noncircumscribed margins. Maximum lesions were noncircumscribed (*n* = 25, 71.42%) and the rest (*n* = 10, 28.57%) were circumscribed.

This study showed a strong positive correlation (Pearson's product-moment correlation coefficient, *r* = 0.730) between malignancy and noncircumscribed (irregular and spiculated) margins. On the contrary, benign lesions were more likely to have smooth edges. These conclusions were highly statistically significant (*p*-value of <0.001).

The sensitivity of margins to detect malignancy approached 100%; however, it had a slightly lower specificity of 66.67%. The positive predictive value, negative predictive value, and accuracy came out to be 75.00, 100, and 83.33%, respectively.

Shape of Lesion

The shape of the margins has a higher specificity for predicting the benign nature of the disease (85%) but lower sensitivity (60%). The positive and negative predictive values were 80.00 and 68.00%, respectively. Accuracy came out to be 72.50% (*p*-value of 0.006).

Diffusion Restriction

In our study, 29 lesions showed diffusion restriction, out of which 18 came out malignant (*n* = 18, 62.06%) and 11 were benign (*n* = 11, 37.93%) on pathological results (Table 1).

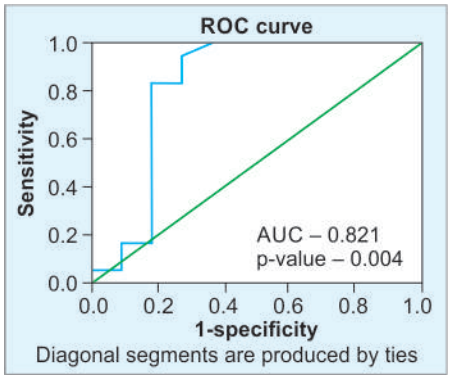


Fig. 1: ROC curve for ADC values in benign and malignant HPE groups

Table 1: Frequency distribution of mean ADC values of diffusion restriction in benign and malignant lesions

Pathological diagnosis	*Frequency*	*Mean*	*SD*	*Median*
Benign	11	1.27	0.44	1.32
Malignant	18	0.79	0.20	0.80
Total	29	0.97	0.39	0.85

Table 2: Distribution of enhancement pattern of the lesion according to HPE

Enhancement	*Benign*	*Malignant*	*Total*
Homogeneous	5	7	12
Heterogeneous	13	11	24
Rim	2	2	4
Total	20	20	40

Table 3: Distribution of early enhancement curve of lesions according to HPE

Enhancement	*Benign*	*Malignant*	*Total*
Fast	3	7	10
Medium	6	9	15
Slow	11	4	15
Total	20	20	40

Receiver operating characteristic (ROC) analysis curves were plotted to find out the maximum cutoff values of DWI between benign and malignant HPE groups (Fig. 1).

The area under the ROC curve was 0.821. At the cutoff value of 0.94, the sensitivity and specificity for predicting the presence of malignant lesions on HPE were 83.33 and 72.73%, respectively. A patient having an ADC value lower than the aforementioned cutoff value had a high probability of having a malignant lesion on HPE.

Enhancement Characteristics

Internal Enhancement Pattern

The enhancement pattern of the lesion was described as one of the following categories—homogeneous, heterogeneous, or rim enhancement. The results of our study showed that heterogeneous enhancement was the most prevalent pattern (*n* = 24, 60%), followed by homogeneous internal enhancement (*n* = 12, 30%), and rim enhancement (*n* = 4,10%).

The distribution of the enhancement pattern of the lesion according to HPE is given in Table 2.

Kinetic Curves

Early Enhancement Curve

A fast curve was seen in 10 lesions (*n* = 10, 25%), out of which 30% (*n* = 3 out of 10) of these cases were benign. A medium curve was seen in 15 lesions (*n* = 15,37.5%), out of which 9 out of the 15 lesions (60%) were cancerous. Around 11 out of 15 lesions (73.33%) with a slow curve (*n* = 15,37.5%) came out to be benign. Early enhancement curves were not statistically significant (*p*-value of 0.065) in predicting the nature of the lesion.

The distribution of the early enhancement curve of lesions according to HPE is given in Table 3.

Late Enhancement Curve

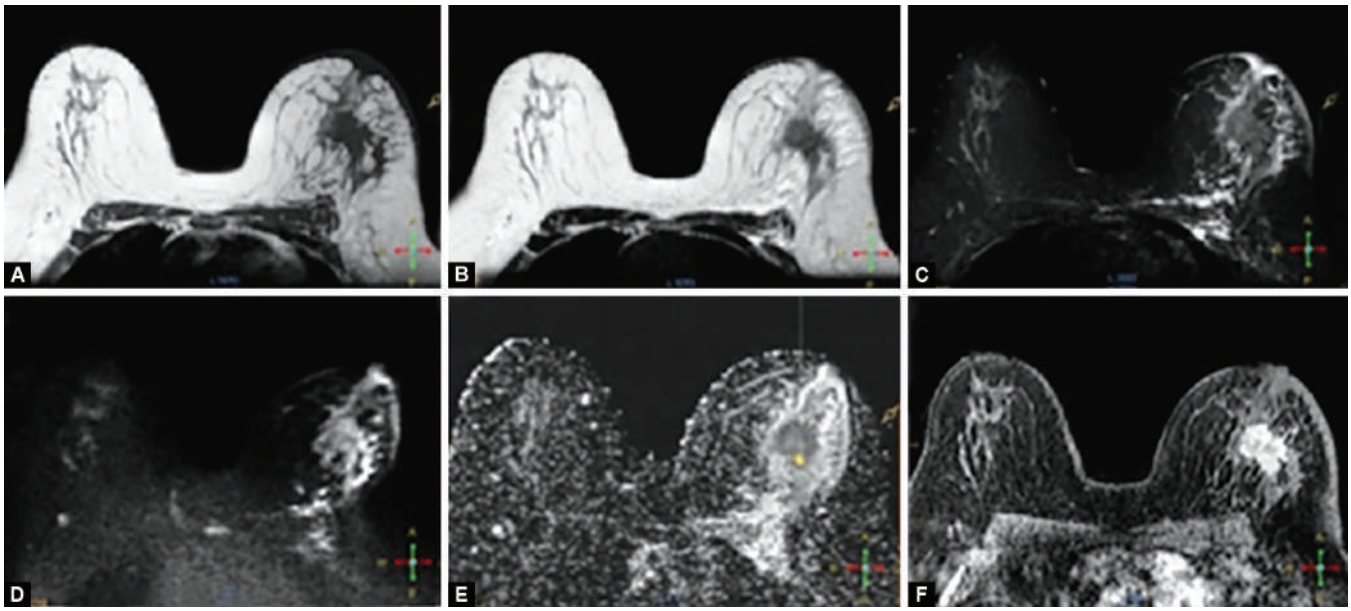
The late enhancement curve is subclassified as persistent, plateau, and washout kinetics. The most common late enhancement kinetic curve was plateau (*n* = 18,45%), followed by persistent curve (*n* = 11,27.5%), and washout kinetic curve (*n* = 11,27.5%).

Kinetic curves for each lesion were defined after choosing the appropriate site for the ROI. A type one/persistent curve was observed in 11 cases, all of which were benign (*n* = 11,100%). 18 lesions with a type two/plateau curve had 12 malignant lesions (*n* = 12 out of 18, 66.67%). Eight of the 11 lesions showed the type three/washout kinetic curve (*n* = 11), out of which 72.72% of them were cancerous.

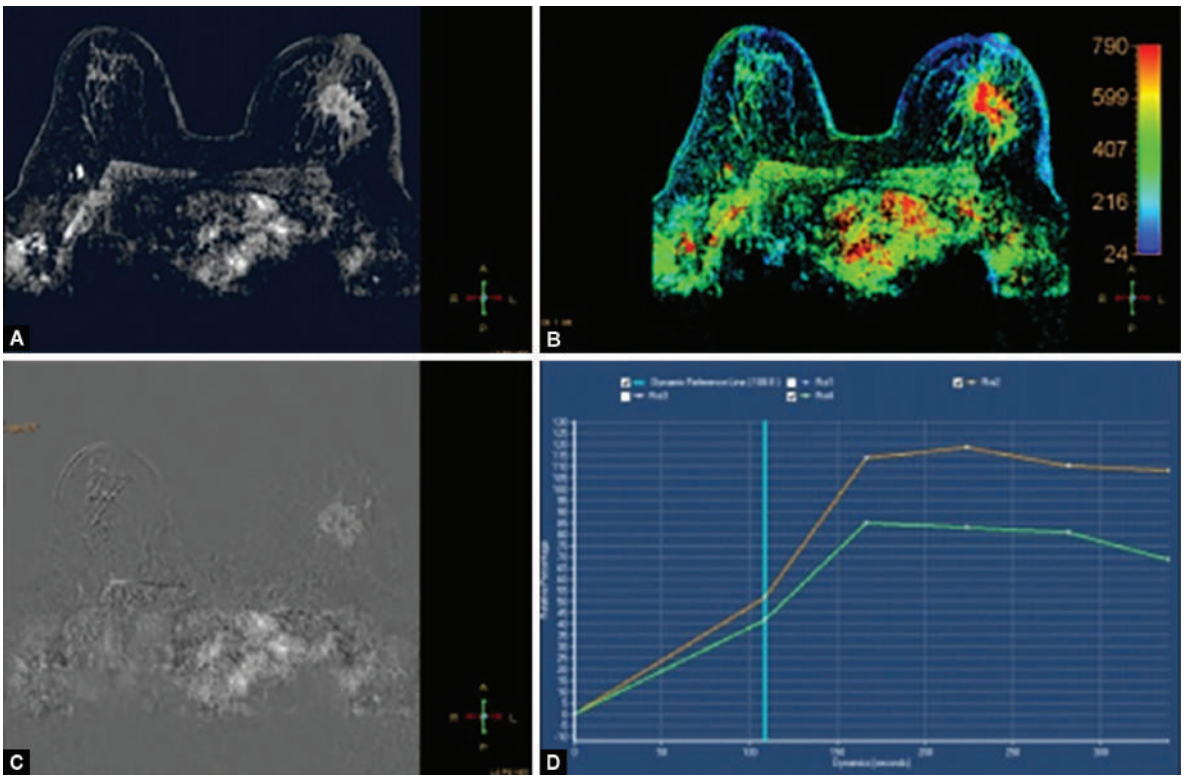
The sensitivity, specificity, positive predictive value, and negative predictive value of MRM in differentiating benign from malignant lesions based on kinetic curves of the lesion came out to be 100, 55, 68.97, and 100%, respectively. These findings were highly statistically significant, with a *p*-value of <0.001.

Based on the persistent kinetic curves of the lesion, the sensitivity, specificity, positive predictive value, and negative predictive value of MRM in distinguishing benign from malignant lesions were found to be 55, 100, 100, and 68.97%, respectively. With a *p*-value of 0.001, these results were highly statistically significant.

In terms of sensitivity, specificity, positive predictive value, and negative predictive value for the identification of malignant lesions, the plateau kinetic curve of a lesion performs better than all other shapes, measuring 60, 77.78, 75, and 63.64%, respectively. Because of this, the plateau kinetics of a lesion is a valuable diagnostic of malignancy with a



Figs 2A to F: Case 1: Irregular lesion appearing. (A) Hypointense on T1WI; (B and C) Hypointense on T2WI and STIR images; (D and E) Showing diffusion restriction with; (F) Heterogeneous enhancement on postcontrast images



Figs 3A to D: Dynamic evaluation shows fast (early phase) and washout (late phase) types of kinetic curves

high level of specificity and accuracy (68.42%) *p*-value of 0.05.

For the purpose of diagnosing malignant lesions, the washout kinetics of a lesion is important and has shown diagnostic performance in terms of sensitivity, specificity, positive predictive value, and negative predictive value, measuring 40, 85, 72.73, and 58.62%, respectively.

Magnetic Resonance (MR) BIRADS

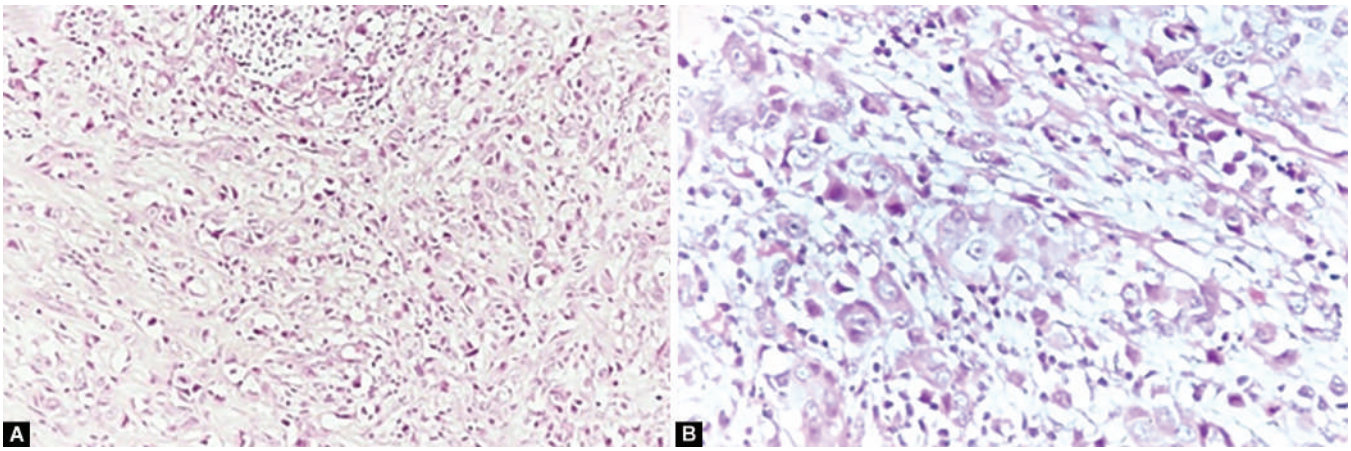
Most of the lesions in our study were upgraded to BIRADS 5 (*n* = 19). The majority of the BIRADS 5 lesions (*n* = 19) were found to be malignant (*n* = 17 out of 19, 89.47%). Lesions categorized as BIRADS 3 (*n* = 8); all came out to be benign (*n* = 8, 100%). Around 13 lesions were categorized as BIRADS 4. A total of 10 lesions (*n* = 10 out of 13, 76.92%) turned out benign and 3 lesions (*n* = 3 out of 13,23.07%) came out to be malignant on pathological diagnosis.

The MR BIRADS lexicon is highly significant in predicting the nature of the breast lesions and thereby characterizing the lesion further (*p*-value <0.001). The sensitivity and specificity, positive predictive value (PPV), and negative predictive value (NPV) of MRM were 85, 90, 89.47, and 85.71%, respectively. Accuracy came out to be 87.50%.

