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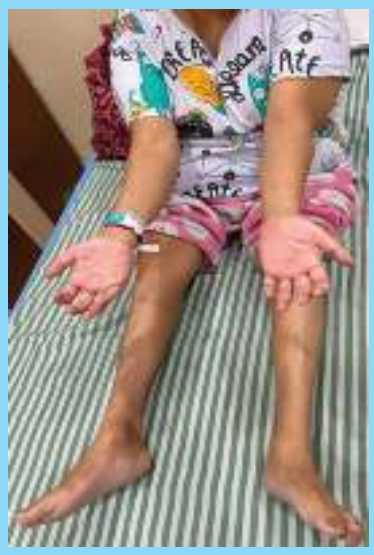

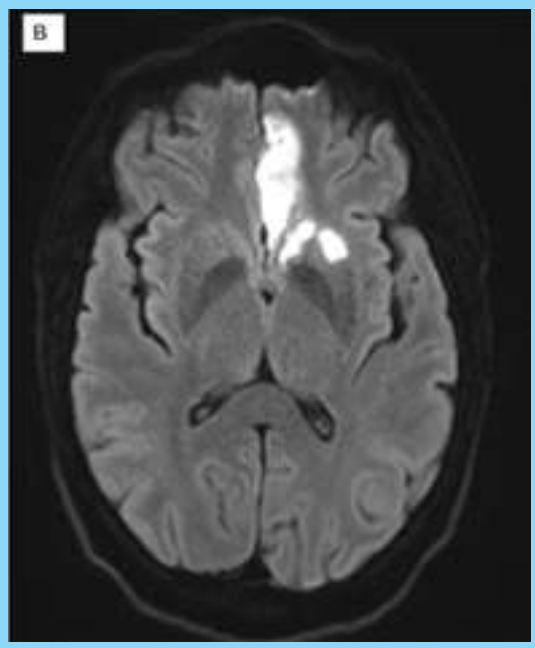

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EDITOR'S NOTE

Dear Friends,

Greetings!

Clearing the haze and smoke surrounding us in our daily lives, we are happy to present the last issue of *JIACM* of 2024. An overwhelming (and unintendedly so) number of articles in this issue pertain to Neurology. It is a happy coincidence that we received and selected most manuscripts from this discipline for our current issue.

Tuberculous Meningitis is explored in two original studies. We also have articles and case reports on neuropathy, meningoencephalitis, acromegaly and Sheehan's syndrome ! Practically, the whole neuraxis has been covered.

The issue carries three eminent and educational reviews on topics and situations we encounter everyday – challenging bronchial asthma, the clinical skill of auditory testing, and how to read the CD nomenclature of cells of the immune system – written by experts in their fields.

The section on *"Images in Clinical Medicine"* brings a very interesting case of Crouzon syndrome – read on to find out. The section on "*Videos in Clinical Medicine*" in the e-journal available at the website *(www.jiacm.in)*, showcases a most dramatic and eye-catching (no pun intended) neurological sign along with a brief write-up. You will get to read about and see this unusual phenomenon, if you visit the website of *JIACM*.

A plethora of rare and unusual cases are presented to add to your knowledge. I encourage you to visit the website *www.jiacm.in* and enjoy all the above offerings and relish the flavours in an electronic format, along with past issues.

The other exciting news is that we may start online submission of manuscripts from the next issue via our website page. Also, the project of archiving past issues is progressing well.

Lastly, I request you *to inform, invite and involve your students, colleagues and friends from India and abroad to read and contribute to JIACM*.

Long live Clinical Medicine, long live *IACM* and long live *JIACM*.

Jai Hind

– Dr Sumeet Singla

CONTENTS

Journal, Indian Academy of Clinical Medicine • Vol. 25, Number 4, October-December, 2024

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is edited by

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ORIGINAL ARTICLE

JIACM 2024; 25 (4): 167-71

Impact of BCG Vaccination on Morbidity and Mortality among Adult Patients with Tuberculous Meningitis: A Prospective Cohort Analysis

Koppuravuri Meher Tej, Amitesh Aggarwal**, Shiva Narang**, Rahul Sharma***, Anish Kumar Gupta*****

Abstract

Background: Tuberculous Meningitis is a severe form of Tuberculosis with high morbidity and mortality. BCG has long been employed to prevent severe forms of tuberculosis, particularly useful in the paediatric population, as suggested by multiple studies. However, there is scarcity of research concerning its efficacy in the adult age group. Consequently, this study was conducted to investigate the impact of BCG vaccination status on Tuberculous Meningitis (TBM) outcomes using the modified Rankin Scale.

Methods: A prospective cohort study was conducted from September 2022 to February 2024, involving adult patients with TBM. Participants were divided into two groups based on BCG vaccination status and followed-up for six months. Morbidity and mortality were assessed using the modified Rankin Scale (mRS). Data were analysed using SPSS, with a Chi-square test to compare outcomes, considering a p-value <0.05 as significant. Nutritional status and BCG vaccination were key factors in the analysis.

Results: In a study of 65 participants with TBM, the mean age was similar between BCG-vaccinated and unvaccinated groups, with more females than males. Clinical symptoms included fever, headache, and seizures, with some participants also having pulmonary TB. Nutritional status varied, with 29% being underweight. Over a six-month follow-up, no significant differences were found between vaccinated and unvaccinated groups or between different BMI categories in terms of functional dependence and mortality. Statistical analysis showed no significant correlation between BCG vaccination or BMI and clinical outcomes.

Conclusion: The findings indicate that morbidity and mortality outcomes in adult and adolescent patients with TBM are similar regardless of BCG vaccination status. Additionally, no significant correlation was found between malnutrition and severe disease or increased mortality. This lack of association may be due to the dominant influence of TBM, which appears to have a consistent impact on all study participants.

Key words: Tuberculous Meningitis (TBM), BCG, adult age group, adolescent age group.

Introduction

Tuberculous meningitis (TBM) occurs when *M. tuberculosis* spreads to the cerebrospinal fluid and meninges, leading to conditions such as ischaemia, hydrocephalus, and increased intracranial pressure, which cause significant brain injury and neurodisability. Key risk factors for TBM include young age and HIV infection. The BCG (Bacillus Calmette-Guérin) vaccine, used for over five decades to prevent tuberculosis (TB), is particularly effective in young children. According to a meta-analysis by Fine[1], the BCG vaccine offers moderate protection - 50% against any form of TB, 64% against TBM, and 71% against TB-related deaths – with greater efficacy against miliary TB and TBM compared to pulmonary TB. While numerous studies confirm the BCG vaccine's effectiveness in reducing morbidity and mortality in children with severe forms of TB, such as miliary tuberculosis and TBM, research on its impact in adults is limited. This study aimed to assess the relationship between BCG vaccination status and the morbidity and mortality outcomes in non-HIV adolescents and adults with TBM, as well as to explore the influence of nutritional status on these outcomes. The primary objective was to compare the morbidity and mortality of TBM based on BCG vaccination status, while the secondary objective was to examine the outcomes in relation to nutritional status.

Material and Methods

An observational prospective cohort study was conducted between September 2022 and August 2023. Patients with TBM were divided into two groups based on their BCG vaccination status and were followed-up over time. Eligible patients, who were newly diagnosed with TBM, over 12 years of age, and HIV-negative, were selected from the wards and ICU of the hospital after meeting the inclusion criteria. Follow-up occurred in person at OPDs or via telephone, weekly for the first two months and biweekly

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for the next four months. Morbidity and mortality were assessed at each follow-up stage using the modified Rankin Scale (mRS), where a score of 0 - 2 indicated functional independence, and a score of 3 - 6 indicated functional dependence, with 6 signifying death.

Participants who met the inclusion criteria and provided informed consent were enrolled in the study. Their demographic and clinical profiles were recorded, including a comprehensive clinical examination within 24 hours of admission, which covered history-taking, examination of BCG scars, and assessment of nutritional status. BCG vaccination was verified by examining for a scar on the upper arms, historical recollection, or available documentation, such as a vaccine card. In the absence of a scar or documentation, patients were classified as unvaccinated. Participants' height and weight were measured at recruitment to calculate their BMI. The sample size was determined using Epi Info 7 software, requiring a minimum of 30 participants per group. Participants were categorised into BCG vaccinated and unvaccinated groups and further divided into underweight and normal/overweight groups based on their nutritional status. Those lost to follow-up were excluded from the final analysis.

Data was entered into an MS Excel sheet and analysed using SPSS 20.0 software. Categorical variables were presented as proportions, and continuous variables as means with Standard Deviation (SD). A Chi-square test was employed to compare morbidity and mortality outcomes between the groups, with a p-value of <0.05 considered statistically significant.

Results

The study included 65 adult and adolescent participants, as shown in Fig. 1. The mean age was 27.5 (± 15.5) years in the unvaccinated group and 29.4 (± 14.7) years in the vaccinated group. Of the participants, 25 (38.4%) were male and 40 (61.6%) were female, with 12 unvaccinated males and 15 unvaccinated females.