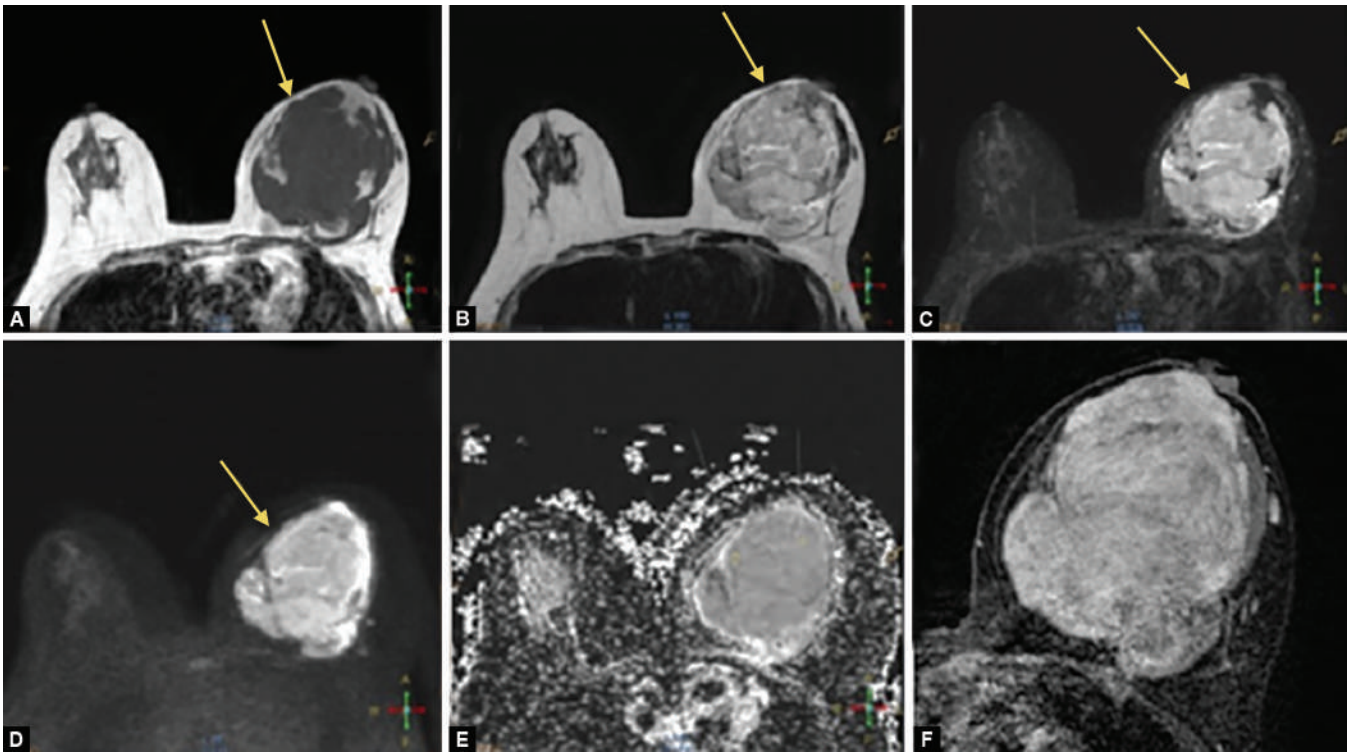
Discussion

Breast cancer and its exponential rise in incidence remains one of the major causes of malignancy-related deaths among women. Epidemiological studies have predicted that the worldwide burden of malignancies related to the breast is expected to cross almost 2 million by the year 2030.[10]

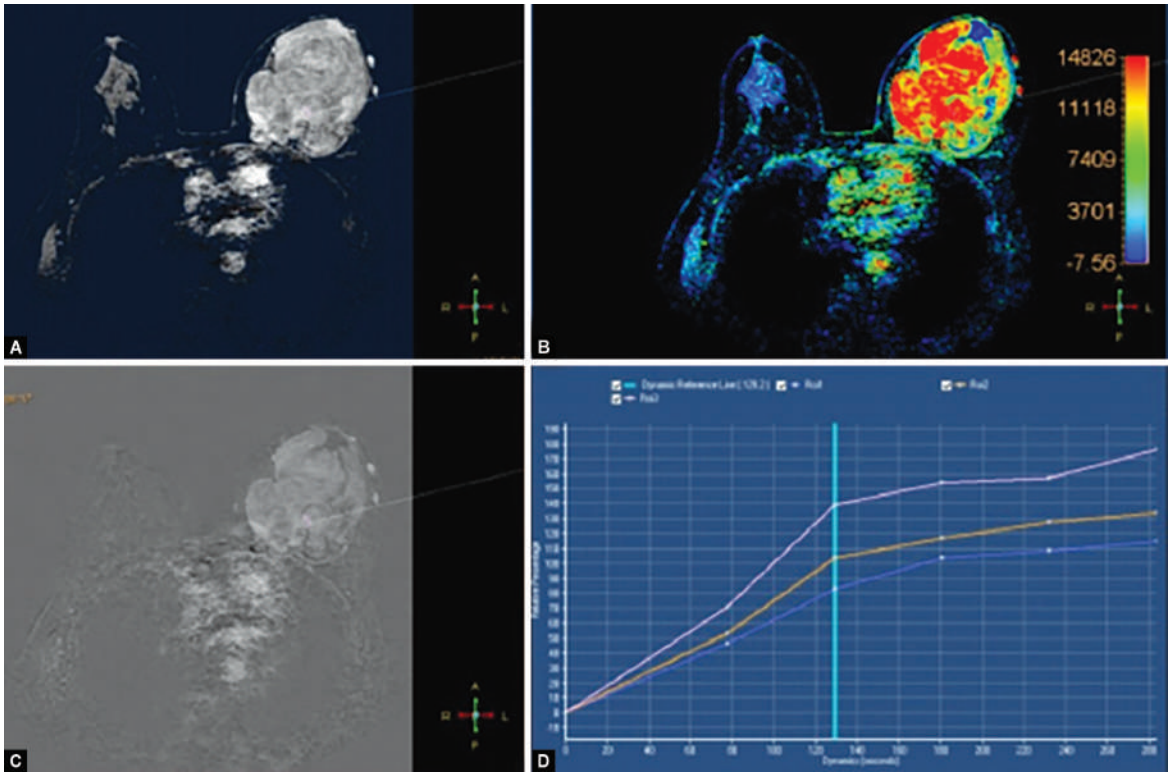
According to recent trends, Indian women experience the disease more frequently and at a younger age than Western women.[11] Imaging is crucial for a precise breast diagnosis and the early identification of



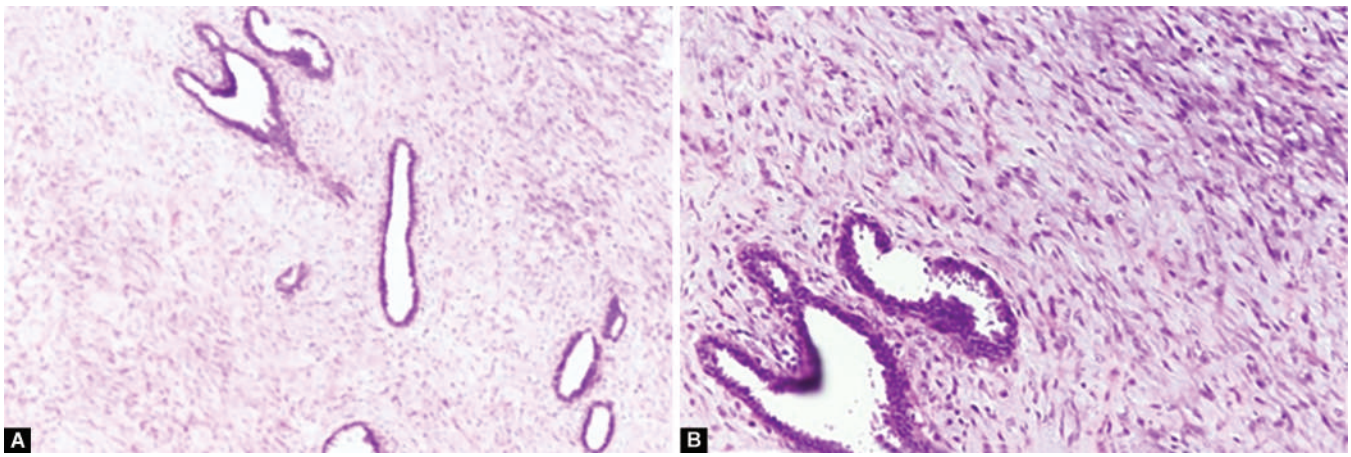
Figs 4A and B: Photomicrograph shows (hematoxylin and eosin) (×200) and (×400) infiltrating ductal carcinoma. Sections show sheets and cords of tumor cells with intervening desmoplastic stroma and moderate lymphocytic infiltrates. Pathological diagnosis—infiltrating ductal carcinoma



Figs 5A to F: Case 2: Large circumscribed lobulated lesion involving almost entire left breast. (A) Hypointense signal on axial T1WI; (B and C) Hyperintense signal on axial T2WI and STIR images with; (D and E) Diffusion restriction; (F) Heterogeneous enhancement on postcontrast images



Figs 6A to D: Dynamic evaluation shows fast (early phase) and persistent (late phase) type of kinetic curves



Figs 7A and B: Photomicrograph (hematoxylin and eosin) (×100) and (×200) benign phyllodes tumor. Sections show tumor cells exhibiting stromal hypercellularity and overgrowth. Pathological diagnosis—phyllodes tumor

breast cancer. In the past, breast imaging has relied heavily on mammography, yet a mammogram that seems normal cannot rule out cancer.[12]

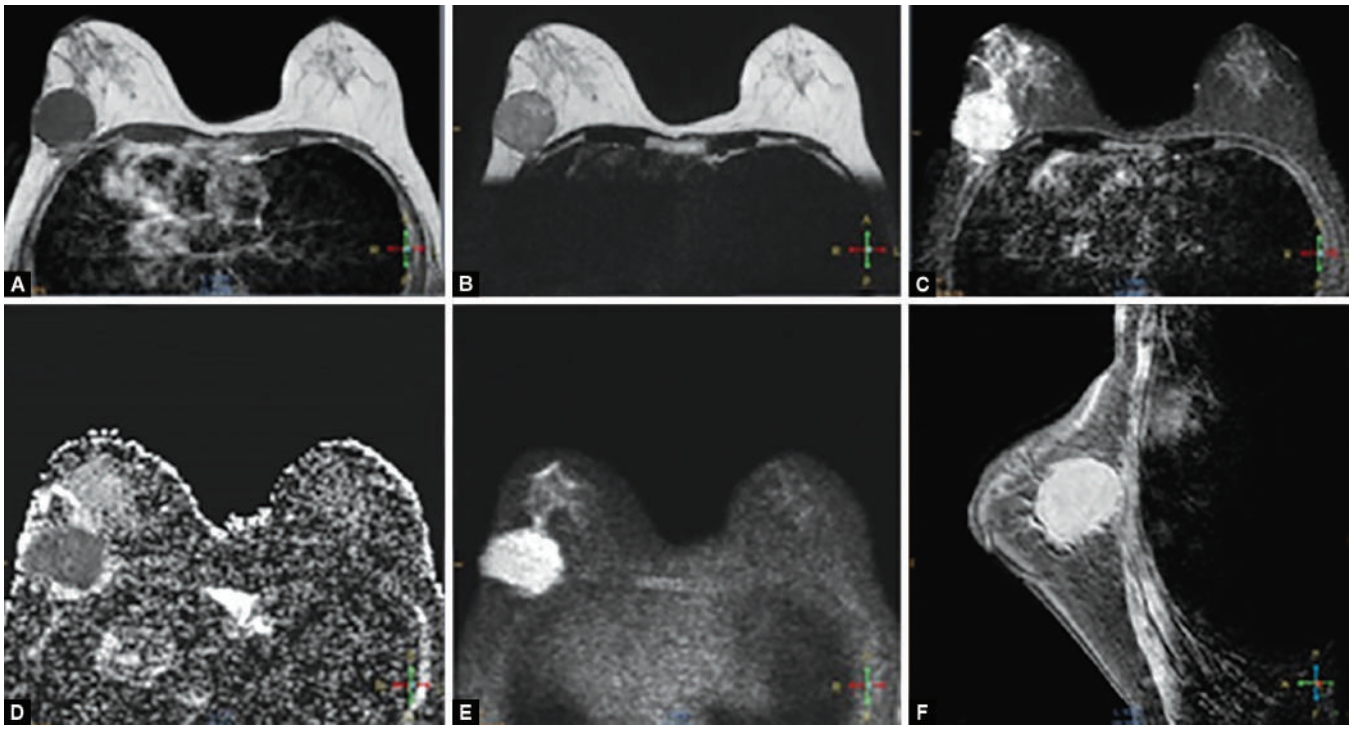
The MRM is quickly moving from the realm of research to the realm of clinical use. Because of its high sensitivity and efficiency in dense breast tissue, MRI can be a valuable addition to the diagnostic workup of a patient with a breast abnormality or biopsy-proven cancer for the detection, characterization, assessment of local extent, evaluation of treatment response, and guidance for biopsy and localization.[13]

The MRM can considerably increase the diagnosis of cancer that is otherwise clinically, mammographically, and sonographically occult, according to clinical trials from the United States and Europe in 2010.[14,15]

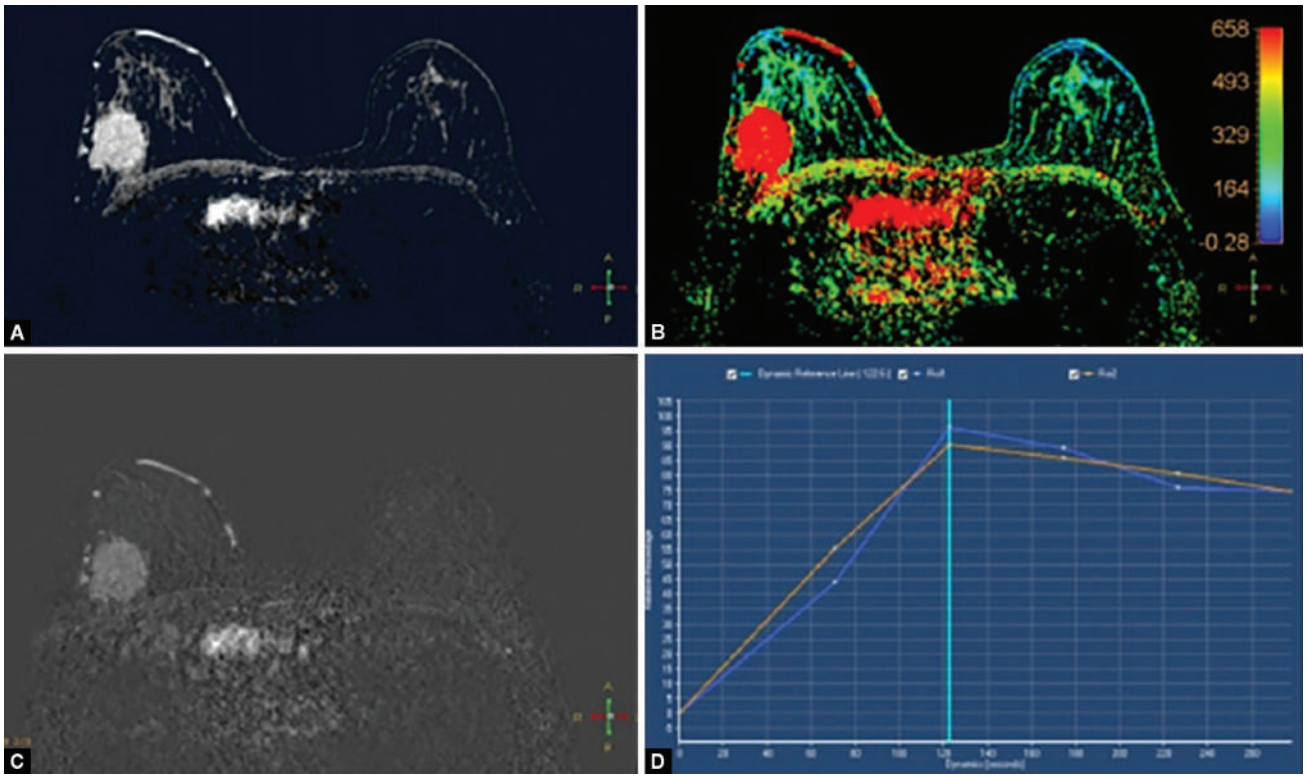
In a total of our 38 patients, there were 35 focal breast lesions and five NME cases.

The age of our 38 female patients ranged between the age group of 18–72 years. The mean age (SD) of the study subjects was 43.9 (+14.180) years. Malignant lesions, like infiltrating carcinoma, had a higher mean age-group (+SD) of 44.95 (+ 13.296) years but were statistically insignificant (*p*-value of 0.831). However, the statistical significance of age and malignancy in our study is not consistent as found in literature in various studies.[16,17]

No statistically significant difference was seen between the mean size of the benign and malignant lesions (*p*-value = 0.307). In literature, various studies have found statistically significant differences between the size of benign and malignant lesions.[18,19] Our results are not consistent with the literature, which may be because of a smaller number of patients in our study. Our cutoff value of 17.5 mm for size was higher in comparison to the studies conducted



Figs 8A to F: Case 3: Round, noncircumscribed lesion in upper outer quadrant of right breast showing (A) hypointense signal on axial T1WI; (B and C) hyperintense on axial T2/STIR images with; (D and E) diffusion restriction; (F) chest wall invasion

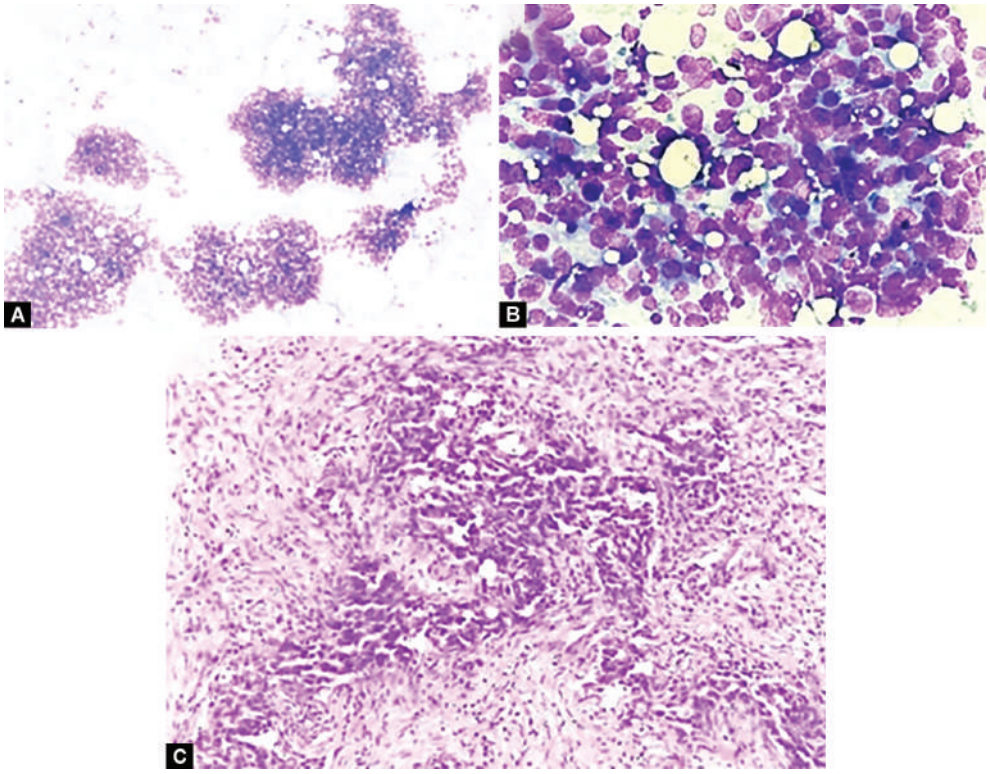


Figs 9A to D: Dynamic evaluation shows washout/type three kinetic curve

by Gutierrez et al. and Kawai et al., who reported the cutoff to be 10 and 12 mm (with a sensitivity of 81.6% and specificity of 50.0%), respectively.[18,19]

Lesions' margins were classified as having circumscribed or noncircumscribed margins. The majority of lesions (n = 25, 71.42%) were noncircumscribed, whereas the remainder (n = 10, 28.57%) were circumscribed. Lesions with circumscribed margins were divided into smooth and noncircumscribed margin categories and were further divided into irregular or spiculated margin categories.

The overall sensitivity of margins on MRM to diagnose malignancy approached 100%; however, had a specificity of 66.67%. The positive predictive value, negative



Figs 10A to C: Photomicrographs showing MGG (×100) and (×200) infiltrating ductal carcinoma. Cytological smears are cellular and show loose clusters of tumor cells. Pathological diagnosis—infiltrating ductal carcinoma

predictive value, and accuracy came out to be 75, 100, and 83.33%, respectively, when considering the margins on MRM. In a study done by Balasubramanian et al., they reported a sensitivity, specificity, positive predictive value, and negative predictive value of MRM in differentiating benign from malignant lesions based on margins of the lesion were 95.45, 84.62, 91.3, 91.67%, respectively.[20] Hence, the sensitivity and NPV were in concordance with the study conducted by Balasubramanian et al.[20]

In our study, smooth margins had a higher specificity for predicting the benign nature of the disease (100%) but lower sensitivity (66.67%). Irregular margins have a higher specificity for predicting malignancy (73.33%) but a lower sensitivity (65%). Spiculated margins showed the highest specificity (93.33%) for predicting malignancy. However, the sensitivity of this finding was low (35%). With the best of literature search and knowledge, no such studies evaluating the individual diagnostic performances of the various types of margins, namely—smooth, irregular, and spiculated, were found.

The overall sensitivity of the shape of the lesion on MRM to diagnose malignancy came out to be 60%; however, it had a specificity of 85%. The findings were similar to a study done by et al.[16]

The mean ADC values in benign and malignant lesions were $1.27 \pm 0.44 \times 10^{-3}$ mm^2/second and $0.79 \pm 0.20 \times 10^{-3}$ mm^2/second, respectively. Our findings are in concordance with studies done by Park et al. and Palle and Reddy.[9,21] Park et al. reported a mean ADC value of invasive ductal carcinoma as $0.89 \pm 0.18 \times 10^{-3}$ mm^2/second.[21]

In our study, the cutoff value of ADC for predicting the presence of malignancy was found to be 0.94×10^{-3} mm^2/second with a sensitivity and specificity of 83.33 and 72.73%, respectively. Our ADC value cutoff is lower but with comparable sensitivity and higher specificity than the cutoff proposed by Kawai et al.[19]

The internal enhancement pattern of lesions, however, had no statistical significance in predicting the nature of lesions in our study. Gul et al. reported that heterogeneous rim and central enhancement patterns in a breast lesion favored malignancy, while homogeneous internal enhancement was mostly a feature of benign breast masses (p-value < 0.001).[16] Our results are not consistent with the literature, which may be because of a smaller number of patients in our study.

Overall, the enhancement kinetic curves showed a statistically significant relationship with the final pathological diagnosis. Findings in our study correspond but with higher sensitivity and NPV compared to the research done by Balasubramanian et al. However, specificity in our research was lower.[20]

All the lesions were scored according to the MR BIRADS lexicon. MR BIRADS lexicon has shown high significance in predicting the nature of the breast lesions in our study (p-value < 0.001). The overall sensitivity and specificity, PPV, and NPV of MRM were 90, 75, 78.26, and 88.24%, respectively, for MRM diagnosing the breast lesions in the present study. The accuracy of MRM came out to be 82.50% when correlated with HPE. We have found a better NPV and similar sensitivity and specificity than reported by Fatima et al. in which they have reported sensitivity, specificity, diagnostic accuracy, PPV, and NPV as 93.9, 73.5, 89.3, 92.3, and 78.1%, respectively, of MRM in diagnosing malignant breast lesions taking histopathology as the gold standard.[22] Our study has also found a better sensitivity and diagnostic accuracy of MRM than in one of the most recent studies conducted by Sedguli et al., in which they reported CE-MRM sensitivity to be 71.7%, specificity—96.6% and diagnostic accuracy 83.7%.[23]

A few MRM cases with associated histopathological findings have been demonstrated below:

- Case 1–(Figs 2 to 4)
- Case 2–(Figs 5 to 7)
- Case 3–(Figs 8 to 10)

Conclusion

This study provides the necessary evidence of the usefulness of an integrated approach for adding MRM and contrast-enhanced MRM to supplement the existing conventional regime using mammography and USG in an effort to reduce the number of unnecessary biopsies and repeated excisions for the benefit of the patients by helping in better preoperative staging and management planning. The various parameters in MRM provide the clinicians with ample information so as to decide on further management.

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Constrictive Pericarditis: An Incidental Finding

Chandrakala[1], Himayatullah Khan[2], Nikhil Purushotham[3*]

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A 41-year-old male presented to medicine outpatient department for physical fitness to undergo hernioplasty. He gave history of breathlessness on exertion since 7 days without any cough, chest pain, or any other symptoms related to cardiovascular or respiratory system. His past, family, and personal history was not significant.

General examination revealed a low-volume pulse with an irregularly irregular rhythm. Systemic examination revealed decreased breath sounds over left mammary region.

Chest X-ray showed calcified pericardium, homogeneous airspace opacities in left lower zone, and bilateral small pleural effusion (Fig. 1). HRCT scan of thorax showed sequelae of old tuberculosis in both lungs, mild cardiomegaly with right and left atrium hypertrophy, and imaging evidence of constrictive pericarditis (Fig. 2). Two-dimensional (2D) echo showed pericardial thickening with abrupt anterior motion following atrial contraction with inspiratory septal shift (Fig. 3). Electrocardiogram (ECG) showed atrial fibrillation with rapid ventricular response with rightward axis and T-wave abnormalities (Fig. 4).

Constrictive pericarditis is the end stage of an inflammatory process involving the pericardium. Virtually any inflammatory process can cause constriction. In developed world, the cause is most commonly idiopathic, postsurgical, or radiation injury. Tuberculosis is the most common cause of constrictive pericarditis in developing countries.[1] Although constriction can follow an initial insult by as little as several months, it usually takes years to develop. The end result is dense fibrosis, often calcification, and adhesions of parietal and visceral pericardium.

The clinical presentation is usually dominated by signs and symptoms of right heart failure.[2] Cross-sectional imaging of computed tomography (CT) scan allows precise identification of pericardial thickening. Definitive diagnosis requires 2D echo and CT imaging.[3] Management mainly involves treating the cause, salt restriction, and diuretics with surgical pericardiectomy being the treatment of choice.[1]

In our case, the patient had no symptoms for a longer time and ultimately presented

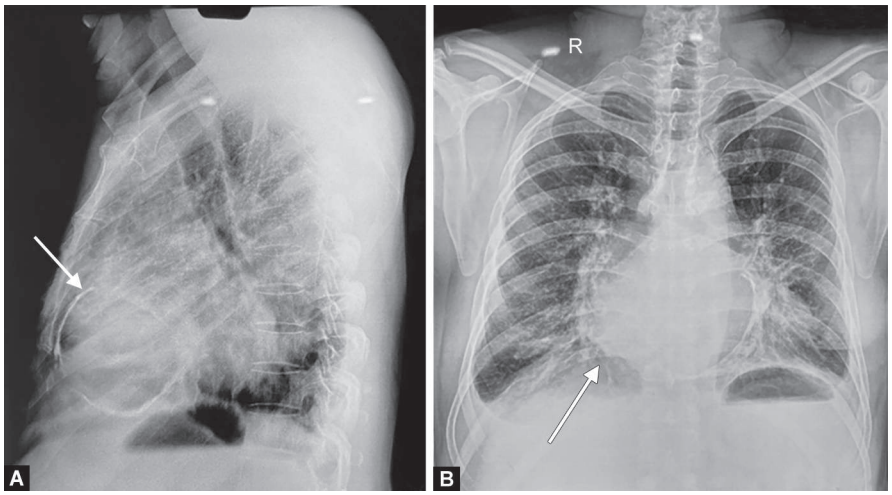

Fig. 1: Chest X-ray showing calcified pericardium, homogenous airspace opacities in left lower zone and bilateral small pleural effusion

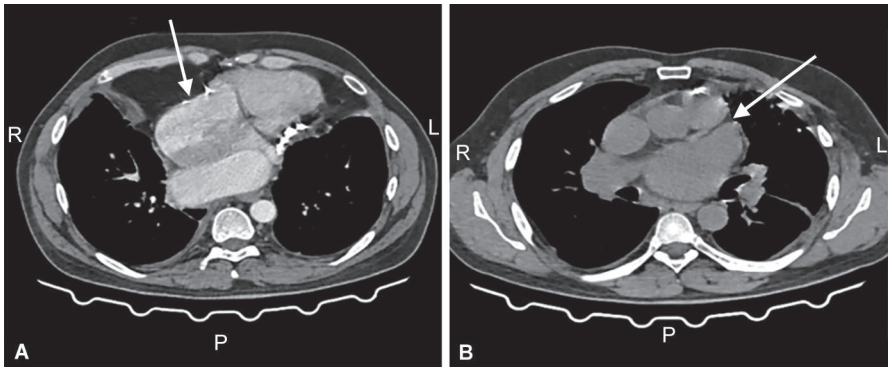

Fig. 2: Computed tomography (CT) scans of thorax showing a sequelae of old tuberculosis in both lungs, mild cardiomegaly with right and left atrium hypertrophy and imaging evidence of constrictive pericarditis

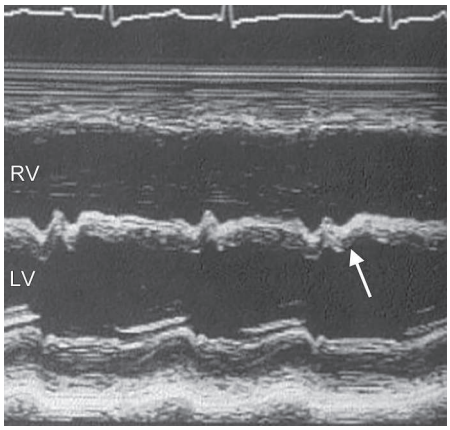

Fig. 3: Two-dimensional (2D) echo showing pericardial thickening with abrupt anterior motion following atrial contraction with inspiratory septal shift

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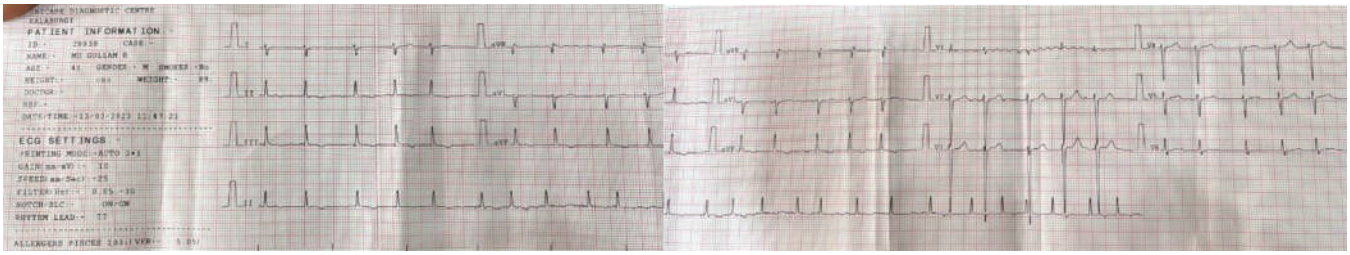

Fig. 4: Electrocardiogram (ECG) showing atrial fibrillation with rapid ventricular response with rightward axis and T-wave abnormalities

with breathlessness of grade-II New York Heart Association without any history of tuberculosis. The patient was referred to higher center for surgical intervention.

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Spontaneous Asymptomatic Chemoport Fracture with Cardiac Migration

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Central venous access devices are usually used nowadays in oncology for administering chemotherapy, of which implantable chemoports are the most common. Spontaneous breakage and migration of the catheters is a very rare complication. Herein, we report a case of spontaneous breakage and cardiac migration of a long tubular part in which the patient was entirely asymptomatic. The patient was successfully managed by an interventional cardiologist. A 56-year-old female patient was diagnosed with acute lymphoblastic leukemia (Philadelphia chromosome detected) on 7th February 2022. She was planned for chemotherapy as per the modified Berlin-Frankfurt-Munich (BFM) protocol. A vascular access chemoport catheter was surgically inserted *via* the right internal jugular vein (IJV) for the administration of chemotherapeutic drugs (BardPort® implantable port with Groshong catheter) under general anesthesia on 11th February 2022 (Fig. 1A). The catheter had remained in the position till 26th March 2023 without any complications (Fig. 1B). During her maintenance chemotherapy on 16th June 2023, it was found that the chemoport was not working and there was no backflow as well as forward flow. The chemotherapy was given through a peripheral vein, and X-ray chest was done to look up for chemo port status (Fig. 1C). It was observed that the distal intravascular part of the catheter wasn't seen, and a faint shadow could be seen within the cardiac silhouette (black arrow, Fig. 1C). It raised a possibility of spontaneous

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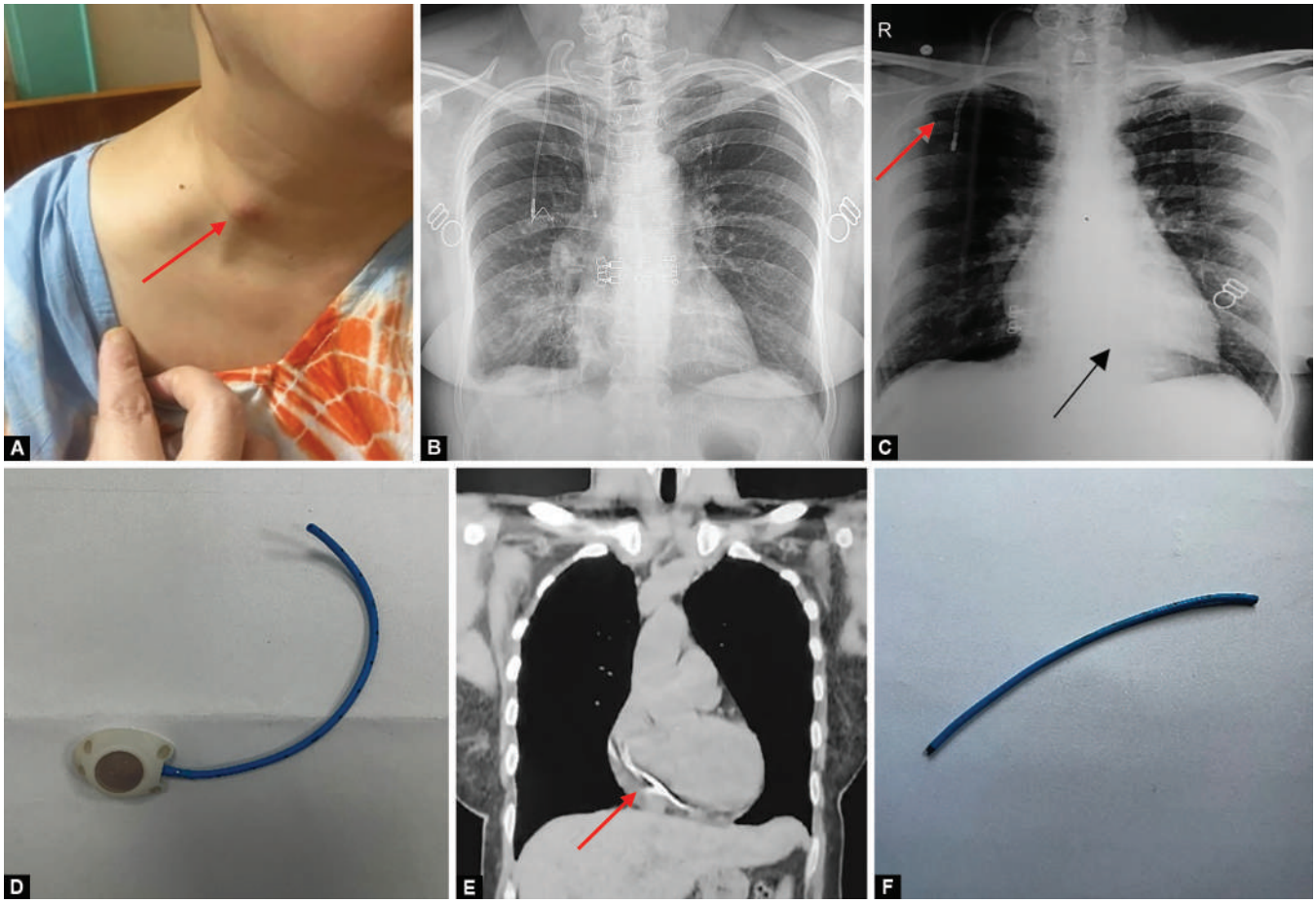