Clinical Profile: Participants presented with symptoms including fever, headache, altered sensorium, nausea, vomiting, and seizures. Some also had co-morbidities, with pulmonary TB being the most common. The distribution of symptoms and co-morbidities between the two groups is summarised in Table I.

Nutritional Profile: Based on Asian BMI criteria, 29% of participants were underweight (BMI <18.5), 51% had normal weight (BMI 18.5 - 22.9), and 20% were overweight (BMI ≥23).

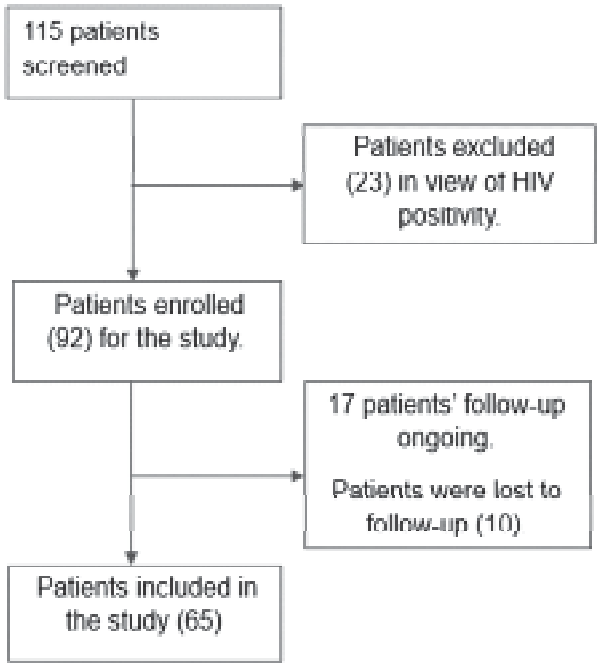

Fig. 1: *Flow diagram for the study.*

Table I: Table depicting the presenting symptoms and comorbidity profile of the participants.

	Unvaccinated Group (n = 30); (Number of participants, percentage)	Vaccinated group (n = 35); (Number of participants, percentage)
Symptom		
Fever ≥30 Days	14 (46.6%)	12 (34.2%)
Headache	28 (93.3%)	34 (97.1%)
Altered Sensorium	30 (100%)	30(85.7%)
Altered Sensorium ≥3 days	13 (43.3%)	13 (37%)
Seizures	9 (30%)	10 (28.5%)
Nausea and Vomiting	26 (86.6%)	31 (88.5%)
Co-morbidity		
Extra Neural TB	15 (50%)	20 (57.1%)
Pulmonary TB	15 (50%)	16 (45.7%)
Past History of TB	5 (16.6%)	7 (20%)
Hypertension	1 (3%)	3 (8.5%)
Diabetes	0 (0%)	2 (5.7%)

Follow-up: Participants were followed for six months. Functional dependency and mortality rates were analysed based on vaccination status (Table II) and nutritional status (Table III) using Chi-square analysis. Initially, 100% of

Table II: Table depicting the follow-up of participants and fraction of participants who were functionally dependent at follow-up and overall mortality according to vaccination status.

	Total Participants (n = 65)		
Follow-up Duration	Unvaccinated Participants (N = 30) Functionally Dependent*	Vaccianted Participants (N=35) Functionally Dependent*	p-Value
Week 4	29 (96.7%)	35 (100%)	0.462
Week 5	26 (86.7%)	31 (88.6%)	1.00
Week 6	26 (86.7%)	28 (80%)	0.475
Week 7	23 (76.7%)	25 (71.4%)	0.63
Week 8	20 (66.7%)	22 (62.9%)	0.74
Week 10	16 (53.3%)	18 (51.4%)	0.87
Week 12	14 (46.7%)	17 (48.6%)	0.87
Week 14	14 (46.7%)	16 (45.7%)	0.93
Week 16	14 (46.7%)	15 (42.9%)	0.758
Week 18	14 (46.7%)	15 (42.9%)	0.758
Week 20	13 (43.3%)	15 (42.9%)	0.96
Week 22	13 (43.3%)	15 (42.9%)	0.96
Week 24	13 (43.3%)	15 (42.9%)	0.96
Overall Mortality	12 (40.0%)	13 (37.1%)	0.81

**During follow-up in case of the death of a participant the mRS(score-6) of that participant is carried forward.*

Table III: Table depicting the follow-up of participants and fraction of participants who were functionally dependent at follow-up and overall mortality according to nutrition status.

	Total Participants (n = 65)		
Follow-up Duration	Unvaccinated Participants (N = 30) Functionally Dependent*	Vaccianted Participants (N=35) Functionally Dependent*	p-Value
Week 4	19 (100%)	45 (97.8%)	1.00
Week 5	17 (89.5%)	40 (87.0%)	1.00
Week 6	16 (84.2%)	38 (82.6%)	1.00
Week 7	16 (84.2%)	32 (69.6%)	0.353
Week 8	14 (73.7%)	28 (60.9%)	0.326
Week 10	10 (52.6%)	24 (52.2%)	0.973
Week 12	8 (42.1%)	23 (50.0%)	0.562
Week 14	8 (42.1%)	22 (47.8%)	0.674
Week 16	8 (42.1%)	21 (45.7%)	0.794
Week 18	8 (42.1%)	21 (45.7%)	0.794
Week 20	8 (42.1%)	20 (43.5%)	0.919
Week 22	8 (42.1%)	20 (43.5%)	0.919
Week 24	8 (42.1%)	20 (43.5%)	0.919
Overall Mortality	7 (36.8%)	18 (39.1%)	0.863

**During follow-up in case of the death of a participant the mRS (score-6) of that participant is carried forward.*

participants were functionally dependent in both vaccinated and unvaccinated groups. Over time, this proportion decreased, with similar percentages of functional dependency at the end of six months in both unvaccinated and vaccinated groups (43.3% vs. 42.9%; p = 0.96). Mortality rates were also comparable (40.0% vs. 37.1%; p = 0.81). Additionally, of the 65 participants, 19 (29.2%) were underweight (BMI <18.5 kg/m^2), and the rest had normal or overweight BMI. The proportion of functionally dependent patients decreased gradually in both groups during the follow-up. By the end of 24 weeks, the proportion of functionally dependent participants was similar between underweight and normal/overweight participants (42.1% vs. 43.5%; p = 0.919), with comparable overall mortality rates (36.8% vs. 39.1%; p = 0.863).

Discussion

This study aimed to evaluate the morbidity and mortality outcomes of TBM in relation to BCG vaccination and nutritional status in non-HIV adolescents and adults. The findings showed that morbidity and mortality rates were similar across both vaccinated and unvaccinated groups, as well as across different nutritional status groups, indicating a consistent and uniform impact on the participants.

While BCG vaccination is known to offer substantial protection against severe TB forms, including TBM, especially in children, evidence regarding its efficacy in adults is less conclusive[2]. The decline in BCG efficacy with age might be due to waning immunity or misclassification. A case-control study by Thilothammal *et al*[3] showed that BCG efficacy was high (79% - 82%) in children under 8 years but dropped to 19% in older children, highlighting diminished immunity with age.

Despite extensive research indicating a significant protective effect of BCG against TBM, our study did not find a significant correlation between malnutrition and severe disease or increased mortality. This discrepancy may be due to the overwhelming impact of TBM, which seems to exert a uniform effect across all participants.

Rodrigues *et al* conducted a meta-analysis demonstrating BCG's strong protective effect against TBM and miliary TB, with 86% protection in randomised trials and 75% in other

studies. However, the protection against pulmonary TB varied, likely due to factors such as patient age, waning BCG effectiveness, and the diverse mechanisms of pulmonary TB[4]. Colditz *et al* in a meta-analysis of 1,264 abstracts confirmed a 64% protective effect of BCG against TBM across various study designs and populations[5].

A cross-sectional study by Kumar *et al* suggested that BCG's protective effect may be influenced by factors such as high infection doses from household contacts, severe malnutrition, and declining immunity over time. In their study of 150 children, vaccinated and unvaccinated groups showed notable differences, particularly in the duration of altered sensorium, which was significantly longer in the unvaccinated group[6].

A case-control study by Kumar *et al* also found that the absence of a BCG scar in TBM cases yielded a crude odds ratio of 2.28. Factors like higher TB contact rates, longer time since BCG vaccination, and older age were observed in some cases, although BCG's protective effect persisted after accounting for these variables[7].

In an observational study by Kelekçi, BCG positivity was a protective factor against mortality, with zero mortality in the vaccinated group, underscoring BCG's protective role in the first two years of life[8]. Barday *et al* in a cohort study spanning 1985 - 2020 found that unvaccinated children with TBM exhibited more severe disease, emphasizing the impact of the global BCG shortage in 2015 on TBM severity in the subsequent years[9].

Zachariah's observational study of 1,181 TB patients found that those with severe malnutrition, age >35 years, and HIV seropositivity had a higher risk of early mortality[10]. Similarly, Kumar *et al* in a case-control study showed significant differences in average weight and height among TBM patients, highlighting the importance of nutritional status on TBM outcomes[7].