Figs 1A to F: (A) Chemoport catheter *in situ*; (B) X-ray dated 26th March 2023 showing chemoport with extra and intravascular parts of a catheter; (C) X-ray dated 16th June 2023 showing proximal part of chemoport *in situ* and tip of the distal part within the cardiac silhouette; (D) Proximal part of chemoport removed under local anesthesia; (E) NCCT chest showing distal part of approximately 11.5 cm lying in the right ventricle and right atrium; (F) Distal embolized long segment removed using a goose-neck snare technique by interventional cardiologist

fracture of the cannula after 26th March 2023 with embolization of its distal part and the proximal part of the chemoport was removed under local anesthesia (Fig. 1D). As it could not be removed completely, it confirmed fracture of the catheter at the insertion site of IJV with embolization of its distal part. Noncontrast computerized tomography (NCCT) chest was done, and it showed a distal long tubular intravascular part of the catheter of approximately 11 centimeters lying in the right ventricle and right atrium (arrow, Fig. 1E). She was admitted for right heart catheterization and its retrieval. She was transferred to the catheterization laboratory, and the fragmented catheter was removed using a goose-neck snare technique on 19th June 2023 by an interventional cardiologist. The length of the migrated piece was 11.5 cm, and no thrombus was observed at the tip (Fig. 1F). No major complication occurred during and after the procedure, and the patient was discharged on 20th June 2023. This is a unique case because spontaneous breakage of venous access devices and cardiac migration after IJV placement is extremely rare, and patients are usually symptomatic. High-pressure infusions for de-obstruction of the catheter or direct catheter injury by guide wire or needle at the time of insertion are likely other causes of catheter fracture.

Acknowledgment

The authors thank Dr Ravinder Singh Rao, Director and Chief Interventional Cardiologist, RHL Heart Center, Rajasthan Hospital, Jaipur, for the intervention and retrieval of the fractured catheter and Dr Virendra Singh, Chairman, Rajasthan Hospital, Jaipur, for his kind help and support.

CASE REPORT

Rare Presentation of a Patient with Cardiac Arrest Due to Cerebral Fat Embolization Following Polytrauma

Nitin Rathod[1], Vishal Rathod[2], Bhakti Parikh[3*], Amishi Rathod[4]

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Abstract

Cerebral fat embolism (CFE) syndrome is a known complication that can occur following polytrauma, particularly in cases involving fractures of long bones, but cardiac arrest is a rare presentation following cerebral fat embolization.[1] Our patient met with a road traffic accident (RTA), sustaining multiple long bones injuries with hypovolemic shock. After 10 hours of admission and achieving hemodynamic stability, the patient developed cerebral fat embolization. He developed sudden cardiac arrest and was resuscitated. We instituted ventilator support, inotropic infusion, antibiotics, and intravenous (IV) fluids. Our patient regained consciousness without neurological deficit over a period of 10 days and underwent surgery for all three major fractures with due precautions. The patient was discharged after 3 weeks of treatment from the hospital.

Journal of the Association of Physicians of India (2023): 10.59556/japi.71.0389

Introduction

The incidence of cerebral fat embolism (CFE) ranges from 0.9 to 11%, with a mean mortality rate of around 10%.[2] Fat embolization syndrome (FES) is characterized by multisystem dysfunction typically presenting 10–72 hours after the initial insult. The classic triad of FES includes hypoxemia, neurological abnormalities, and petechiae. The clinical manifestations of respiratory failure, petechiae, and a diffuse or focal cerebral disturbance are characteristic but not pathognomonic of the syndrome. A petechial rash is considered pathognomonic of FES and is reportedly present in up to 60 % of patients, usually on the conjunctiva, oral mucous membranes, skin folds of the neck, and axillae.[3]

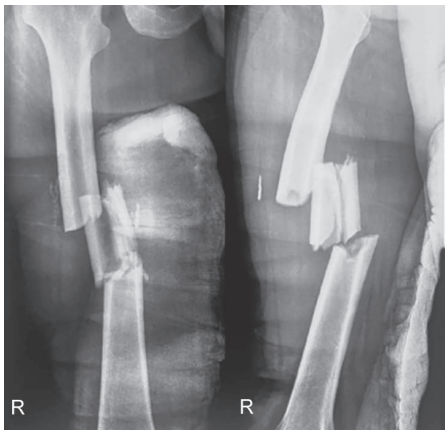

Fig. 1: Right humerus fracture

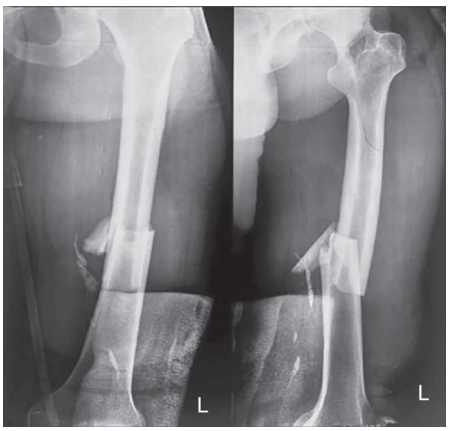

Fig. 2: Left femur fracture

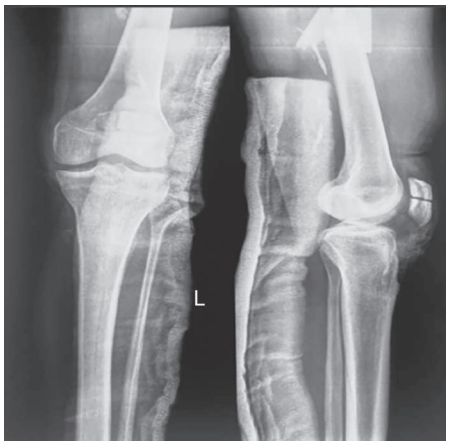

Fig. 3: Left patellar fracture

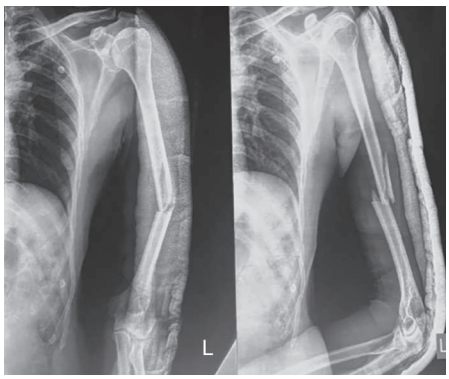

Fig. 4: Left humerus fracture

Case Description

A 51-year-old male, with no comorbidities and addiction, presented to the emergency department with an alleged history of road traffic accidents (RTA). He was travelling in the auto-rickshaw which collided with a car, sustaining trauma to his face, bilateral lower limbs, and left arm.

Upon admission to the emergency department, he was fully conscious and oriented. His pulse rate was 130 beats per minute, with a blood pressure of 90/60 mm Hg measured in his right arm on arrival. Blood oxygen saturation (SpO_2) was 90% on room air, with a respiratory rate of 28 breaths per minute and patent airways. Clinical examination revealed multiple abrasions on the right arm and right shoulder, and a contused laceration wound over the right side of the chin measuring 1 × 0.5 × 0.5 cm. X-rays of anteroposterior and lateral view of bilateral lower extremity showed: (1) right femur shaft segmental fracture (Fig. 1), (2) left femur shaft segmental fracture (Fig. 2), and (3) left patellar fracture (Fig. 3). X-rays of the left upper limb and shoulder revealed a displaced fracture of the shaft of the humerus (Fig. 4). X-ray of the head revealed a left zygomatic bone fracture and a left maxillary sinus fracture. After initial resuscitation with intravenous (IV) fluid,

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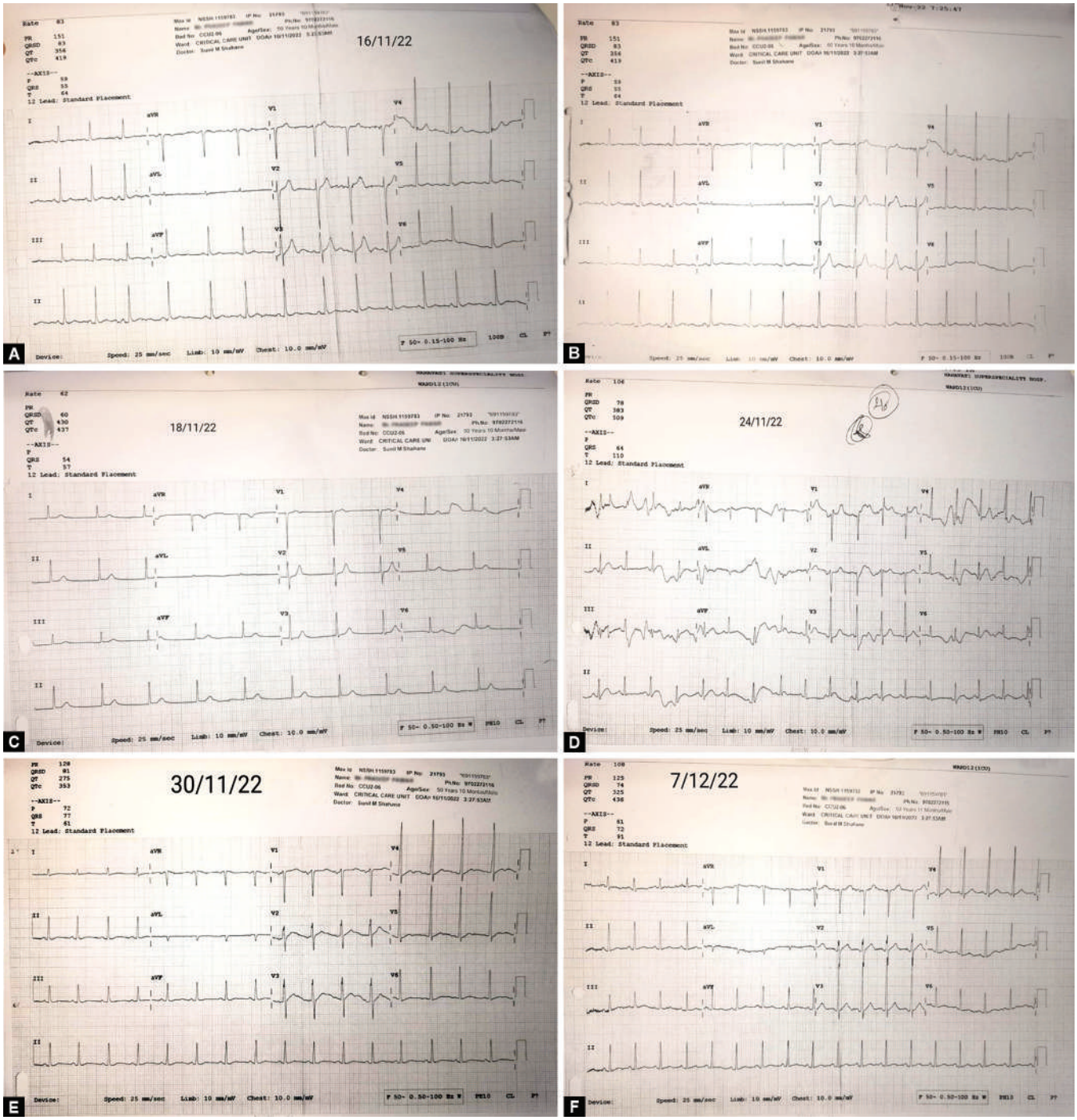
Table 1: Routine investigations

	16/11/22	*17/11/22*	*19/11/22*	*25/11/22*	*1/12/22*	*19/12/22*
HB (gm/dL)	14.1	8.8	9.7	8.8	10.4	10.2
HCT (%)	43.9	26.6	29.7	27.1	32.3	31.4
TLC/mm^3	14860	6680	11760	15770	7760	11050
Platelets/mm^3	419000	138000	126000	255000	578000	570000
PT (second)	10.8	14.5	19.5			
INR	1.04	1.39	1.87			
NA (mEq/L)	137	136	144	134	133	134
K (mEq/L)	3.69	4.13	4.26	3.78	4.16	4.5
CL (mEq/L)	96	102.6	110.7	100.4	94	95.5
ESR (mm/hour)						
CREAT (mg/dL)	0.98	0.75	0.95		0.45	
CPK (U/L)		2689	4424			
BIL (mg/dl)			1.005			
SGOT (U/L)			25.53			
SGPT (U/L)			21.06			
Protein (gm/L)			3.98	4.5		
Albumin (gm/L)			2.3	2.3		
pH		7.38	7.36	7.43		
$PaCo_2$ (mm Hg)		30.4	40.5	37.1		
PaO_2 (mm Hg)		526	73	163		
HCO_3 (mmol/L)		17.5	22.3	23.9		
Lactate (mmol)	1.6	5	2	1		
Urea (mg/dL)			37.5	39.4		
BUN (mg/dL)			17.52	18.41		
CRP (mg/L)				163.72	156.63	30.07
ESR (mm/hour)	10		1.79			
Mg (mg/dL)			6.82			
Ca (mg/dL)						
PCT (ng/mL)						
Urine routine						
Sputum culture				No growth	*Klebsiella pneumonia* *Pseudomonas aeruginosa*	
Urine culture					No growth	
Blood culture					*Pseudomonas aeruginosa* *Staphylococcus aureus*	

inotropes, and analgesics, a plaster of Paris Slab was applied to the left arm. Temporary stabilization of the bilateral femur fracture was done using Thomas's splint. Routine blood investigations (Table 1) were carried out, which indicated a picture suggestive of acute blood loss with the initial rise in hemoglobin (Hb), hematocrit levels, and total leukocyte counts due to hemoconcentration, followed by an eventual drop in (Hb, hematocrit, and total leukocyte count) parameters.

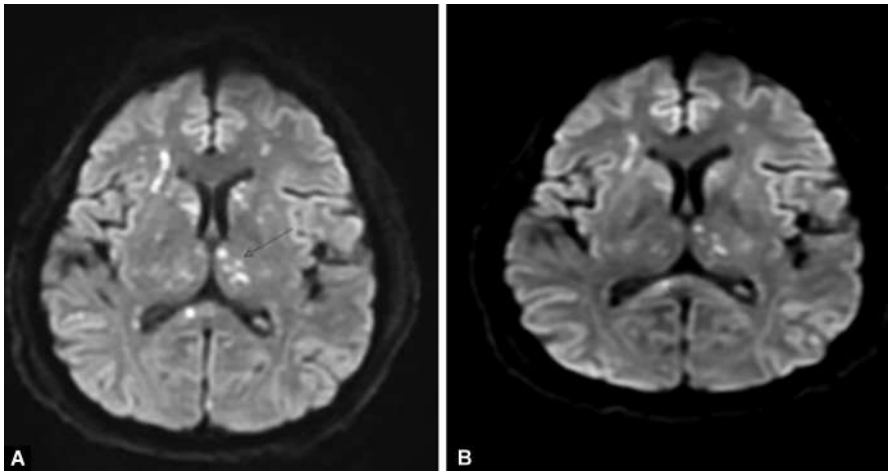
The patient was immediately shifted to the intensive care unit (ICU) in view of hemodynamic instability associated with multiple long bone fractures. Inotrope and vasopressor support were continued. Multiple (four) units of packed red blood cells were transfused. The patient was having a right conjunctival bleed and small petechial hemorrhages over the right axilla and chest wall. After 8 hours of stabilization, the patient developed a convulsion which was followed by cardiac arrest (Fig. 5**).** He was immediately resuscitated, intubated and put on a ventilator. Postresuscitation and stabilization computerized tomography (CT) scan of the head, chest, and abdomen was performed. The CT scan of the brain did not show any evidence of acute infarct or bleeding. The CT scan of the chest did not reveal evidence of ground-glass opacity or pulmonary embolism. The next day, in view of the deteriorating neurological status and reduced power in the limbs, magnetic resonance imaging (MRI) with MR angiography (brain) was done which revealed (Figs 6A, 7A and 8A) multiple small lesions in the whole brain consistent with a "star field" pattern.[4] It showed numerous tiny foci of restricted diffusion in the bilateral frontal, parietal, occipital and temporal gray-white matter junction and basal ganglia, thalami, selenium of corpus callous and both cerebellar hemispheres. These findings represent the sequel of fat embolism. On three-dimensional arterial spin labeling (noncontrast perfusion) there was symmetric and maintained cerebral perfusion. A two-dimensional-echo was done which revealed good left ventricular function with left ventricular ejection fraction = 55%, no regional wall motion abnormality, and no clot or vegetation in the left ventricle. There was no intra-atrial



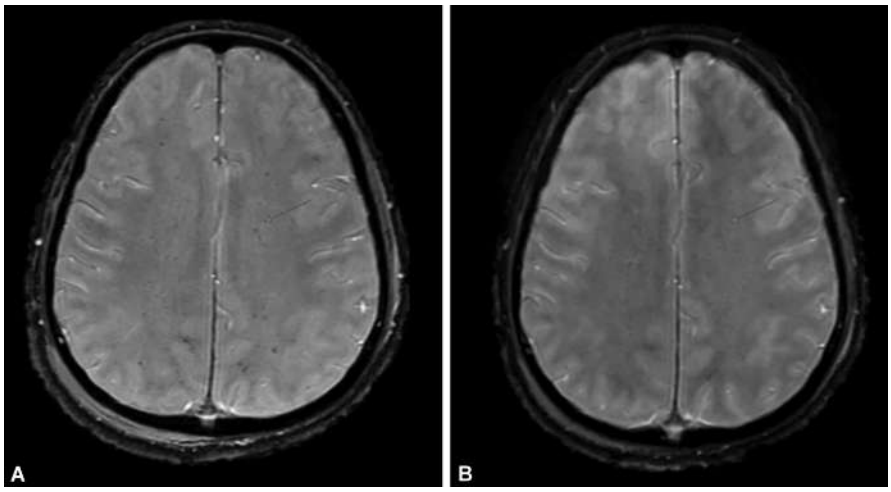
Figs 5A to F: Electrocardiogram findings of the patient

septal or intraventricular septal defect or shunt. There was neither right ventricular enlargement or hypertrophy, nor evidence of pulmonary artery hypertension, thus ruling out pulmonary embolization. A bilateral lower limb Doppler was done, which showed evidence of echogenic thrombus seen from the left distal superficial femoral vein-popliteal junction extending into the left popliteal vein. He was started on deep vein thrombosis prophylaxis with low molecular weight heparin (LMWH). Antiepileptic was started. Ventilator and inotrope support was continued along with IV antibiotics. Ryle's tube was inserted and the patient was given tube feeds. Hemodynamic stability was achieved in 4 days and an MRI brain (Figs 6B, 7B and 8B) was repeated which revealed partial resolution of the previously seen areas of restricted diffusion and blooming in both cerebral hemispheres. No new areas of altered signal intensity were seen in brain parenchyma on repeated scans.

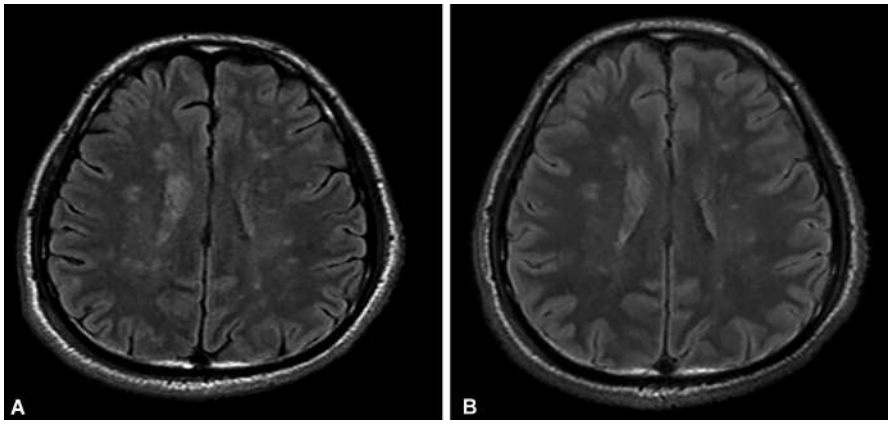
As soon as hemodynamic stability was achieved, the patient was planned and taken up for surgical repair (closed reduction and internal fixation with nailing) of the right mid-shaft femoral fracture. LMWH was withheld 12 hours prior to surgery. The surgery was



Figs 6A and B: (A) Diffusion-weighted images show multiple areas of restricted diffusion; (B) These show temporal evolution



Figs 7A and B: (A) Tiny subtle areas of blooming are seen throughout bilateral cerebral parenchyma; (B) These show reduction



Figs 8A and B: (A) Flair images show hyperintensity in affected areas; (B) These show a reduction

uneventful. The patient was vitally stable in the postoperative period. LMWH was restarted after 12 hours of surgery. He was then tracheotomized for further oxygen delivery and subsequently patient was weaned off the ventilator with tracheostomy *in situ*. He maintained a blood oxygen saturation of 97% on 2 L O_2/minute through a T-tube (tracheostomy tube). Arterial blood gas was repeated which showed improvement in all the parameters (pH = 7.43, pCO_2 = 37.1, HCO_3 = 23.9 mmol/L, lactate = 1 mmol/L). Physiotherapy was initiated, with careful monitoring of all vital parameters.

Subsequently, the patient underwent two more surgeries for (1) the left femur shaft, fracture closed reduction and internal fixation, open reduction and internal fixation with tension band wiring for left patellar fracture and (2) the left shaft of humerus fracture open reduction and internal fixation with plating. The patient's neurological status started improving. He was fully conscious and obeying verbal commands. The patient continued to receive room temperature feeds. Slowly and gradually oral clear liquids were initiated and were well tolerated by him.

With symptomatic improvement and hemodynamic stability, the patient was shifted to the ward from the ICU. Physiotherapy sessions were continued in the form of active therapeutic movements, active release technique, static quadriceps exercise, sitting over the edge of the bed with support and bed-to-chair mobilization, and breathing exercises.

The patient's condition improved symptomatically with well-maintained hemodynamic stability, with a novel oral anticoagulant and was discharged from the hospital.

Discussion

After a "bone burst," according to the accepted pathophysiology, "fat droplets" are shot into the systemic circulation, giving rise to emboli. There is no univocal explanation existing to describe how the syndrome develops. The mechanical theory postulates that fat microemboli enter venous sinusoids, collect in the pulmonary microvasculature, and migrate into the systemic circulation *via* the pulmonary capillary bed. The fat embolism syndrome is believed to be caused by the toxic effects of free fatty acids liberated at the endothelial layer, which cause capillary disruption, perivascular hemorrhage, and edema.[5]

Our patient developed fat embolization after 10–12 hours of injury (long bone fractures). The patient also developed cardiac arrest following cerebral fat embolization. After adequate resuscitation, the patient showed improvement without any neurological deficit.

Conclusion

When patients have long bone and pelvic fractures or multiple bone fractures and a deteriorated neurological status, CFE should be considered. CFE may occur without the presence of a patent foramen

oval. Diffusion-weighted imaging often demonstrates the characteristic "star field pattern," while diffuse microhemorrhages on susceptibility-weighted imaging may be the only characteristic finding.

Clinical Significance

The early diagnosis and appropriate management of FES are important, and patients should be monitored comprehensively in the intensive care unit. With appropriate treatment, CFE patients may achieve good results.

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CASE REPORT

Familial Ectopia Lentis: Looking Beyond Marfan's Syndrome

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Abstract

Ectopia lentis has a myriad of causes, with Marfan's syndrome and homocystinuria being well-known causes. Here, we report two siblings with ectopia lentis and tall stature presenting with a diagnostic challenge.

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Introduction

Traboulsi syndrome is a rare autosomal recessive disorder caused by a mutation in the aspartate β-hydroxylase (ASPH) gene. It is also known as facial dysmorphism–lens dislocation–anterior segment abnormalities and spontaneous filtering blebs syndrome.[1] Here, we report two siblings with similar clinical features, in whom exome sequencing identified a mutation in the ASPH gene, which has been reported as pathogenic previously.[2]

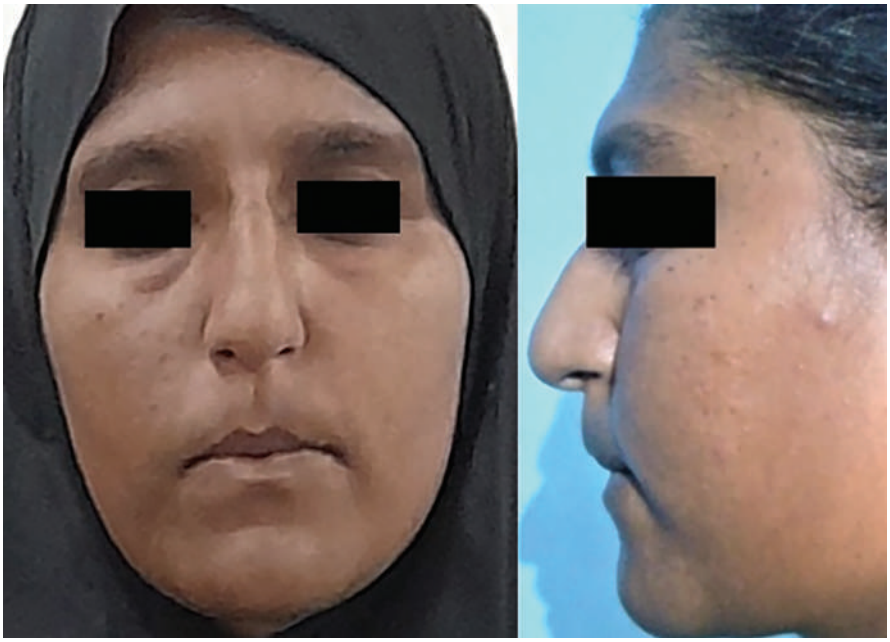
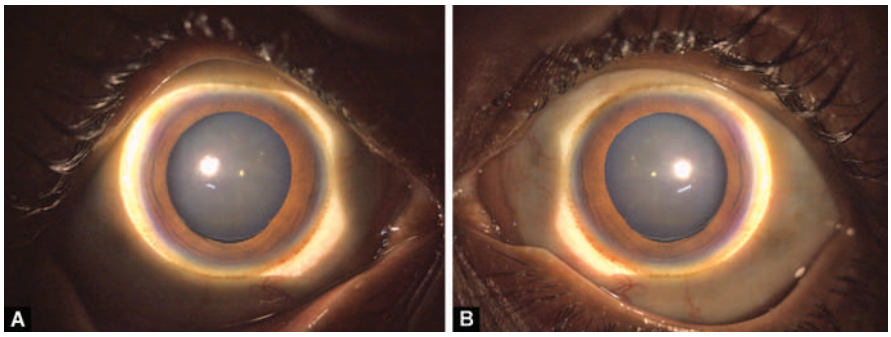


Fig. 1: Photograph (front and side profile) showing the dysmorphic facial features of the proband with elongated facies, beaked nose, retrognathia, and malar hypoplasia



Figs 2A and B: (A) Slit lamp examination shows superonasal subluxation of the lens in the right eye; (B) Left eye in the proband

Case Description

The proband, a 20-year-old lady born of a consanguineous marriage, presented with complaints of tall stature and significant myopia from 5 years of age. Clinical examination revealed dysmorphic facies in the form of a beaked nose with an elongated face with malar flattening (Fig. 1). Her height was 184 cm, arm span to height ratio of >1.05, and upper segment to lower segment ratio of 0.85. She had a high arch palate with malalignment of teeth. Her best corrected visual acuity was six out of nine in both eyes, with ocular examination showing bilateral superonasal lens dislocation (Fig. 2) with myopic fundi (Fig. 3).

Her younger brother (15 years of age) was also tall (178 cm) and had similar facies with superotemporal lens dislocation and a normal fundus examination. Both parents were of normal height with no ocular abnormalities (Table 1). Initially, they were referred to as suspected Marfan's syndrome/homocystinuria. However, a normal echocardiogram and normal serum homocysteine levels ruled out the other diagnoses. A genetic evaluation was performed as the siblings were born out of a consanguineous marriage, suspecting an autosomal recessive inheritance.

Whole exome sequencing showed homozygous missense variation in exon 25 of the ASPH gene [chr8:g.61503432C>T, (RefSeq NM_004318.3)] leading to substitution of arginine by glutamine at codon 735 (p.Arg735Gln, R735Q) in both the siblings. This condition is called Traboulsi syndrome and is characterized by lens subluxation, spontaneous filtering blebs, and marfanoid habitus. This variant has been previously

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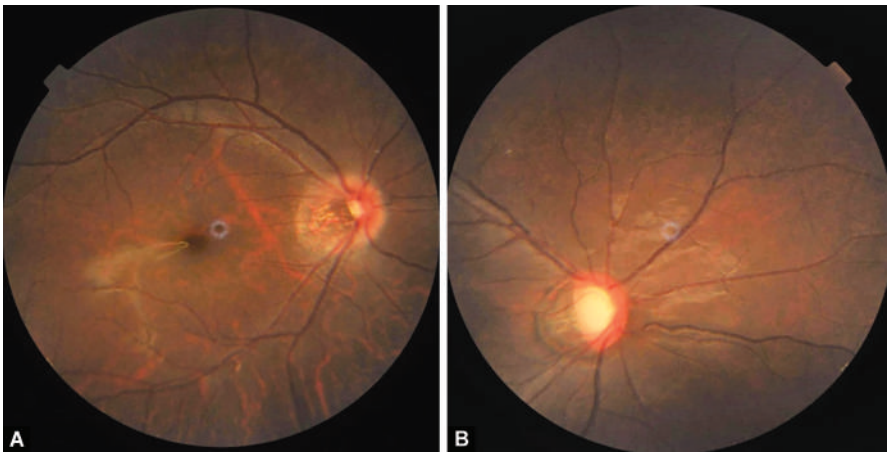

Figs 3A and B: (A) Fundus examination showing myopic changes in right; (B) Left eyes of the proband

Table 1: Clinical features and investigations of the proband and her sibling

Parameters	*Proband*	*Sibling*
Gender	Female	Male
Age (in years)	20	15
Height (cms)	184	178
Facies		
Elongated face	+	+
Malar hypoplasia	+	+
Retrognathia	+	+
Ocular findings		
Ectopia lentis	+	+
Conjunctival blebs	–	–
Nose		
Beaked nose	+	+
High nasal bridge	+	+
Mouth		
Malocclusion of teeth	+	–
High arched palate	+	+
Bifid uvula	–	–
Others		
Arachnodactyly	–	–
Pes excavatum	–	–
Flat feet	–	–
Investigations		
Echocardiography	Normal	Normal
Serum homocysteine levels	Normal	Not done
ASPH mutation	+	+

shown to be affecting the active site of ASPH and has been classified as a pathogenic variant as per American College of Medical Genetics and Genomics guidelines.

Discussion

The most common causes of ectopia lentis with marfanoid habitus are Marfan's syndrome and homocystinuria. However, the absence of cardiac abnormalities and intellectual disability leads to a diagnostic dilemma in such cases. In our cases, a whole exome sequencing revealed the diagnosis of Traboulsi syndrome, which is a rare autosomal recessive disorder characterized by spontaneous lens subluxation, shallow anterior chamber, and facial dysmorphism in the form of the beaked nose, elongated face, maxillary hypoplasia, and overcrowding of teeth.[3] Though rare, skeletal and cardiac abnormalities have been reported.[4–6] This syndrome was originally described in a consanguineous Druze family in Lebanon in 1995, and the pathogenic mutation was identified in 2014.[1,3] Since then, only 21 genetically proven cases of this syndrome have been reported to date, including five patients from India.[6–8]

This syndrome occurs due to biallelic variants in the ASPH gene in chromosome 8q12. This gene is found to be highly expressed during the development of the lens. The gene encodes an enzyme called aspartyl/asparaginyl β-hydroxylase, which hydroxylates aspartic acid and asparagine residues on epidermal growth factor domain-containing proteins. Arginine residue at the 735th position in the catalytic domain is critical for the function of the ASPH enzyme. Hence, the substitution of arginine by tryptophan (R735W) or glutamine (R735Q) may lead to a loss of ASPH enzyme activity.[1] The R375Q variant has been previously reported by Siggs et al.[2] However, their patient did not have any skeletal abnormality.

The presence of this clinical phenotype can be explained by studies that found fibrillin-1 (FBN1) and latent transforming growth factor β-binding protein-2 to be ASPH substrates. Mutation in the FBN1 gene leads to Marfan's syndrome, which is a genetic cause of ectopia lentis. However, asparagine hydroxylation of FBN1 protein is observed only during the development of the embryo, and so its functional significance of hydroxylation is yet to be ascertained.[9] Although the role of FBN1 in Traboulsi syndrome is unknown, it may be proposed that the overlapped phenotypes may indicate a molecular link between ASPH and FBN1.

Conclusion

We would like to conclude that Traboulsi syndrome must be considered as a differential diagnosis for familial ectopia lentis with Marfanoid habitus.