In a retrospective cohort study by Bhargava *et al*, lower pre-treatment weight was significantly associated with TB death in men but not in women. They concluded that nutritional support should be provided to severely underweight TB patients to reduce mortality risk[11].

Feleke *et al* in a cross-sectional study found that malnutrition odds were 47% higher in extra-pulmonary TB patients compared to pulmonary TB patients, with a high prevalence of underweight individuals among TB patients[12].

Ren M *et al* in a case-control study on nutritional risk in TBM children found that those at nutritional risk had higher rates of complications, cranial nerve damage, and drug-induced liver injury, highlighting the economic burden and health challenges associated with nutritional deficiencies in TBM patients[13].

Our study has limitations, including being a single-center study in the national capital, which may limit generalisability. The six-month follow-up period, while informative, may be insufficient for a comprehensive understanding of TBM outcomes. Accurately determining BCG vaccination status was challenging, particularly among older participants, leading to potential misclassification and impacting the study's precision regarding BCG vaccination and TBM outcomes.

Exploring BCG's potential protective efficacy in adults against TBM remains relatively underexplored. Conducting a multicentric study with an extended follow-up period could provide a more robust understanding, allowing for broader generalisation and extrapolation of findings.

Conclusion

This prospective cohort study examined TBM outcomes in relation to BCG vaccination and nutritional status. Sixty-five newly diagnosed TBM patients were divided into groups based on vaccination status (unvaccinated vs. vaccinated) and nutritional status (underweight vs. normal/overweight). The overall morbidity and mortality were similar across both vaccination and nutritional status groups. No substantial difference in outcomes was observed among adult TBM patients based on BCG vaccination or nutritional status.

Tuberculosis and its complications have had a significant and enduring impact on global health. Despite medical advancements, TB remains a formidable challenge, emphasizing the need for ongoing research, prevention, and treatment efforts to alleviate its global burden.

References

1. Fine PEM. Variation in protection by BCG: implications of and for heterologous immunity. *The Lancet* 1995; 346.
2. Kumar R, Dwivedi A, Kumar P, Kohli N. Tuberculous meningitis in BCG vaccinated and unvaccinated children. *J Neurol Neurosurg Psychiatry* 2005; 76.
3. Thilothammal N, Krishnamurthy P V, Runyan DK, Banu K. Does BCG vaccine prevent tuberculous meningitis? *Arch Dis Child* 1996; 74.
4. Rodrigues LC, Diwan VK, Wheeler JG. Protective effect of bcg against tuberculous meningitis and miliary tuberculosis: A meta-analysis. *Int J Epidemiol* 1993; 22.
5. Colditz GA, Brewer TF, Berkey CS *et al*. Efficacy of BCG Vaccine in the Prevention of Tuberculosis: Meta-analysis of the Published Literature. *JAMA: J Amer Med Asso* 1994; 271.
6. Kumar R, Singh SN, Kohli N. A diagnostic rule for tuberculous meningitis. *Arch Dis Child* 1999; 81.
7. Kumar P, Kumar R, Srivastava KL, Kumar M. Protective role of BCG vaccination against tuberculous meningitis in Indian children: A reappraisal. *National Med J India* 2005; 18.
8. Kelekci S, Karabel M, Karabel D *et al*. Bacillus Calmette-Guérin is a preventive factor in mortality of childhood tuberculous meningitis. *Inter J Infectio Dis* 2014; 21.

9. MA Barday. The impact of BCG non-vaccination on TBM severity in a TBM-endemic setting, Cape Town: A Case-Controlled Study 2021.
10. Zachariah R, Spielmann MP, Harries AD, Salaniponi FML. Moderate-to-severe malnutrition in patients with tuberculosis is a risk factor associated with early death. *Trans R Soc Trop Med Hyg* 2002; 96.
11. Bhargava A, Chatterjee M, Jain Y *et al*. Nutritional Status of Adult Patients with Pulmonary Tuberculosis in Rural Central India and Its Association with Mortality. *PLoS One* 2013; 8.
12. Feleke BE, Feleke TE, Biadglegne F. Nutritional status of tuberculosis patients, a comparative cross-sectional study. *BMC Pulm Med* 2019; 19.
13. Ren M, Chen L, Shu M *et al*. Relationship between nutritional factors and clinical outcome in children with tuberculous meningitis. *Zhonghua Er Ke Za Zhi* 2022; 60.

ORIGINAL ARTICLE

JIACM 2024; 25 (4): 172-76

Prevalence and Characteristics of Neuropathy in Hyperthyroidism: A Cross-Sectional Study

Vani Singh, Sathish Mandala**, Santosh Kumar Singh***, Samir Samadarshi****, Sandeep Kumar*****, Abhishek Kumar******, Satish Kumar*******, Rohit Vashisht*********

Abstract

***Background:** Hyperthyroidism can cause various neurological manifestations, including neuropathy. However, the prevalence and characteristics of neuropathy in hyperthyroidism are not well understood.*

***Methods:** This cross-sectional study included 65 adult patients with newly diagnosed or established hyperthyroidism. Patients underwent clinical neurological examination, nerve conduction studies (NCS), and laboratory investigations. Patients with other possible causes of neuropathy, i.e., diabetes mellitus, chronic alcoholism, nutritional deficiencies, chronic liver or kidney disease, malignancies, Human Immunodeficiency Virus (HIV) infection, family history of neuropathy and use of drugs known to cause neuropathy were excluded.*

***Results:** 40% of patients had neurological symptoms, while 10.7% had abnormal NCS findings (n = 7). Axonal neuropathy was diagnosed in 6 out of 7 cases, with 1 case of mixed axonal and demyelinating neuropathy. Older age and longer disease duration were significantly associated with abnormal NCS results. 30% of patients with normal NCS results complained of paresthesias, suggesting that NCS may miss subtle neurological changes.*

***Conclusion:** Neurological symptoms are predominant in hyperthyroidism. NCS may miss subtle neurological changes despite significant neurological symptoms. The study also showed that older age and longer disease duration are the two factors which are linked to abnormal NCS result among the study population.*

***Key words:** Hyperthyroidism, Nerve conduction studies, neurological symptoms.*

Introduction

The thyroid gland secretes two major hormones: Thyroxine (T4) and Triiodothyronine (T3), with T4 accounting for 93% of the secretion and T3 for 7%. T4 acts as a prohormone and a reservoir for the more active T3, which is formed as needed in the tissues. Thyroid hormones play a crucial role in normal growth and metabolism, acting on nearly all nucleated cells[1]. Thyroid hormones are essential for normal brain development as they influence neurogenesis, neuronal and glial cell differentiation and migration, synaptogenesis, and myelination[2].

Hyperthyroidism is defined as suppressed thyroid stimulating hormone (TSH) and high serum concentration of T3 and/or free T4. Thyrotoxicosis refers to a hypermetabolic state that results from excessive amounts of circulating thyroid hormones including extrathyroidal sources such as exogenous intake or release of preformed hormone. The clinical presentation of hyperthyroidism or thyrotoxicosis varies from asymptomatic (subclinical) to life-threatening (thyroid storm). Common symptoms include palpitations, unintentional weight loss, hyper defaecation and heat intolerance[3]. Thyroid storm is an endocrine emergency, and when undiagnosed, can lead to serious complications such as delirium, muscle weakness, atrial fibrillation, congestive heart failure (CHF), cardiovascular collapse, and death[4].

Neurological manifestations of hyperthyroidism include seizures, encephalopathy, neuropsychiatric manifestations, tremors, choreoathetosis, myopathy, hypokalaemia periodic paralysis, and neuropathy. These conditions may be related to the effects of thyroid hormone on mitochondria, cytoskeletal elements, and neurotransmitter systems. Treatment of hyperthyroidism usually leads to the resolution of these neurological symptoms[5]. The pathogenesis of neuropathy in hyperthyroidism is not fully understood, but several mechanisms have been proposed. One theory suggests that excessive thyroid hormones have a direct neurotoxic effect, disrupting nerve function due to abnormal metabolic activity. Another possibility is an immune-mediated process, where the immune response

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that targets the thyroid in conditions like Graves' disease also affects the nerves. Additionally, the hypermetabolic state of hyperthyroidism may deplete nerves of essential nutrients and energy, impairing their function. Vascular changes affecting blood flow to the nerves have also been suggested as a contributing factor. Overall, neuropathy in hyperthyroidism likely results from a combination of these factors[6]. In a prospective study of hyperthyroid patients 19% had sensory-motor axonal neuropathy in nerve conduction studies (NCS)[7].

Neurophysiological features of axonal degeneration typically include normal or near normal nerve conduction velocity with reduced compound muscle action potential (CMAP) amplitudes, or reduced sensory nerve action potential (SNAP) amplitudes, and the presence of fibrillation in denervated muscles. On the other hand, neurophysiological features of segmental demyelination include conduction block, slowed nerve conduction velocity (NCV) across the affected segment, prolonged distal latency, and temporal dispersion[8].

We decided to carry out a study to find the prevalence of neurological signs and symptoms in newly diagnosed patients of hyperthyroidism and to evaluate the electrophysiological evidence of neuropathy in these patients.