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CASE REPORT

Apparent Dengue Fever Turned Out to be Hemophagocytic Lymphohistiocytosis

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Abstract

Hemophagocytic lymphohistiocytosis (HLH) is an extremely uncommon hematologic condition that is potentially fatal. It is a disease of histiocyte or lymphocyte hyperactivity, which can be inherited (primary) or acquired (secondary). Dengue fever and HLH both can present with fever, lethargy, and a blood profile of pancytopenia, which makes it difficult to diagnose HLH promptly in a region with dengue endemicity. Clinical and supportive biochemistry findings help clinicians to suspect and diagnose HLH. This article presents a case report of a patient who was diagnosed with dengue fever during initial presentation with subsequent swerves toward HLH. Diagnosing HLH associated with dengue can be difficult. However, it is of utmost importance to diagnose it early, as an early diagnosis and management can lead to significantly improved outcomes.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is an uncommon and life-threatening condition marked by excessive inflammation. Its key characteristics include fever, enlarged liver and spleen, pancytopenia, and hemophagocytosis, primarily in the bone marrow and also in the liver and lymph nodes.[1] HLH is classified as primary (inherited) with predominance in children and infants or secondary (acquired) with predominance in adults. Primary HLH has inherited defects in lymphocytes, causing uncontrollable proliferation of macrophages and T-cells, producing excessive amounts of inflammatory cytokines. The etiology of primary HLH includes Griscelli syndrome, Chediak–Higashi syndrome, X-linked lymphoproliferative syndrome, and known genetic defects in *PFRI*, *UNC13D*, and *STX11* genes, to name a few.[2] The mechanism of acquired HLH is still not fully determined, but it is possibly due to immune system activation following infections from a variety of microorganisms or also due to certain autoimmune conditions and malignancies.[3] The most frequently associated infectious cause is Epstein–Barr virus, but during recent times, HLH has been found to be increasingly associated with dengue fever, cytomegalovirus, bacterial, fungal, protozoal, and even found to be associated with severe coronavirus (severe acute respiratory syndrome coronavirus 2) infections.[4–6] Malignancies, including leukemias (acute lymphocytic leukemia and acute myeloid leukemia), lymphomas (B-cell and T-cell lymphoma), and myelodysplastic syndrome as well as autoimmune conditions, including systemic lupus erythematosus, juvenile idiopathic arthritis, rheumatoid arthritis, Kawasaki disease, and Still's disease are also found to be secondary causes for HLH.[7]

Cytotoxicity of the natural killer (NK) cells is the cornerstone behind the development of this syndrome. Due to some pathologic mutations, these cells are unable to remove infected cells as well as antigen-presenting cells, which leads to unregulated activation of the immune response and release of excessive cytokines. The unchecked multiplication and infiltration of these cells in the organs with the subsequent release of more cytokines, including interleukin (IL)—1, IL-6, and tumor necrosis factor-α (TNF-α), is responsible for the clinical picture of HLH. Release of IL-1 and 6 is responsible for fever, TNF-α causes suppression of hematopoiesis, which results in cytopenia, and macrophage activation releases ferritin along with activators of plasminogen, which is responsible for excessive fibrinolysis.[8] The Histiocyte Society introduced diagnostic criteria for HLH in 1994, which were subsequently updated in 2004 and are now widely accepted as the standard guidelines for identifying HLH.[9] According to this criterion, HLH can be diagnosed by establishing molecular confirmation (pathogenic mutations in genes *PRF1*, *MUNC18-2*, *RAB27A*, *BIRC4*, etc.) or by establishing five or more of the following clinical findings: fever ≥38.5°C, hepatosplenomegaly, cytopenia impacting at least two blood cell lines (hemoglobin <9 gm/L, platelet count <100,000/mm^3, absolute neutrophil count <1000 b/L), ferritin elevation (>500 µg/L), hemophagocytosis in bone marrow biopsy, elevated soluble cluster of differentiation (CD25) (α-chain of SIL-2 receptor), low/absent NK cell activity, hypertriglyceridemia, hyperfibrinogenemia, and hyponatremia.[10]

Nowadays, dengue fever is becoming an important cause of secondary HLH.[5] Dengue infection and HLH share overlapping and nonspecific clinical features, so it becomes difficult to suspect HLH in a patient of dengue. HLH aggravates quickly if left untreated with extremely poor outcomes. Hence, it becomes important to keep a high suspicion for HLH in patients of dengue who are unresponsive to standard treatment and initiate prompt management.[5,11]

Case Description

A 42-year-old male patient presented with a primary complaint of experiencing a high-grade fever accompanied by chills for the past 7 days. Additionally, he reported multiple episodes of vomiting and headache. Upon initial evaluation, he exhibited fever with a temperature of 101.3°F, blood pressure at 112/60 mm Hg, a heart rate of 98 beats/minute, respiratory rate of 20 breaths/minute, and oxygen saturation of 99% while breathing room air. Pallor was noted on general examination. On abdominal examination, 1 cm below the right costal margin and mild splenomegaly. Respiratory and cardiovascular system examination findings were within normal limits.

Lab investigation showed pancytopenia [hemoglobin (Hb), 8.2 gm/dL; total count, 1,300/mm^3; platelet count, 48,000/mm^3]. Elevated ferritin levels were observed from the iron studies along with an iron of 10.0 µmol/L, transferrin levels of 1.6 gm/L, and 73% transferrin saturation. The results of renal function test, liver function test, and urinalysis

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Table 1: Blood investigation—blood reports throughout the course of management

Investigation	*Patient values*					*Units*	*Normal value*
	On admission	*Day 5*	*Day 10*	*Day 15*	*Day 20*		
Complete blood count							
Total RBC count	2.43	2.17	2.10	1.49	1.46	Million/mm^3	4–5.5
Hb	8.2	7.1	6.8	4.9	3.9	gm/dL	13–17 (adult male)
Packed cell volume	27.4	21.5	22.0	14.2	13.4	%	40–50
Mean corpuscular volume	88	99	78	95.3	82	fL	80–100
Mean corpuscular hemoglobin	33.0	32.8	32.0	32.9	32.0	Pg	27–32
Mean corpuscular hemoglobin concentration	33.2	33.1	32.6	34.5	33.4	%	31–35
Total WBC count	1,300	800	500	800	5,300	Cells/mm^3	4,000–11,000
Neutrophils	40	34	32	60	64	%	40–80
Lymphocytes	58	64	66	38	34	%	20–40
Eosinophils	1	1	1	1	1	%	1–6
Monocytes	1	1	1	1	1	%	2–10
Basophils	0	0	0	0	0	%	0–1
Platelet count	0.48	0.15	0.38	0.55	0.4	Lakhs/mm^3	1.5–4.5
Erythrocyte sedimentation rate 1 hour	20					mm	0–22/hour
Coagulation profile							
Prothrombin time	14		14		58.6	Seconds	10–15
Activated partial thromboplastin time	34		38		>1 minute	Seconds	21–38
International normalized ratio	1.22		1.23		4.78		2–3
Serum fibrinogen			72.4			mg/dL	150–400
Renal function test							
Serum urea	58	71	132	109	146	mg/dL	15–45
Serum creatinine	1.35	1.45	2.76	1.81	2.21	mg/dL	0.80–1.30
Sodium	131	136	130	132	168	mmol/L	136–145
Potassium	6	3.9	3.9	3.3	3.2	mmol/L	3.5–5.1
Bicarbonate			26		18	mmol/L	21–30
Liver function tests							
Total serum bilirubin	1.6	4.2		4.0	4.8	mg/dL	0.1–1.2
Direct bilirubin	0.7	2		1.8	2.2	mg/dL	<0.3
Indirect bilirubin	0.9	2.2		2.2	2.6	mg/dL	<0.7
Serum glutamic pyruvic transaminase	56	60		88	100	units/L	<40
Serum glutamic oxaloacetic transaminase	162	253		210	132	units/L	<40
Alkaline phosphatase	386	363		348	360	IU/L	60–160
Total serum protein	6.1				5.4	gm/dL	5.5–8
Serum albumin	3.2				2.8	gm/dL	3.5–5
Lipid profile							
Total cholesterol			194			mg/dL	<200
Serum triglycerides			609			mg/dL	<150
Iron profile							
Serum iron		266				µg/dL	65–175 (male)
Total iron-binding capacity		361				µg/dL	250–450
Iron saturation		73.6				%	16–50
Serum ferritin		>2,000				ng/mL	13–400
Plasma fibrinogen		72.4				mg/dL	150–400

were within normal limits (Table 1). The blood, urine, and stool cultures were also negative. Chest X-ray was normal. Ultrasonography of the abdomen was suggestive of hepatomegaly and splenomegaly. Considering the residence of the patient from a dengue-endemic region, dengue fever serology was checked. Dengue nonstructural protein 1 antigen was nonreactive, but immunoglobulin M dengue antibodies were reactive, suggesting a recent infection with the dengue virus (DENV). Treatment was started with intravenous fluids and supportive measures considering dengue fever with pancytopenia.

In view of persistent fever, pancytopenia, and lack of improvement despite treatment, further evaluation for underlying hematologic disorder was considered. A bone marrow

biopsy from the right posterior superior iliac spine was performed along with other necessary blood investigations on the 5th day of admission. Bone marrow biopsy was suggestive of a relative hypercellularity as per age, megaloblastic changes such as giant metamyelocytes and dysmegakaryopoietic changes, and abnormally scattered, loose clusters of histiocytes/macrophages. Few of these histiocytes demonstrated hemophagocytosis with engulfment of red Blood cells (RBCs), white blood cells (WBCs), and platelets (Fig. 1). These cumulative biopsy findings, in accordance with clinical correlation, were suggestive of HLH. The diagnosis was supported by lab findings like hypertriglyceridemia (609 mg/dL), decreased serum fibrinogen (72.4 mg/dL), and raised ferritin (>2000).

The patient was treated with immunosuppression in the form of methylprednisolone pulse therapy and supportive management in the form of blood component transfusion, antibiotics, antipyretics, and intravenous fluids. The patient initially responded to methylprednisolone, but on the 6th day of starting immunosuppressants, the patient had massive hematemesis. Despite aggressive management, the patient's condition deteriorated with the development of acute disseminated intravascular coagulation (DIC) and hemorrhagic shock, and the patient succumbed to his illness.

Discussion

Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory condition that can be primary or secondary. Primary or familial HLH is an autosomal recessive inherited disorder, whereas secondary HLH results from an aberrant immune response triggered by factors like severe infection, autoimmune diseases, or malignancies. Secondary HLH can occur in individuals of all ages and does not exhibit a gender preference. HLH almost has a 100% mortality rate without timely diagnosis and prompt management. However, some studies have shown survival rates of up to 60% with prompt HLH-directed treatment.[5] Some patients with severe dengue develop HLH despite timely and adequate management, and in a country like India, which accounts for almost one-third of the global burden for dengue infection,[12] this associated fatal condition should not be overlooked. Dengue usually manifests suddenly, typically after an incubation period of 5–7 days. The initial 2–6 days of the disease is the febrile phase with typical symptoms like fever, muscle and bone pain, headache, and retro-orbital pain, or even mild bleeding manifestations such as petechiae and bleeding gums. From here, the disease can worsen and go into a critical phase, or the condition may improve, and the patient goes into a convalescent/recovery phase.[13] An underlying condition like HLH can possibly divert the course of dengue toward the critical phase. In our case, the patient's condition wasn't showing any signs of improvement after initial management, and later, the patient deteriorated over a brief span of time and developed shock and DIC-related hemorrhagic manifestations.

Previous reports exist for dengue-associated HLH in children as well as in adults. Dengue is a significant contributor to arthropod-borne viral illnesses in humans. Clinically, it can either present as an asymptomatic condition or progress to more severe forms, such as dengue hemorrhagic fever and dengue shock syndrome. There are four identified DENVs (DENV1, DENV2, DENV3, and DENV4) responsible for the disease. Among these, DENV1, 3, and 4 have been linked to development of HLH. As the number of cases continues to rise each year, the incidence of dengue associated with HLH has also been on the increase.[14,15] The greatest hurdle toward prompt management of HLH is delay in diagnosis because of its variable presentation and lack of specific clinical and lab findings.[2,3] In our patient, the initial clinical presentation was suggestive of dengue infection in a dengue-endemic region. But subsequently focus shifted toward alternative diagnosis due to nonresponsiveness to prior treatment. The histiocyte society has developed a set of diagnostic guidelines to help in the rapid diagnosis of HLH. This diagnostic criterion includes clinical and laboratory findings (Table 2).[16] Our patient had fever, splenomegaly, pancytopenia, hepatitis, elevated ferritin, hypertriglyceridemia, hypofibrinogenemia, and hemophagocytosis in bone marrow biopsy (Table 1).

The treatment for HLH is aimed toward managing the life-threatening inflammation by suppressing the immune system. Corticosteroids such as methylprednisolone or dexamethasone are considered the first line of management, but recently, new immunotherapies like monoclonal antibodies, etoposide, intravenous immunoglobulin have also emerged as a potential and more effective treatment modality.[17] The use of dexamethasone in treating patients with HLH secondary to dengue has shown successful outcomes in several previous case reports.[11,18] Chang et al. reported two cases of HLH in dengue fever. In those cases, suspicion of HLH arose due to the recurrence of high-grade fever following an initial period of improvement. The first was characterized by elevated serum ferritin level and abrupt thrombocytopenia, while the second case involved the persistence of fever for over 1 week with declining liver functions. Intravenous dexamethasone was initiated in both of these patients due to strong clinical suspicion of HLH. They responded positively to treatment, experiencing resolution of fever and improvement in blood parameters. Early detection and diagnosis of dengue-associated HLH, coupled with swift intervention, played a crucial role in observing positive outcomes.[17]

Munshi et al. reported a case of HLH secondary to dengue fever. The patient was clinically stable without life-threatening complications throughout the course of illness and improved with supportive measures only.[11] In the study conducted by Ellis et al., eight out of 10 patients with severe disease

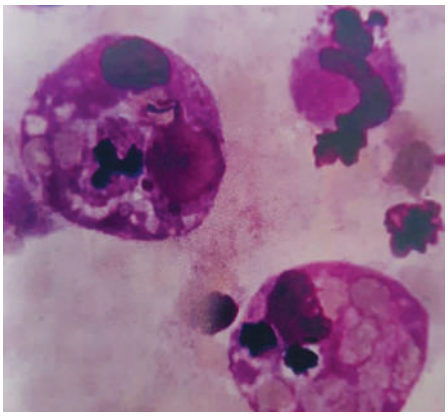

Fig. 1: High power field—bone marrow aspiration shows hemophagocytosis of RBCs and WBCs

Table 2: Hemophagocytic lymphohistiocytosis (HLH) 2009 criteria

At least three of the following
Fever
Splenomegaly
Cytopenia (affecting at least two cell lines): Hb <9 gm%, platelets <1 lakh/mm^3, absolute neutrophil count <1,000/mm^3
Hepatitis
At least one of the following
Ferritin elevation
Elevated soluble CD25 (IL-2 receptor)
Hemophagocytosis on tissue biopsy
Low or absent NK cell activity
Other supportive features
Hypertriglyceridemia
Hypofibrinogenemia
Hyponatremia

who received dexamethasone treatment survived.[19] Whereas in our case, despite treatment with high-dose systemic steroids, the patient developed fatal complications and succumbed due to the same. Due to its infrequency, diverse clinical presentations, and broad spectrum of symptoms, there is an urgent need for randomized controlled trials to assess the effectiveness of immunosuppressants and immunotherapy in the management of dengue fever complicated by HLH.

Conclusion

Hemophagocytic lymphohistiocytosis (HLH) is a rare, aggressive, and potentially life-threatening syndrome that is likely to go underdiagnosed. Hence, a high index of suspicion for the diagnosis of HLH should be kept for patients presenting with dengue fever with pancytopenia, hepatosplenomegaly, and lack of improvement with usual supportive measures. Early diagnosis and intervention can improve a patient's outcome. A delay in diagnosis and management can lead to fatal outcomes.

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CASE REPORT

Acquired Amegakaryocytic Thrombocytopenia Misdiagnosed as Immune Thrombocytopenia in a Patient with Seronegative Arthritis: A Case Report

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Abstract

Acquired amegakaryocytic thrombocytopenia (AATP) is an uncommon cause of severe thrombocytopenia with preserved cells of other lineages, which can present with severe bleeding episodes. We report a case of a 45-year-old male with seronegative arthritis who was diagnosed with idiopathic thrombocytopenic purpura (ITP) and was being treated with steroids for ITP. Despite aggressive treatment, the patient had persistently low levels of platelets. In view of persistent thrombocytopenia, bone marrow biopsy was done and was diagnosed as Acquired Amegakaryocytic Thrombocytopenia (AATP). Patient was successfully treated with cyclosporine. Correct identification of AATP is essential because it can lead to life threatening bleeding manifestations and advance into Aplastic anemia or MDS.

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Introduction

Acquired amegakaryocytic thrombocytopenia (AATP) is an uncommon cause of thrombocytopenia, which occurs due to the marked reduction or absence of thrombocyte precursors in the bone marrow while preserving the hematopoiesis of other cell lineages.[1] While the precise prevalence of AATP is not known,[2] it could be higher than reported as many of the cases are being wrongly identified as idiopathic thrombocytopenic purpura (ITP) (immune thrombocytopenia). The mainstay of treatment for ITP are steroids and intravenous immunoglobulin (IVIg), whereas AATP poorly responds to these. Hence, it becomes very pertinent to differentiate between ITP and AATP.[3]

The deregulated immune response is considered to be the major mechanism behind AATP. Currently, there are no standard treatment guidelines for the treatment of AATP.[4] The treatment currently is dependent on the various case reports illustrating successful management of AATP. We present a patient who was treated for ITP for a couple of years, despite which his platelet counts worsened. Later, the patient was identified to have AATP, which was successfully managed with cyclosporine.

Case Description

A 45-year-old male with a previous diagnosis of seronegative arthritis presented to the emergency room complaining of bleeding from gums to easy fatigability for 2 months. The patient reported that he was detected with ITP in May 2022, and a complete blood count was done, which showed a platelet count of 20,000 cells/mm^3. He received a 15-day course of oral prednisolone daily; after this, his platelets improved to 30,000 cells/ mm^3. Following this, his platelets remained stable between 30,000 and 40,000 cells/mm^3. He denied any history of hematoma formation or major bleeding. He had no personal or family history of bleeding diathesis, autoimmune diseases like lupus erythematosus, rheumatoid arthritis, or malignancy.

An initial clinical examination of the patient revealed stable vitals. Head-to-toe examination showed swan neck deformity of both hands (Fig.1). Initial tests on arrival at our center revealed anemia with hemoglobin of 11.5 gm/dL, thrombocytopenia with a platelet count of 19,000 cells/mm^3 and white blood cell within normal limits. A peripheral smear done had unremarkable findings except for thrombocytopenia with no giant platelet. The reticulocyte count was 1.4%. Tests, including renal function tests, liver function tests, human immunodeficiency virus and hepatitis C virus, antinuclear antibody profile, coagulation panel, anti-cyclic citrullinated peptide, rheumatoid factor, and both direct and indirect Coomb tests, yielded unremarkable or negative levels. Folic acids and vitamin B_{12} levels were within normal limits. Dengue immunoglobulin M (IgM), IgG, and nonstructural protein 1 were negative. As the patient had severe thrombocytopenia with bleeding manifestation, the patient received platelet transfusions. Abdominal imaging did not show any organomegaly or malignancy.

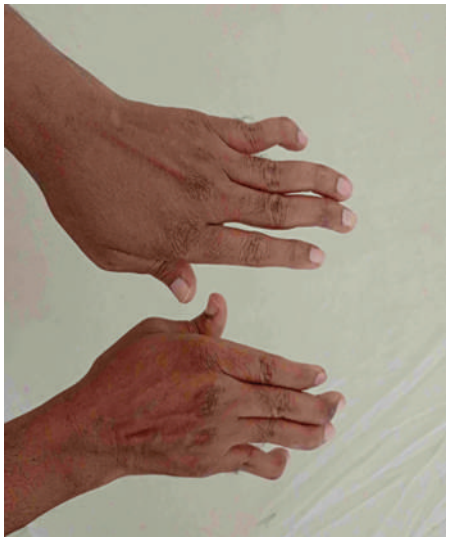

Fig. 1: Image showing swan neck deformity in the patient

The patient received a short course of oral steroids for 4 days, but despite the steroid supplement, his platelets remained at about 20,000 cells/mm^3. A biopsy of the bone marrow was done, which showed megakaryocytic hypoplasia with few hypolobulated forms. Otherwise, the bone marrow was normocellular, without dysplasia or other cytogenetic abnormalities. The myeloid to erythroid ratio

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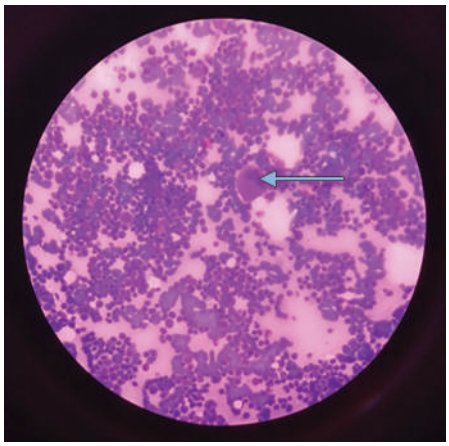

Fig. 2: Image showing varying cellularity in the bone marrow of the patient with amegakaryocytic thrombocytopenia; this 40× image shows a rare megakaryocyte in the bone marrow

was 0.83:1 (Fig. 2). There was no morphologic evidence of myelodysplastic syndrome (MDS). These features are more suggestive of amegakaryocytic thrombocytopenia instead of chronic ITP because, in ITP, there is a compensatory increase in thrombocyte precursors in the marrow. Agglutinins directed against thrombopoietin were not detectable, nor were any antibodies against platelets detected. The patient was initiated on cyclosporine 200 mg daily in two divided doses. After the initiation and continuation of the treatment for a couple of months, the patient experienced an increase in the platelet count above 50,000 cells/mm^3.

Discussion

The etiology for acquired thrombocytopenia is quite vast and includes defective thrombocyte production with sufficient marrow cellularity, flawed thrombopoietin action with adequate marrow cellularity, acquired marrow hypocellularity, and increased platelet extermination.[2]

Our patient was previously diagnosed with chronic immune thrombocytopenic purpura and received oral prednisone with minimal furtherance in his platelet count. Nonetheless, the gradual depletion of his thrombocyte levels over time and lamentable response to high-dose oral glucocorticoids raised the possibility of an alternative cause for thrombocytopenia. Moreover, the absence of thrombocyte precursors in the biopsy of bone marrow in the patient is incompatible with the diagnosis of immune thrombocytopenic purpura and was in favor of an alternative diagnosis since in immune thrombocytopenic purpura; there is a compensatory increase in megakaryocytes in the bone marrow. This case signifies the value of doing a biopsy of bone marrow in patients with inscrutable, isolated thrombocytopenia or in patients with the diagnosis of idiopathic thrombocytopenic purpura not responding to glucocorticoids or intravenous Ig (IVIg).

Amegakaryocytic thrombocytopenia (AMT) is a frightful cause of thrombopenia with lowered or no platelet precursor in the bone marrow. AMT can be acquired or congenital. Eventually, there is a concatenation to involve and hamper all of the three cell lines.[1]

Acquired amegakaryocytic thrombocytopenia (AATP) can be a primary disorder or idiopathic, or it may be seen in consortium with immune-mediated diseases such as systemic lupus erythematosus, rheumatoid arthritis, viral infections such as Epstein–Barr virus, hepatitis C or parvovirus B19, vitamin B_{12} deficiency, subjection to environmental toxins such as benzene and lymphoproliferative disorders. It might eventually lead to aplastic anemia, MDS, or acute leukemia.[4,5]

The exact mechanism of AATP is not known; however, it is resolutely reckoned to be an immune-mediated process. Thrombocyte production is primarily regulated by thrombopoietin, the majority being produced by the hepatocytes. It acts on every single stage of megakaryocyte production, including the production, differentiation, and maturation of megakaryocytes into platelets. Impaired antibody-mediated immunity is described as one of the mechanisms for AATP.[5,6] This has been put across as there is the presence of the anti-thrombopoietin IgG agglutinins and agglutinins against the cellular-myeloproliferative leukemia receptor in patients with AAMT, hampering the role of thrombopoietin. T-lymphocytes obtained from a patient with AATP selectively inhibited thrombocyte lineage *in vivo*, indicating the role of T-cell-mediated immunity. Improvement after immunosuppressant administration further subsists the immune-mediated pathogenesis of AATP.

Currently, there are no established management guidelines for AATP.[7] Contrary to immune thrombocytopenic purpura, treatment with prednisolone and IVIg has been found to be predominantly unproductive. Even though there is no expert consensus, many case reports have shown cyclosporine to be effective, including our patient. Cyclosporine has to be taken for many days to weeks for remission. The strength of the dose can be tapered gradually after the platelets have normalized. In patients with severe bleeding due to low thrombocyte counts or refractory to cyclosporine alone, treating patients with cyclosporine in conjunction with ATG has been found to be effective. Other treatment modalities that are used with varying success include rituximab,[3] mycophenolate mofetil,[8] danazol, and azathioprine.[9] In patients refractory to the above treatment or disease progressing to MDS or aplastic anemia, an allogeneic bone transplant must be strongly considered. Roy et al. showed alemtuzumab, a T-cell depleting agent, to be quite effective in patients with refractory AATP. Even thrombopoietin receptor agonist agonists like romiplostim and eltrombopag have also been shown to summon an admissible response in people with refractory acquired autoimmune thrombocytopenia.[10]

The clinical trial and prognosis of acquired autoimmune thrombocytopenia are quite variable, with few having a durable response, although the rest have relapsing-remitting disease courses. In addition, there are those who advance rapidly to MDS,[11] aplastic anemia,[12] or even leukemia in spite of bellicose immunosuppressive treatment, which makes well-ordered follow-up a necessity.

Conclusion

Amegakaryocytic thrombocytopenia (AATP) is an uncommon cause of thrombocytopenia, which requires a high degree of clinical intuition for identification before consequential complications like lethal hemorrhage occur. Successful identification of the patient requires an integrative approach between physicians and pathologists.

Enlistment of patients in international and national trials is necessary as the disease is rare, and currently, there are no established treatment guidelines; hence, the treatment is dependent on case reports describing success with various therapies for congenital AATP and AATP.

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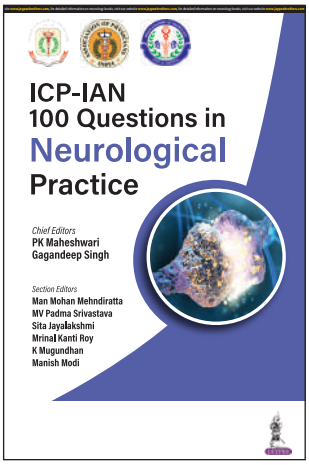
Book Review

ICP-IAN 100 Questions in Neurological Practice



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The book "ICP-IAN 100 Questions in Neurological Practice" is an extremely useful guide for all practicing physicians and students. Although there is abundant literature available in Neurology, this book written in simple question and answer format will be a valuable addition to the existing armamentorium. The book has very lucidly covered three important areas in Neurology namely CNS Infections, Stroke, and Epilepsy. This interrogative approach to common issues in Neurological practice will be immensely beneficial to all postgraduate students of medicine and Neurology. The editors of the book have taken great efforts to simplify complex topics in Neurology and deserve appreciation for compilation of this book. This handy monograph will be an excellent asset to the libraries of all institutions as it proves to be a ready reckoner for all medical professionals while making important decisions in clinical practice.

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CASE REPORT

AMAN with Ophthalmoparesis: A Rare Presentation

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Abstract

Acute motor axonal neuropathy (AMAN) is a variant of Guillain–Barré syndrome (GBS), characterized by acute areflexic flaccid quadriparesis with motor axonal changes and absence of demyelinating findings in electrophysiological studies. A 30-year-old man presented with acute onset flaccid type of weakness involving all four limbs, along with drooping of eyelids. Examination revealed ptosis with restricted horizontal and vertical eye movements. Spinomotor system examination revealed acute flaccid areflexic quadriparesis. Nerve conduction studies (NCS) showed features suggestive of motor axonal neuropathy changes. Cerebrospinal fluid (CSF) revealed albuminocytological dissociation. The diagnosis of AMAN was made, and the patient was treated with intravenous immunoglobulin (IVIg). His weakness gradually improved over 1 month, with partial improvement in ptosis and eye movements. This case highlights the occurrence of ophthalmoparesis in the AMAN variant of GBS. The presence of ophthalmoparesis and areflexia makes it necessary to exclude Miller-Fisher syndrome. But, the presence of axonal changes in nerve conduction study and the profound weakness with negative serum anti-GQ1b antibody profile, supports the diagnosis of AMAN.

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Introduction

Acute motor axonal neuropathy (AMAN) is characterized clinically by a pure motor syndrome without sensory involvement.[1] The electrophysiological findings show a decreased amplitude of compound muscle action potential (CMAP) without any evidence of demyelination or changes in sensory nerve action potential (SNAP).[2] We report a case of the AMAN variant presenting with external ophthalmoparesis and symmetric proximal and distal weakness without sensory abnormalities.

Case Description

A 30-year-old man without any comorbidity presented with acute onset flaccid-type weakness of both lower limbs. Within 1 day, the weakness progressed to involve both upper limbs. The next day, he developed drooping of both eyelids. The patient had a history of fever 3 days before these symptoms. There was no history of neck pain, sensory disturbances, urinary retention, ileus, or respiratory compromise.

Examination revealed ptosis (Fig. 1) with restricted horizontal and vertical eye movements, initially in the left eye followed by the right eye. He also had bilateral lower motor neuron (LMN) facial paresis (Fig. 2). Spinomotor system examination revealed hypotonia and areflexia of all limbs. Muscle power examination showed bilateral symmetrical proximal and distal weakness, with the lower limbs being affected more than the upper limbs (Table 1). Plantar reflex showed bilateral flexor response. Sensory and cerebellar examinations were normal.

Complete hemogram, random blood sugar, liver function tests, renal function tests, serum electrolytes, thyroid function test, urine analysis, and vasculitic workup were normal. Serology tests for human immunodeficiency virus, hepatitis B, and hepatitis C were negative. Magnetic resonance imaging scans of the brain and spine were normal. Cerebrospinal fluid (CSF) analysis revealed elevated protein levels with albuminocytological dissociation (Table 2). The serum ganglioside GQ1b antibody was negative. Nerve conduction studies (NCS) done on the day of admission showed normal findings (Table 3), with no denervation potential and motor unit potentials detected in needle electromyography (EMG) (Table 4).

The patient's clinical findings did not show improvement at the end of 2 weeks. On the 14th day, repeat electrophysiological studies revealed a marked reduction in CMAP amplitude in all four limbs, with normal distal latencies and conduction velocities. Sensory nerve conduction velocities and sensory

Fig. 1: Ptosis of both eyes (left > right)

Fig. 2: Absence of both nasolabial folds

Table 1: Grading of muscle power on admission, discharge, and follow-up

Power	*On admission (day 01)*	*On admission (day 14)*	*Discharge (day 45)*	*Follow-up (day 72)*
UL[#]—proximal	4/5	3/5	4/5	4+/5
UL—distal	4/5	3/5	4/5	4+/5
LL*—proximal	1/5	0/5	3/5	4–/5
LL—distal	1/5	0/5	3/5	4–/5

[#]UL, upper limbs; * LL, lower limbs

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action potentials were normal. F-waves were impersistent in the bilateral median and peroneal nerves (Table 3).

The diagnosis of the AMAN variant of Guillain–Barre syndrome (GBS) was made based on clinical presentation, which was further supported by NCS findings. The patient was treated with 0.4 gm/kg/day intravenous immunoglobulin (IVIg) for 5 days. His weakness gradually improved during the next 4 weeks, with partial improvement in ptosis and eye movements.

Table 2: Cerebrospinal fluid (CSF) analysis

CSF	*Findings*
Opening pressure	15 cm of H_2O
Sugars	48 mg/dL
Proteins	108 mg/dL
Cell count	Acellular
Gram stain	Negative
Acid-fast bacilli stain	Negative
Culture sensitivity	Negative

Discussion

Acute motor axonal neuropathy (AMAN), characterized by decreased CMAP and the absence of demyelinating findings in electrophysiological studies, is a variant of GBS.[3] Patients diagnosed with AMAN typically experience a more rapid progression of weakness, reaching an earlier nadir compared to AIDP, resulting in prolonged paralysis and respiratory failure over a few days.[4] In our case, the patient had a rapid worsening of symptoms with prolonged motor weakness, without any respiratory failure.

Table 3: Comparison of nerve conduction study findings

NCS	*On day 01*	*On day 14*
CMAP* amplitude	Normal	Reduction noted in bilateral median, ulnar, tibial, and peroneal nerves
Distal latency	Normal	Normal
Conduction velocity	Normal	Normal
SNAP** amplitude	Normal	Normal
F waves		
	Normal	Impersistent in bilateral median and peroneal nerves
Impression	Normal study	Findings suggestive of motor axonal neuropathy of all four limbs

*CMAP, compound muscle action potential; **SNAP, sensory nerve action potential

Table 4: Electromyography (EMG) findings

Electromyography (EMG) findings (right deltoid and right vastus lateralis)	
Spontaneous activity	No activity
Insertional activity	Normal
MUAP*	Biphasic/triphasic in morphology 10 ms in duration 200 µv in amplitude
Recruitment	Normal
Interference pattern	Normal

*MUAP, motor unit action potential

Usually, the AMAN variant presents as a pure motor weakness without any sensory or cranial nerve involvement. However, in our case, the patient had bilateral ophthalmoparesis with bifacial weakness, which is a rare presentation. The close differential diagnosis is Miller-Fisher syndrome, characterized by a triad of ataxia, ophthalmoparesis, and areflexia. The presence of ophthalmoparesis and areflexia makes it necessary to exclude Miller-Fisher syndrome. However, the presence of axonal changes in nerve conduction study and profound weakness, with the absence of ataxia and negative serum anti-GQ1 antibody profile favors the diagnosis of AMAN more likely.