Material and Methods

This cross-sectional observational study was conducted at a tertiary care teaching hospital for a period of two years from July 2021 to June 2023. Adult patients of newly detected or established hyperthyroidism (supressed TSH and high free T4) attending internal medicine, endocrinology, or neurology out-patient departments were included in the study. Patients with other possible causes of neuropathy, i.e., diabetes mellitus, chronic alcoholism, nutritional deficiencies, chronic liver or kidney disease, malignancies, Human Immunodeficiency Virus (HIV) infection, family history of neuropathy and use of drugs known to cause neuropathy were excluded.

All patients fulfilling inclusion criteria were asked about any neurological symptoms and underwent a clinical neurological examination. NCS was carried-out for all participants according to standardised protocols. Laboratory investigations included complete blood count (CBC), erythrocyte sedimentation rate (ESR), red blood cell (RBC) indices, liver function test (LFT), kidney function test (KFT), serum vitamin B12, serum folate, TSH, free T4 and free T3.

Axonal neuropathy was diagnosed if compound muscle action potentials (CMAP) were reduced in motor nerves, or reduced sensory nerve action potentials (SNAP), with normal or slightly reduced nerve conduction velocities. Demyelinating neuropathy was diagnosed if distal motor latencies were increased along with slowing of nerve conduction velocities, and absent or delayed F-waves[9].

Sample size was calculated by assuming an estimated prevalence of neuropathy in hyperthyroidism (p) to be 50%, and applying finite correction factor (N) as 75 (based on the average number of patients with hyperthyroidism likely to report during the study period). For 95% confidence level and a confidence limit of 5%, the sample size calculated was 63.

Data was analysed using open-source statistical software. Numerical variables were expressed as mean and categorical variables were reported as proportions. The two-sample t-test (Student's t) was used for analysing quantitative variables with normal distribution and Chi ($\chi 2$) square test was used for categorical variables. For all tests of significance, p-values less than 0.05 were considered to be statistically significant.

Results

Fig. 1 shows the flow of participants in the study. A total of 65 newly diagnosed or previously established cases of hyperthyroidism were included in the study. 29.23% were females (n = 19) and 70.74% were males (n = 46). The mean age of the study population was 42.3 ± 15 years. The most common diagnosis was Graves' disease (n = 44). 16 participants had toxic multi-nodular goitre and 5 patients had thyroiditis.

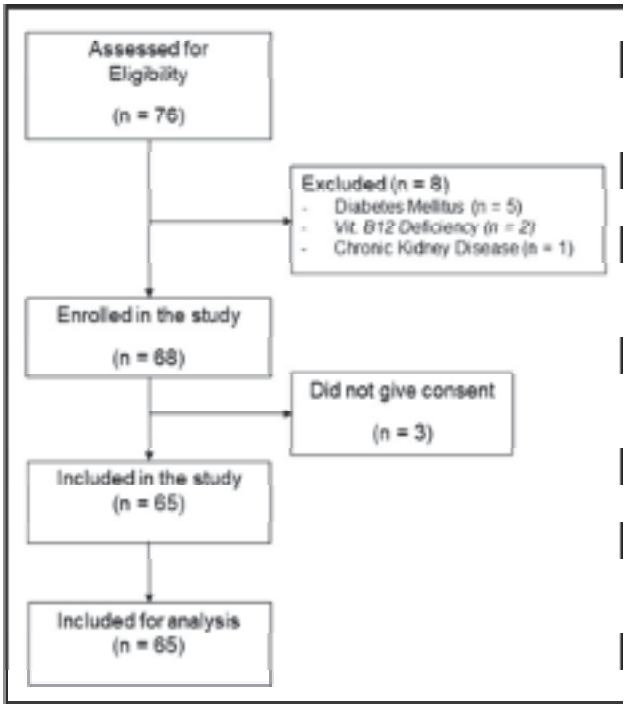

Fig. 1: *Flow of Participants in study.*

Commonest symptom which led the participants to seek medical attention were tremors and palpitations followed by weight loss. Fig. 2 depicts the presenting complaints with which the study participants reported to our hospital. The commonest symptoms were weight loss and tremors. 62 participants were treated with a combination of antithyroid medication (Carbimazole) and/or a non-selective beta blocker (Propranolol), while 3 participants did not require any treatment.

Table I lists the neurological symptoms and signs observed among the study participants. The predominant symptom seen among the study participants was paraesthesia (n = 22) and the most common neurological sign observed was graded sensory loss in the lower limbs (n = 9). 2 patients had hypokalaemia induced flaccid quadriparesis and 2 patients had sensory ataxia.

Table I: Neurological symptoms and signs among the study participants.

Neurological Symptoms/Signs	Number of participants
Paraesthesia in one or more limbs	22
Flaccid paralysis of all 4 limbs	2
Ataxia	2
Graded sensory loss in lower limbs	9
Attenuated deep tendon reflexes	4
Loss of proprioception	2

10.7% participants (n = 7) had abnormal NCS findings. Table II summarises the abnormal NCS findings observed. A diagnosis of axonal neuropathy was made in 6 out of 7 cases. 3 had sensory axonal neuropathy, 2 had sensory motor axonal neuropathy and 1 participant had pure motor axonal neuropathy. 1 participant was found to have mixed axonal and demyelinating sensory neuropathy in the ulnar nerve distribution.

Table II: Abnormal NCS findings among the study participants.

NCS Findings		Number of participants
Axonal Neuropathy	Sensory axonal neuropathy involving bilateral lower limbs	3
	Pure motor axonal neuropathy bilateral common peroneal nerve	1
	Sensory motor axonal neuropathy in all 4 limbs	2
Axonal + Demyelinating Neuropathy	Axonal + demyelinating sensory neuropathy bilateral ulnar nerves	1

The mean age of participants with abnormal NCS was 61.6 ± 16.1 years as compared to those with normal NCS being 39.7 ± 12.9 years (p value <0.0001). 6 out of 7 participants with abnormal NCS had duration of illness more than 4 years, compared to 15 out of 58 participants with normal NCS (p value 0.00067). There was no statistically significant

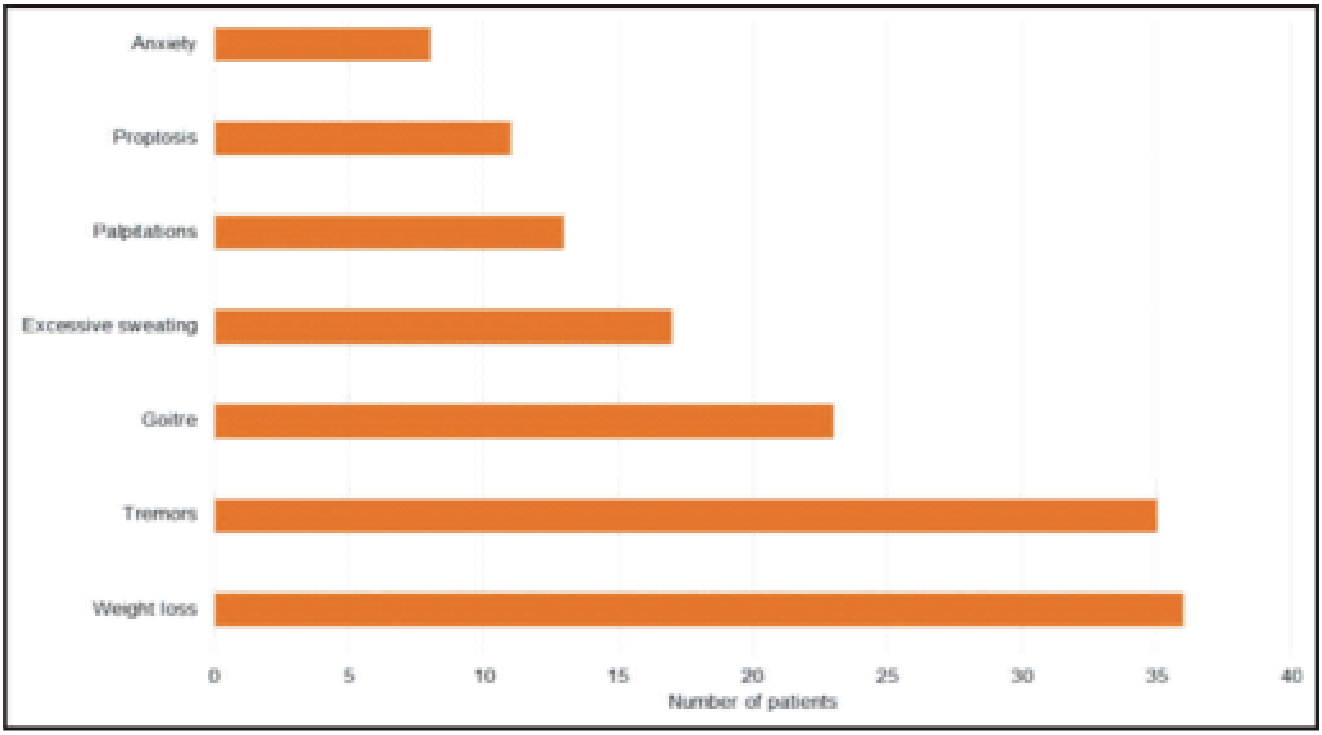

Fig. 2: *Presenting complaints among the study participants.*

difference in the thyroid function test between those with normal NCS and abnormal NCS findings.

Discussion

In our study, among 65 participants with newly diagnosed or previously established hyperthyroidism, 7 had abnormal NCS findings (10.7%). Duyff *et al* evaluated 21 patients with hyperthyroidism and found that symmetric distal sensory disturbances in the limbs combined with depressed ankle tendon jerks, clinically consistent with a polyneuropathy, was present in 4 patients (19%) and 2 patients had subclinical sensory carpal tunnel syndrome[10]. In a similar study by Berlit *et al* 8 out of 27 hyperthyroid patients (29.6%) were found to have sensory neuropathy of the Sural nerve in NCS[11].

Out of the 7 participants with abnormal NCS, 4 had sensory and 1 participant had pure motor axonal neuropathy. 2 participants were found to have thyrotoxic paralysis associated with hypokalaemia. Their NCS showed sensory motor axonal neuropathy in all 4 limbs. Kumar *et al* studied 400 patients with thyroid disorder and reported that 144 of them had neuropathy, of which 4 patients had hyperthyroidism and 56 had hypothyroidism[12]. Kelly *et al* reported that 7 out of 10 patients with thyrotoxic periodic paralysis showed reduced CMAP on NCS[13].

Proximal myopathy has predominantly been associated with hyperthyroidism especially Graves' disease[12]. However, our study demonstrates that electro-physiologically confirmed neuropathy can also be found in patients with hyperthyroidism. Review of literature showed few case reports from India, demonstrating the occurrence of polyneuropathy in hyperthyroid patients. Mohamed *et al* reported that in 45-year-old male patient with Basedow's paraplegia, NCS showed mixed motor and sensory polyneuropathy[14]. Malakar *et al* reported thyrotoxic neuropathy in a 47-year-old female with NCS showing mixed sensory motor neuropathy[15].

Age and duration of illness were the two factors which were found to be significantly associated with the presence of abnormal NCS results in the study population. The age of participants with abnormal NCS was significantly higher than those with normal NCS (p value <0.0001). Majority of patients with abnormal NCS has disease duration longer than 4 years as compared to those with normal NCS (p value 0.00067).

There are few limitations of the present study. We did not have a control group of normal individual to compare the results of NCS. Electromyography was not carried out as part of electro-diagnostic studies. This was a cross-sectional study and the participants were not followed-up over time to look for resolution or worsening of symptoms. Longitudinal case control studies are required to further establish the natural course of neuropathy in hyperthyroid patients.

Conclusion

To conclude, in our study of 65 hyperthyroidism patients, 40% had neurological symptoms. However, only 10.7% had abnormal NCS result. 6 out of 7 had axonal neuropathy and 1 had mixed axonal and demyelinating neuropathy. 30% patients had normal NCS results, despite complaining of paresthesias, suggesting that NCS may miss subtle neurological changes. The study also showed that older age and longer disease duration are the two factors which are linked to abnormal NCS result among the study population.

References

1. Stathatos N. Thyroid physiology. *Med Clin North Am* 2012; 96 (2): 165-73.
2. Bernal J. Thyroid Hormones in Brain Development and Function. In: Feingold KR, Anawalt B, Blackman MR *et al*. eds. Endotext. South Dartmouth (MA): MDText.com, Inc.; January 14: 2022.
3. Lee SY, Pearce EN. Hyperthyroidism: A Review. *JAMA* 2023; 330 (15): 1472-83.
4. Chiha M, Samarasinghe S, Kabaker AS. Thyroid storm: an updated review. *J Intensive Care Med* 2015; 30 (3): 131-40.
5. H Mod. Salah, Faisal Mohammed Alyahya, Barradah HFK *et al*. Overview on Neurological Manifestations of Thyroid Disease. *J Pharmaceutic Res Inter* 2021; 33 (56B): 171-8.
6. Al-Wahaibi AK, Kumar S, Al-Risi A, Wali F. Thyrotoxic Neuropathy: A rare cause of acute flaccid paraplegia. *Sultan Qaboos Univ Med J* 2017; 17 (4): e460-e463.
7. Duyff RF, Van den Bosch J, Laman DM *et al*. Neuromuscular findings in thyroid dysfunction: a prospective clinical and electrodiagnostic study. *J Neurol Neurosurg Psychiatry* 2000; 68 (6): 750-55.
8. Huynh W, Kiernan MC. Nerve conduction studies. *Aust Fam Physician* 2011; 40 (9): 693-7.
9. Lehmann HC, Wunderlich G, Fink GR *et al*. Diagnosis of peripheral neuropathy. *Neurol Res Pract* 2020; 2: 20.
10. Duyff RF, Van den Bosch J, Laman DM *et al*. Neuromuscular findings in thyroid dysfunction: a prospective clinical and electrodiagnostic study. *J Neurol Neurosurg Psychiatry* 2000; 68 (6): 750-55.
11. Berlit P, Mahlberg U, Usadel KH. Zur Frage der Polyneuropathie bei Hyperthyreose – eine klinisch-neurophysiologische Studie [Polyneuropathy in hyperthyroidism – a clinical neurophysiologic study]. *Schweiz Arch Neurol Psychiatr (1985)* 1992; 143 (1): 81-90.
12. Kumar MSS, Ganesan R, Nithyanandham A, Prabhu E. Evaluation of prevalence of neuromuscular disorders in thyroid disorders in tertiary care institution - An interdepartmental inter institutional study. *IAIM* 2019; 6 (3): 176-81.
13. Kelley DE, Gharib H, Kennedy FP *et al*. Report of 10 cases and review of electromyographic findings. *Arch Intern Med* 1989; 149

(11): 2597-600.

14. Mohamed Saqlain, Jayachandra; Nanjundaiah, Nagaraj. Basedow's Paraplegia, A Rare Presentation of Severe Untreated Hyperthyroidism. *APIK J Inter Med* 2022; 10 (2): 131-2.
15. Malakar S, Sharma KN, Rana A *et al*. Thyrotoxic neuropathy - a case report. *Ind J Case Reports* 2019; 28: 515-7.
16. Ganesan R, Arumugam M, Pachaiappan A *et al*. A study of assessment in peripheral neuropathy in patients with newly detected thyroid disorders in a tertiary care teaching institute. *J Evolution Med Dent Sci* 2016; 5 (54): 3671-82.

MEDICAL COUNCIL OF INDIA (MCI)/NATIONAL MEDICAL COMMISSION (NMC) GUIDELINES FOR AUTHORS (AMENDED), 2020

As per notification No. MCI-12(2)/2019-Med. Misc./189334 dated 12 February, 2020 published in Extraordinary Gazette of Govt. of India, the MCI/NMC has made changes to amend the "Minimum Qualifications for Teachers in Medical Institutions Regulations, 1998". These will be part of "Minimum Qualifications for Teachers in Medical Institutions (Amendment) Regulations, 2019" and shall come into force from the date of their publication in the Official Gazette.

1. Original papers, meta-analysis, systematic reviews, and case series that are published in journals included in Medline, Pubmed Central, Citation index, Sciences Citation index, Expanded Embase, Scopus, Directory of Open access journals (DoAJ) will be considered.
2. The author must be amongst first three or should be the Corresponding author.

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ORIGINAL ARTICLE

JIACM 2024; 25 (4): 177-80

Prevalence of Hepatitis C Infection among Patients of Chronic Kidney Disease in a Tertiary Care Hospital of Western Uttar Pradesh

Abha Gupta, Shweta Sharma**, Anupam Nirala****

Abstract

***Introduction:** Chronic kidney disease (CKD) and Hepatitis C, both are menacing public health problems, Hepatitis C virus (HCV) can easily be transmitted via haemodialysis. HCV leads to greater mortality and morbidity due to cirrhosis, while also increasing the progression of CKD. The aim of this study was to determine the prevalence of HCV infection among patients of CKD.*

***Methods:** It was a cross-sectional study of CKD patients who were admitted in Medicine department in LLRM Medical College, Meerut, which is a tertiary care center in Western UP. Proper clinical history and laboratory investigations were noted and testing for HCV infection was conducted in all patients. Diagnosis of HCV was confirmed by HCV RNA (RT PCR) and positive Anti HCV IgG antibody.*

***Results:** Total 146 patients of CKD were included in the study out of which 54.50% were males and 44.50% were females. Most of the patients were in age group 35 - 50 years (52.05%). Our study showed prevalence of HCV in CKD patients to be about 6.84% with male predominance.*

***Conclusions:** Prevalence of HCV infection in CKD patients is high as compared to the general population. Screening for HCV with HCV RNA (RT PCR) is recommended. Strict precautions should be taken in hospitals and dialysis units to prevent it's transmission.*

***Key words:** Chronic kidney disease, prevalence, Hepatitis C.*

Introduction

Currently India has around 10 - 15 million people infected with Hepatitis C virus (HCV) with a prevalence of 0.5 - 1.5%[1]. In renal replacement therapy units, HCV infection is a significant cause of morbidity and mortality among haemodialysis (HD) patients, and management of such patients becomes complicated in view of HCV infection. The prevalence of HCV among dialysis patients in India is reported to range between 10% and 40%[2-7].