Conclusion

This case highlights the occurrence of ophthalmoparesis in the AMAN variant of GBS. To the best of our knowledge and literature search, no case reports are available yet for the same. The presence of ophthalmoparesis and areflexia makes it necessary to exclude Miller-Fisher syndrome. However, the presence of axonal changes in nerve conduction study and profound weakness, with a negative serum anti-GQ1 antibody profile support the diagnosis of AMAN.

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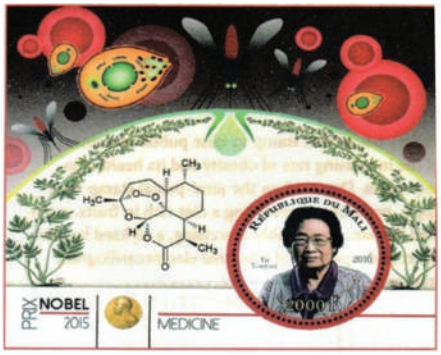
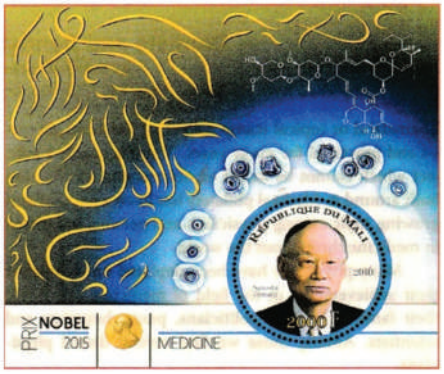
Nobel Prize 2015: Newer Antiparasitic Agents

J V Pai-Dhungat



Tu You You, malaria-infected red blood cells, wormwood tree and chemical structure of artemisinin M/S-(size reduced) Republic of Mali, 2016



Satoshi Omura with a chemical structure of ivermectin and onchocerciasis worm cross-section M/S Republic of Mali, 2016 (size reduced)

Three scientists using modern laboratory techniques discovered antiparasitic drugs long hidden in nature's herbs and soil and won the Nobel Prize in Physiology or Medicine in 2015. Nobel Committee of the Karolinska Institute said their drug therapies "have revolutionized the treatment of some of the most devastating parasitic diseases." In announcing the winners—William C. Campbell (1930) of New Jersey, Satoshi Omura (1935) of Japan, who shared one-half of the award, and Tu You You (1930) of China, won the other half.

Campbell and Omura developed avermectin, the parent of ivermectin, a medicine from the soil in Tokyo that has nearly eradicated river blindness.

Satoshi Omura, Tu You You, Campbell Nobel Prize 2015. Togo, 2015

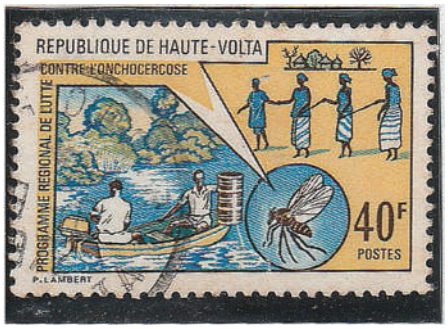
Tu You You (1930) was inspired by Chinese traditional medicine in discovering artemisinin, a drug that is now part of standard antimalarial regimens and that has reduced death rates from *falciparum* malaria. You you is the first mainland Chinese scientist to win the Nobel Prize in the history of science in China.

Tu You You was born in Ningbo, a city in Eastern China. She studied pharmacy at the Beijing Medical College. She attended Peking University Medical School in 1955; Tu You You graduated from Beijing Medical University School of Pharmacy and continued her research on Chinese herbal medicines. In the Academy of Chinese Medical Sciences. Later Tu was trained for 2.5 years in traditional Chinese medicine.

Tu You You worked at the Academy of Traditional Chinese Medicine. By 1971, her team had screened over 2,000 traditional Chinese recipes and made 380 herbal extracts, from some 200 herbs, which were tested on mice malaria.

One compound was effective, sweet wormwood (*Artemisia annua*), which was used for "intermittent fevers," a hallmark of malaria. Its preparation was described in a 1,600-year-old text, in a recipe; at first, it was ineffective because they extracted it with traditional boiling water. Tu discovered that a low-temperature extraction process could be used to isolate an effective antimalarial substance from the plant; sweet wormwood contains the largest amount of artemisinin at the beginning of the flowering period.

She and her colleagues obtained the pure substance and named it qinghaosu or artemisinin. They determined the chemical structure of artemisinin.



Three blind women with black fly below staff spraying chemical agent on the river. Republic of Upper Volta, 2019**

In 1973, Campbell and Omura developed avermectin, the parent of ivermectin, a medicine that is highly effective in onchocerciasis causing river blindness and radically reduces the incidence of filariasis, which causes the disfiguring elephantiasis. In India, the schedule of single annual doses of Diethyl carbamazine citrate + Albendazole for 5 years is reduced to 3 years by the addition of ivermectin to the regime.

Campbell was born in Ramelton, Ireland, in 1930 and earned a Ph.D. at the University of Wisconsin. He worked for decades at the Merck Institute for Therapeutic Research before moving to Drew University in Madison, Campbell and Omura collaborated but worked independently on different aspects of the discovery of avermectin. Omura, who was born in 1935, earned two Ph.D.s from the University of Tokyo, in pharmaceutical sciences and chemistry. He is an emeritus professor at Kitasato University in Tokyo. Omura applied "extraordinary skills in developing unique methods" He isolated *Streptomyces* avermactin from the soil next to the Tokyo tennis court.

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The Enemy within—Multifocal Tuberculous Abscesses Complicating Dermatomyositis

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Sir,

Tuberculosis can present with diverse manifestations, particularly in immunocompromised hosts. Although cold abscesses can complicate spinal tuberculosis, subcutaneous abscesses due to tuberculosis are considerably uncommon and, unlike cold abscesses, necessitate surgical drainage.[1] We present an extremely rare case of disseminated tuberculosis in a patient with subcutaneous involvement mimicking cellulitis.

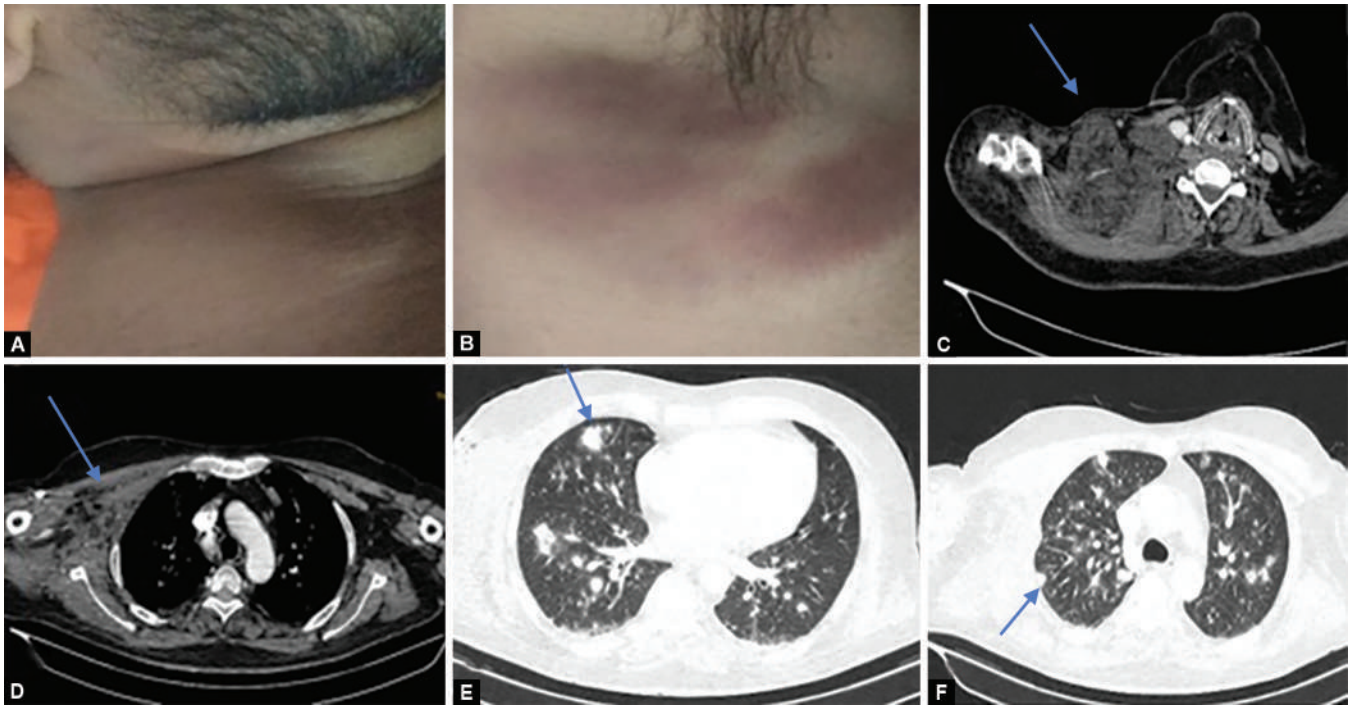
A 42-year-old male presented with progressive proximal lower and upper limb weakness, associated with a facial rash typical for dermatomyositis, developing over 2 months. Elevated creatine phosphokinase, electromyography findings, and muscle biopsy were consistent with dermatomyositis. He was initiated on oral prednisolone (1 mg/kg) and azathioprine. He showed improvement in muscle strength over the next 2 months. In the 3rd month, he presented with fever and painful swelling of the left thigh, associated with overlying erythematous induration of the skin, developing over 5 days. Similar swelling was also noted in the right neck and axilla (Fig. 1). He had enlarged axillary and left inguinal nodes.

He was initially managed for suspected pyogenic cellulitis with intravenous antibiotics for 7 days. Venous Doppler lower limbs showed extensive subcutaneous edema. However, he showed no clinical response. Reexamination on the 8th day revealed fluctuant swellings in the left thigh, right axilla, and right neck. Computed tomography (CT) chest demonstrated large abscesses in the right neck and axillary region, along with pulmonary nodules and consolidation (Fig. 1). Ultrasound (left thigh) showed a large collection. Surgical drainage of the left thigh and right axillary collections yielded frank pus. Ziehl–Neelsen stain showed abundant acid-fast bacilli (AFB). Aerobic and fungal cultures were sterile. Sputum for AFB was negative.

He was managed with extensive surgical drainage, along with antituberculosis therapy (ATT). Steroids and azathioprine were reduced as myositis was stable. Surgical drainage of the left thigh was required twice more over the next 2 weeks due to repeated abscess formation. The fever subsided after 2 weeks. ATT was continued for 12 months in total. The patient showed gradual but complete clinical recovery.

The common sites of development of tuberculous abscesses include the thoracic and abdominal wall and paravertebral region.[1–3] Subcutaneous tuberculous abscesses are highly uncommon and have been previously reported only in six cases.[1] Of these, concomitant pulmonary involvement was seen in five, four were immunocompromised, and one had dermatomyositis. The usual site of predilection was the thigh, and these collections were extensive in immunocompromised patients. All responded well to a combination of surgical and medical management. Unusually, our patient demonstrated simultaneous multifocal involvement, suggesting extensive hematogenous dissemination.

Due to overlying cutaneous changes, tuberculous abscesses may simulate pyogenic abscess or cellulitis, leading to the institution of antibiotics. Suspicion for an alternative etiology must be maintained in patients with compromised immunity and in the absence of clinical response. Combinational therapy is increasingly advocated. Surgical drainage seems crucial because ATT drugs have poor



Figs 1A to F: (A) Presence of a swelling on the right side of the neck; (B) Erythematous induration and swelling seen in the right axillary region; (C) CT demonstrating collection in the neck (arrow); (D) CT demonstrating collection in the axilla (arrow); (E) Organizing collection; (F) Multiple, variable-sized nodules in the lungs (arrows)

penetrability across the abscess wall. Multiple drainage procedures may be required as new locules emerge.

Our case highlights that extrapulmonary tuberculosis may present with atypical features, particularly in immunosuppressed individuals. Early diagnosis and aggressive surgical management, in combination with appropriate ATT, ensure excellent recovery.

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Letter to Editor in Response to Case Report Titled "A Novel Treatment for Malignant Cough Syncope" Published in JAPI 2020;68:83–85

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We read with interest the article titled "A Novel Treatment for Malignant Cough Syncope" published in JAPI 2020;68:83–85. We would like to offer the following comments:

- Authors have used the term "malignant cough syncope" and have also defined it without quoting any reference. Cough syncope is a well-defined term but the use of the term malignant cough syncope has not been found in the literature search except in one case report,[1] wherein the term has not been defined. The justification for using this term needs to be elaborated by the authors.
- The patient in the reported case presented with a chronic cough of 1-year duration. Idiopathic pulmonary arterial hypertension (IPAH) is a very uncommon cause of chronic cough and he should have been worked up to exclude most common causes of chronic cough like, asthma including cough variant asthma, nonasthmatic eosinophilic bronchitis, gastroesophageal reflux, and upper airway cough syndrome.[2] Except for high-resolution computed tomography (which was done to exclude diffuse parenchymal lung disease), other investigations like spirometry, allergy workup, bronchoscopy, rhino sinus imaging, and gastrointestinal endoscopy were also required to be done before attributing the cause of chronic cough, leading to repeated syncope, solely to IPAH.
- Since the diagnosis of IPAH is one of exclusion, arterial blood gas analysis, polysomnography to rule out sleep-disordered breathing, abdominal ultrasound to rule out portopulmonary hypertension, color Doppler of lower limbs to rule out deep vein thrombosis (DVT) should also have been done to label it as IPAH. Authors have mentioned New York Heart Association (NYHA) class III for IPAH which instead should have been the World Health Organization (WHO) functional class (which is NYHA revised functional classification for patients with pulmonary hypertension) and which is one of the important criteria for risk stratification and also for planning specific treatment.[3] Also the risk stratification should have been done for assessment of prognosis and response to treatment and should have included a 6-minute walk test, N-terminal pro-B-type natriuretic peptide (NT-pro BNP) apart from the workup done in the present case.[3]
- Considering his symptomatology, this patient was probably in WHO functional class IV in view of right-sided failure and syncope. He was started on specific therapy with increasing dosages of sildenafil and in view of no significant response, he was put on tablet ambrisentan which also did not yield significant response. The recommended approach, in this case, should have been using upfront combination therapy with tadalafil, ambrisentan, and intravenous epoprostenol (class IIa evidence).[3] Since intravenous epoprostenol is expensive and considering the affordability of the patient he could have been instead given a combination of ambrisentan with tadalafil (class IIb evidence).[3] A multicentre, multinational, blinded, and placebo-controlled trial[4] compared first-line monotherapy with tadalafil or monotherapy with ambrisentan with upfront combination therapy with tadalafil and ambrisentan in *de novo* WHO-FC II and III IPAH patients. The primary endpoint was a composite of clinical failure events (including death, hospitalization, IPAH progression, and unsatisfactory clinical status). The study was positive with a 50% reduction in events in the combination group. In the absence of a significant response to combination therapy, upfront or sequential triple combination therapy with bosentan, sildenafil, and intravenous epoprostenol has also been recommended for such patients. A pilot study[5] on an initial triple combination in 19 WHO-FC class III and IV patients has provided preliminary evidence of the long-term benefits of upfront triple combination therapy in patients with severe IPAH.
- The authors have used atrial septal stenting as a palliative procedure in this case. This invasive procedure with anecdotal evidence of its effectiveness may be offered to patients who have failed maximal drug therapy and as a bridge to lung transplantation.[6] Patients with atrial septal stenting combined with optimum medical therapy fare better with reduced events of spontaneous closure of atrial septostomy and repeat surgeries and improved survival rate compared to patients with atrial septostomy alone.[7] However, the case report does not mention any postprocedure pharmacotherapy. The reported case did not receive maximal drug therapy for IPAH and usage of this intervention in this case may give a wrong message about the indications of this intervention in IPAH.
- The authors have mentioned in the discussion (without quoting any reference) that specific drug therapy in IPAH has minimal effect on long-term survival and is very expensive. This may not be true since comprehensive analysis of survival from the time of diagnosis in a large cohort of patients with IPAH suggests considerable improvements in survival in the past 2 decades.[7] So far as cost is concerned, except for epoprostenol, most of the drugs are affordable by the majority and would have been more cost-effective in the given setting, considering the lack of studies on the long-term prognosis, and course of disease postatrial septostomy and stenting.
- The title of the case report should have conveyed the correct purpose of this novel therapy primarily for IPAH and not malignant cough syncope. Also, the references quoted by the authors are very old.

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