For patients with severe renal impairment, acute renal failure, and stage IV chronic kidney disease, HD is a simulated way of maintaining homeostasis in the body. Many patients undergo dialysis for prolonged periods of time and are exposed to various side-effects as a consequence of dialysis. The spread of HCV to patients on HD is generally hospital acquired and potential risk factors include failure to disinfect devices, sharing of single-use vials for infusion, improper techniques, contaminated dialysis equipment, and supplies, contamination by attending personnel. However, long-standing vascular exposure and manifold blood transfusions also increases the risk.

The aim of these study was to estimate the prevalence of Hepatitis C infection among patients of CKD and to compare the prevalence of HCV infection in CKD patients on maintenance HD with those CKD patients not on maintenance HD.

Material and Methods

The study was conducted in SVBP Hospital, Meerut, (UP) during the period of one year. It was cross-sectional, single centre study.

All cases in the study were admitted in the hospital and evaluated for hepatitis C infection. The study included consenting adult patients of CKD and excluded CKD patients who had any haematological disorder or HIV infection.

A questionnaire was made to ensure proper data collection. The data collected included age, sex, occupation, duration of haemodialysis, number of blood units transfused, history of hypertension or diabetes mellitus.

1. Cut-off for CKD was defined as:

   GFR (eGFR) less than 60 mL/min/1.73 m^2 (GFR category G3a - G5) or,

   Persistent proteinuria by urinary dipstick for 3 months or more[8].

2. eGFR was calculated by CKD-EPI creatinine equation

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141 × min (SCr/, 1) α × max/κ × max (SCr/≥, 1) -1.209 × 0.993 age (× 1.018 if female) (× 1.159 if black).

Scr = standardised serum creatinine in mg/dL

κ = 0.7 (females) or 0.9 (males)

α = -0.329 (female) or -0.411 (male)

min (Scr/κ, 1) is the minimum of Scr/κ or 1.0

max (Scr/κ, 1) is the maximum of Scr/κ or 1.0 age (years)[8].

3. The diagnosis of HCV was made via HCV RNA (RT - PCR) and /or positive AntiHCV IgG serology (3rd Gen ELISA) and was done in all patients.

Results

A total of 146 patients were included in the study. Majority of the patients were in the 18 - 65 year age group. Of all the patients of CKD, 54.50% were males and 44.50% females (Table I and II).

Out of all CKD patients most were in the age group 35 - 50 years (52.05%), followed by those >65 years (17.80%) followed by 50 - 65 years (22.60%) and the least number of patients were in age group >18 - 35 years of about 7.53%.

Out of these patients, 51.36% were on MHD and 48.63% were not on MHD (Table XIII). In the patients of CKD on MHD, 18 - 65 year age group was about 81.70% and 18.70% were in age group >65 years. Males in this group contributed about 50.70% and females were about 49.30% (Table III and V). In the patients of CKD not on MHD, 18 - 65 year age group was about 83% and 17% were in age group >65 years. Males in this group contributed about 60.60% and females were about 39.40% (Table IV and VI).

In the patients of CKD on MHD, HCV positive status was found in 9.33%, out of which, 18 - 65 year age group was 100% and no patients were in age group >65 years. Males in this group contributed about 57.10% and females were about 42.90% (Table IX and X).

In patients of CKD not on MHD, 4.22% patients were found to be HCV positive, out of which all patients were of age group 18 - 65 years. 66.7% were males and 33.3% were females (Table VII and VIII).

Total number of patients found to be HCV positive were 6.84%, all were from age group 18 - 65 years. Of this group males were about 60% and females were about 40% (Table XI and XII).

There was no association between being on MHD and HCV status (p = 0.062).

Table I: Age distribution of CKD patients (n = 146).

Age (years)	Patients
>18 - 35	11 (7.53%)
35 - 50	76 (52.05%)
50 - 65	33 (22.60%)
>65	26 (17.80%)
Total	146

Table II: Gender distribution of CKD patients (n = 146).

Gender	Patients
Male	81 (54.50%)
Female	65 (44.50%)
Total	146

Table III: Age distribution of CKD patients on MHD (n = 75).

Age (years)	Patients
>18 - 65	61 (81.30%)
>65	14 (18.70%)
Total	75

Table IV: Age distribution of CKD patients not on MHD (n = 71).

Age (years)	Patients
>18 - 65	59 (83.0%)
>65	12 (17.0%)
Total	71

Table V: Gender distribution of CKD patients on MHD (n = 75).

Gender	Patients
Male	38 (50.7%)
Female	37 (49.3%)
Total	75

Table VI: Gender distribution of CKD patients not on MHD (n = 71).

Gender	Patients
Male	43(60.6%)
Female	28(39.4%)
Total	71

Table VII: Age distribution of CKD patients not on MHD with HCV positive status (n = 3).

Age (years)	Patients
>18 - 65	03 (100%)
>65	00 (0%)
Total	03

Table VIII: Gender distribution of CKD patients not on MHD with HCV positive status (n = 3).

Gender	Patients
Male	02(66.7%)
Female	01(33.3%)
Total	03

Table IX: Age distribution of CKD patients on MHD with HCV positive status (n = 7).

Age (years)	Patients
>18 - 65	07 (100%)
>65	00 (0%)
Total	07

Table X: Gender distribution of CKD patients on MHD with HCV positive status (n = 7).

Gender	Patients
Male	04(57.10%)
Female	03(42.90%)
Total	07

Table XI: Gender distribution of CKD patients with HCV positive status (n = 10).

Gender	Patients
Male	06(60%)
Female	04(40%)
Total	10(100%)

Table XII: Age distribution of CKD patients with HCV positive status (n = 10).

Age (years)	Patients
>18 - 65	10 (100%)
>65	00 (0%)
Total	10 (100%)

Table XIII: Total distribution of CKD patients with respect to HCV status.

Status	HCV+	HCV (-ve)	Total
On MHD	07 (9.33%)	68 (90.66%)	75 (51.36%)
Not on MHD	03 (4.22%)	68 (90.66%)	71 (48.63%)
Total	10 (6.84%)	136 (93.15%)	146 (100%)

Discussion

This study shows a prevalence of HCV infection among CKD patients of about 6.84%. In patients of CKD not on MHD 4.22% of the patients were HCV reactive and in patients of CKD on MHD 9.33% of patients were HCV reactive. Various other studies have been conducted in India on the prevalence of HCV infection in CKD patients on Renal Replacement Therapy. All these studies indicate high prevalence (10 - 40%)[2-7] which do not corroborate with the prevalence of 9.33% in this study. This study shows prevalence of HCV in CKD patients to be about 6.84%.

In the context of India, the prevalence of Hepatitis C infection in CKD patients in this study is significantly higher than the general population (0.5 - 1.5%)[1]. In a study done in Pakistan, by Shafi *et al*, the frequency of Hepatitis C in CKD patients was about 27.2%[9]. In another study done by Fabrizi *et al* in Italy, hepatitis C antibody was seen in 20% of CKD patients[10]. Male predominance was seen in our study with 60% of HCV positive patients which is comparable to 69% males in a study done by Arora *et al*, this can be due to higher incidence of CKD per se, and in males owing to higher incidence of diabetes and hypertension. Most common age group was 35 - 50 years, as in other studies, again attributed to high incidence of CKD in this age group[11]. In this study the prevalence of HCV infection in females was found to be around 40%.

Conclusions

In conclusion, a significant number of chronic kidney disease (CKD) patients (6.84%) have hepatitis C virus (HCV) infection. Studies done previously in India have also shown that prevalence of HCV in CKD patients is about 10% to 40%, which is not seen in this study. This could be due to more advanced screening methods and increased awareness in people about Hepatitis C and CKD in the recent years. This indirectly also shows the increased efficiency of screening methods for Hepatitis C in recent times. Furthermore, this decline could also be due to availability

of separate Dialysis machines for seropositive patients in recent years in dialysis units.

To prevent HCV infection, it is critical to test patients before they start dialysis. Hospitals and dialysis centers should also follow strict safety measures and separate machines should be used for seropositive and seronegative patients.

References

1. Bhattacharya PK, Roy A. Management of hepatitis C in the Indian context: an update. *J Liver* 2015; 4 (187): 2167-0889.
2. Jaiswal SP, Chitnis DS, Naik G. Prevalence of anti-HCV antibodies in central India. *Indian J Med Res* 1996; 104: 177-81.
3. Gosavi MS, Shah SK, Shah SR *et al*. Prevalence of hepatitis C virus (HCV) infection in Mumbai. *Indian J Med Sci* 1997; 51 (10): 378-85.
4. Chandra M, Khaja MN, Hussain MM. Prevalence of hepatitis B and hepatitis C viral infections in Indian patients with chronic renal failure. *Intervirol* 2004; 47 (6): 374-6.
5. Reddy AK, Murthy KV, Lakshmi V. Prevalence of HCV infection in patients on haemodialysis: survey by antibody and core antigen detection. *Indian J Med Microbiol* 2005; 23 (2): 106-10.
6. Medhi S, Potukuchi SK, Polipalli SK. Diagnostic utility of hepatitis C virus core antigen in haemodialysis patients. *Clin Biochem* 2008; 41 (7-8): 447-52.
7. Jasuja S, Gupta AK, Choudhry R. Prevalence and associations of hepatitis C viraemia in haemodialysis patients at a tertiary care hospital. *Indian J Nephrol* 2009; 19 (2): 62-7.
8. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int Suppl* 2013; 3 (1): 1-150.
9. Shafi ST, Hassan MZ, Saleem M *et al*. Frequency of Hepatitis C in hospitalised patients with chronic kidney disease. *Pak J Med Sci* 2017; 33 (1): 18-21.
10. Fabrizi F, Marcelli D, Bacchini G *et al*. Antibodies to hepatitis C virus (HCV) in chronic renal failure (CRF) patients on conservative therapy: prevalence, risk factors and relationship to liver disease. *Nephrol Dial Transplant* 1994; 9 (7): 780-4.
11. Arora A, Bansal N, Sharma P *et al*. Hepatitis C Virus Infection in Patients with End-Stage Renal Disease: A Study from a Tertiary Care Centre in India. *J Clin Exp Hepatol* 2016; 6 (1): 21-5.

ORIGINAL ARTICLE

JIACM 2024; 25 (4): 181-86

Infarction Patterns among Patients with Tuberculous Meningitis: An Entity with Diversity in Itself

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Introduction

Tuberculosis (TB) is one of the most common infectious diseases of developing countries. As per World Health Organisation (WHO) Global TB report 2022, the incidence of TB increased. The number had increased to 10.6 million people worldwide with a total mortality of approximately 1.6 million (~15.1%)[1]. Tuberculosis is caused by *Mycobacterium tuberculosis*, an acid-fast bacillus producing granulomatous lesions. Primary infection evokes protective response in the human body whereas Post-primary TB evokes destructive response. When the host has insufficient immunity as seen in children, elderly, immunocompromised (primary or secondary) or immunosuppressed persons, primary TB occurs where granulomas are formed. There can occur dissemination to other organs via lymphatics or blood which produces a whole clinical spectrum of disseminated TB leading to meningitis, miliary tuberculosis, renal and adrenal TB, third space effusions, abscesses etc[2]. Amongst all the systemic manifestations, Central nervous system Tuberculosis (CNS TB) accounts for approximately 1 - 2% of cases but with high morbidity and mortality burden. In CNS TB, small mycobacteria rich foci form in one of the three sites namely brain, spinal cord, or meninges. Most common presentation of CNS TB is tuberculous meningitis (TBM), whereas encephalitis, tuberculoma/s, vasculitis, ventriculitis, myelitis or tuberculous abscess can also be seen[3]. Infarcts are not uncommon in patients with TBM. Back in 1992, Hsieh and colleagues observed that amongst all the infarctions which occur in TBM, 75% involved what is known as tubercular zone. This Tubercular zone (also known as TB zone) is the area which is supplied by the medial lenticulostriate and thalamo-perforating arteries. In contrast to this, the ischaemic zone is the area which is supplied by the lateral lenticulostriate, anterior choroidal and thalamo-geniculate arteries. As described classically by Hsieh *et al*, the "TB zone" involves (1) Head of caudate nucleus, (2) Genu of internal capsule (3) Anterior limb of internal capsule and (4) Anteromedial Segment of thalamus whereas lentiform nucleus, posterior limb of internal capsule, posterolateral segment of thalamus contribute to the "Ischaemic zone"[4]. Recent case reports also show these two classical patterns and outcome of infarctions in tuberculous meningitis[5,6].

Material and Methods

This was a retrospective study conducted in the Department of Neurology and General Medicine from a period of March 2019 till March 2024. Only patients with TBM having infarction were included in this series. Clinico-demographic profile and Magnetic Resonance Imaging (MRI) findings were studied. CSF study was conducted on the day of the admission and MRI was carried-out within 48 hours of hospital admission. Co-morbidities and risk factors contributing to ischaemia were not excluded from the study. Patients with ischaemic stroke prior to onset of TBM were excluded from the study. The outcome and disability at the time of discharge were studied. Since this was a retrospective study, waiver of consent and institutional ethics committee was taken.

Results

Amongst all 20 (100%) total patients studied, there were 13 (65%) males and 7 (35%) females. The average age was found to be 47.6 years. Major co-morbidities in these patients were hypertension, diabetes mellitus and hypothyroidism. 6 (30%) patients had pulmonary TB. Most common site involvement other than CNS was abdominal. Mean duration of illness was 21.95 days. 6 (30%) patients had history of contact with TB patient. None of the patients had immunocompromised status in the form of HIV seropositivity. Five (25%) patients had modified Rankin Scale (mRS) of 2, five (25%) had mRS of 3, two (10%) had mRS of 4, four (20%) patients had mRS of 5 and three (15%) had mRS of 6 indicating death. All the patients received antitubercular treatment as per National guidelines along with corticosteroids and antiplatelets. 6 patients had seizures warranting the addition of antiseizure medications. Ventriculoperitoneal shunt was required in 1 patient and 2 patients needed decompressive craniotomy due to mass effect of the lesion. Table I. Shows the clinico-demographic

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profile of patients with TBM with infarction.

The Magnetic Resonance Imaging (MRI) Brain findings of these patients along with CSF findings are depicted in Table II. Fig. 1 and Fig. 2 show the various infarction patterns that were found in these patients. Eleven out of 20 patients had multiple infarctions and 17 out of 20 were supratentorial (Table III). 10 patients had TBM grade III according to Palur grading whereas 7 had Palur grade IV and only 3 had Palur grade II (Table IV).

Table I: Showing the clinico-demographic profile of patients of CNS tuberculosis.

S. No.	Age	Sex	Co-morbidities	History of Pulmonary Tuberculosis	Extra-pulmonary involvement besides CNS	Duration of illness	Treatment for tuberculosis taken or not before arrival to hospital	Treatment (duration)	Contact with patients with tuberculosis	HIV Status	Back-ground	Treatment Received	mRS at discharge	Outcome
1.	62	Male	Coronary artery disease	Yes	Abdominal lymphade-nopathy	3 months	Yes (1 month)	HRZE	No	Negative	Urban	Antitubercular treatment Antiplatelets Corticosteroid	5	Alive
2.	69	Male	Hypertension, diabetes mellitus	No	No	15 days	No	No	No	Negative	Urban	Antitubercular treatment Antiplatelets Corticosteroid Antiseizure medication	5	Alive
3.	29	Female	None	No	No	20 days	No	No	No	Negative	Urban	Antitubercular treatment Antiplatelets Corticosteroid	2	Alive
4.	25	Female	Hypothyroidism	No	No	20 days	No	No	No	Negative	Urban	Antitubercular treatment Antiplatelets Corticosteroid Antiseizure medication	2	Alive
5.	52	Male	None	Yes	No	5 days	No	No	No	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid Antiedema measures Ventriculoperitoneal shunt	5	Alive
6.	60	Female	Diabetes mellitus, Hypertension, COPD, DCMP	Yes	No	7 days	No	No	No	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid Diuretics Antiseizure medication	5	Alive
7.	34	Female	None	No	No	10 days	No	No	No	Negative	Urban	Antitubercular treatment Antiplatelets Corticosteroid	3	Alive
8.	22	Female	None	No	No	15 days	No	No	No	Negative	Urban	Antitubercular treatment Antiplatelets Corticosteroid	6	Death
9.	47	Male	Diabetes mellitus	No	No	27 days	No	No	Yes	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid	3	Alive
10.	38	Female	None	No	No	21 days	No	No	Yes	Negative	Urban	Antitubercular treatment Antiplatelets Corticosteroid	3	Alive
11.	55	Male	None	No	No	60 days	No	No	No	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid	3	Alive
12.	54	Male	Hypertension, Hypothyroidism, Diabetes Mellitus	No	No	18 days	No	No	Yes	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid	2	Alive
13.	65	Male	Diabetes Mellitus	Yes	Abdominal	7 days	No	No	Yes	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid Antiseizure medication Decompressive craniotomy	6	Death
14.	39	Male	None	No	No	10 days	No	No	No	Negative	Urban	Antitubercular treatment	2	Alive

											Antiplatelets Corticosteroid		
15.	27	Female	None	No	No	24 days	No	No	No	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid	4	Alive
16.	30	Male	None	No	No	15 days	No	No	No	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid	3	Alive
17.	44	Male	None	Yes	No	15 days	No	No	No	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid	2	Alive
18.	67	Male	None	Yes	No	23 days	No	No	Yes	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid Antiseizure medication Decompressive craniotomy	6	Death
19.	81	Male	Hypertension, Diabetes Mellitus	No	No	7 days	No	No	Yes	Negative	Rural	Antitubercular treatment Antiplatelets Corticosteroid Antiseizure medication	4	Alive
20.	52	Male	Hypertension	No	No	30 days	No	No	No	Negative	Urban	Antitubercular treatment Antiplatelets Corticosteroid	2	Alive

Table II: Showing the Radiological (MRI Brain) and CSF findings profile of patients of CNS tuberculosis.

S. No.	MRI Brain Findings	CSF Glucose	Blood Sugar	CSF Protein	CSF Cbnaat for MTB	Rifampicin Resistance	ZN Stain	Gram Stain	Cytology
1.	Acute infarct in left pons	73	104	12	Detected	Not Detected	NEG	NEG	N30L70
2.	Acute infarct in right cerebellar hemisphere, left basal ganglia and right medial temporal lobe	56	96	41	Detected	Not Detected	NEG	NEG	N10L90
3.	Acute infarcts left capsuloganglionic region and left gyrus rectus and basifrontal areas.	70	82	112	Detected	Not Detected	NEG	NEG	N40L60
4.	Acute infarcts in bilateral capsuloganglionic regions, right corona radiata, bilateral basifrontal and right temporal lobe	61	112	101	Detected	Not Detected	NEG	NEG	N30L70
5.	Acute infarct left anteromedial thalamus	79	105	102	Not Detected	Not Detected	NEG	NEG	N60 L40
6.	Acute infarcts in the right thalamus, thalamocapsular region, right medial temporal lobe, right cerebral peduncle and left cerebellar peduncle	56	89	112	Detected	Not Detected	NEG	NEG	N30L70
7.	Acute infarcts in bilateral anteromedial thalamus (Right> Left)	78	100	100	Detected	Not Detected	NEG	NEG	N30L70
8.	Acute infarct in left dorsal pons	59	115	46	Detected	Not Detected	NEG	NEG	N10L90
9.	Areas of diffusion restriction in splenium of corpus callosum suggestive of acute infarct	155	88	48	Detected	Not Detected	NEG	NEG	N10L90
10.	Left Cerebellar infarct	114	98	90	Not Detected	Not Detected	NEG	NEG	N30L70
11.	Acute Vasculitic infarct in basal ganglia	39	120	59	Not Detected	Not Detected	NEG	NEG	N40L60
12.	Brainstem infarcts	47	126	66	Detected	Not Detected	NEG	NEG	N30L70
13.	Large Right MCA territory infarct with haemorrhagic transformation	46	188	81	Detected	Not Detected	NEG	NEG	N10L90
14.	Acute Vasculitic infarct in basal ganglia	113	156	10	Not Detected	Not Detected	NEG	NEG	N10L90
15.	Acute Vasculitic infarct in basal ganglia	30	106	271	Not Detected	Not Detected	NEG	NEG	N10L90
16.	Acute infarct in posterior limb of internal capsule	34	98	32	Detected	Not Detected	NEG	NEG	N30L70
17.	Acute infarct in posterior limb of internal capsule	89	130	66	Detected	Not Detected	NEG	NEG	N30L70
18.	Large right MCA territory infarct	58	90	25	Detected	Not Detected	NEG	NEG	N10L90
19.	Right frontal infarct	206	450	84	Not Detected	Not Detected	NEG	NEG	N10L90
20.	Acute infarcts in bilateral anteromedial thalamus and anterior limb of internal capsule	67	103	78	Not Detected	Not Detected	NEG	NEG	N40L60

Table III: Distribution of infarctions

Number of infarctions	Single infarction	9
	Multiple infarctions	11
Distribution of infarctions	Supratentorial	17
	Infratentorial	3
Duration	Acute	20
	Chronic	0

Table IV: TBM Palur grade

Palur grade	Clinical features	GCS Score	Number of patients
I	No neurological deficit	15	0
II	Neurological deficit	15	3
III	Altered sensorium, easily arousable	9-14	10
IV	Deeply comatose	3-8	7

Discussion

The prevalence of stroke in TBM ranges from 15% to 67%, and when it does occur, it is typically linked to unfavourable outcomes[7]. Infarction patterns associated with TBM have been previously documented. Infarcts in the TB zone have been the subject of numerous research and case studies as a poor prognostic indicator. In our study, a worse prognosis was indicated by the subcortical location of infarcts in the tubercular zone. Hsieh and colleagues (1992) examined the location of infarcts in patients with TBM, as was previously mentioned. According to their observations, of 14 patients, 75% had an infarction in the "TB zone" and only 11% in the "Ischaemic zone." They observed that TB zone infarctions that were bilaterally symmetrical were more common in TBM (71%)[4]. A different study conducted in 2016 by Tai MLS *et al* found that 34 patients, or 67% of the 51 patients examined, had cerebral infarction. Using Hsieh's patterns to categorise the infarctions, 20 out of 34 patients (or 59%), had infarcts in both the "TB zone" and the "ischaemic zone." Conversely, infarcts in the "ischaemic zone" and "TB zone" were seen in 12 patients (35%) and 2 patients (6%), respectively[8]. In 2019, Soni *et al* examined 90 TBM patients. They discovered that cerebral infarcts occurred in 57.7% of the participants in the study. There were 35%, 13%, and 15% of these infarctions in the ischaemic, tubercular, and both zones, respectively. In TBM, there can be microscopic or macroscopic vascular involvement. Subcortical lacunar infarcts, large blood vessels occlusions, and various other presentations might result from the involvement of small, medium, and large vessels. It has been observed that MTB-induced direct vessel wall

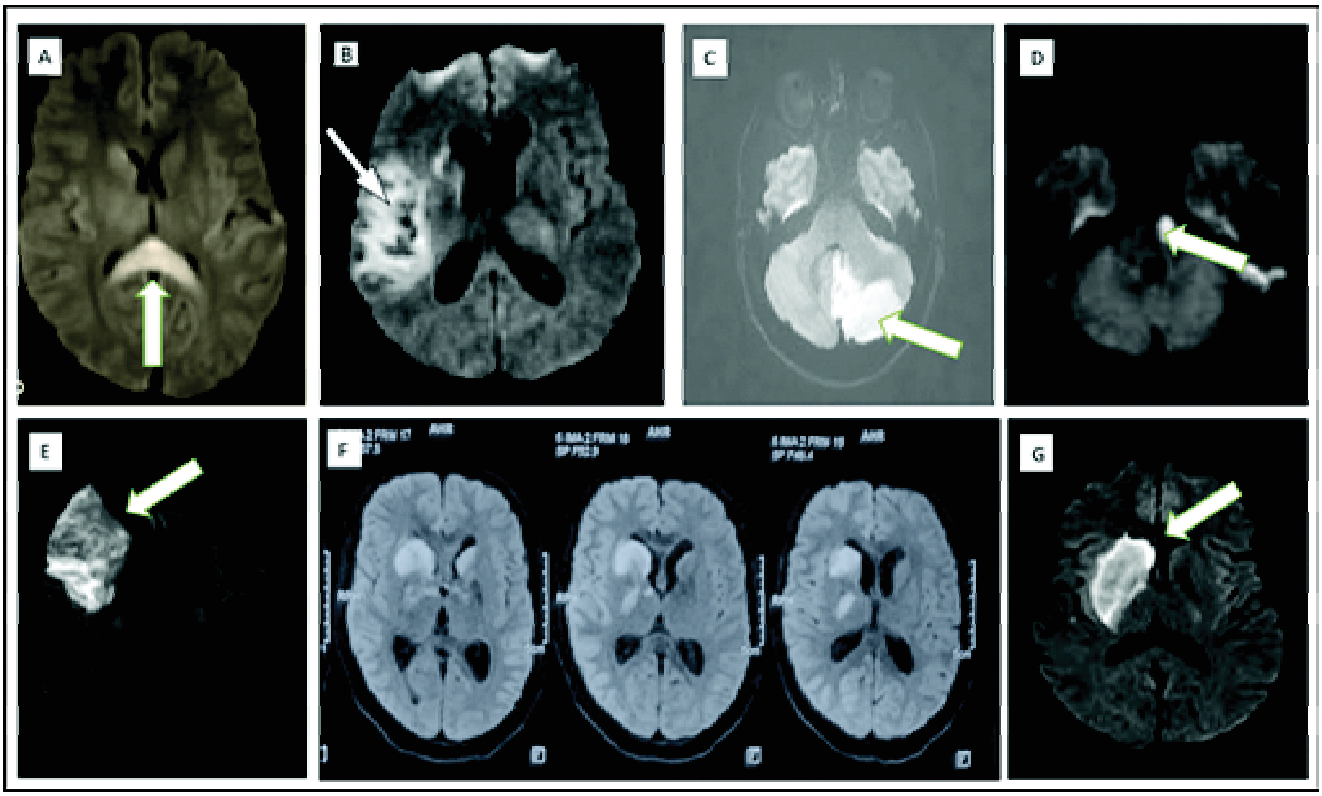

Fig. 1: *A. Splenium Infarct B. Right MCA Infarct with haemorrhagic transformation C. Left Cerebellar Infarct D. Left Pontine Infarct E. Right MCA Infarct F. Multiple Thalamoganglionic Infarcts G. Right Caudate and Putamen Infarcts.*

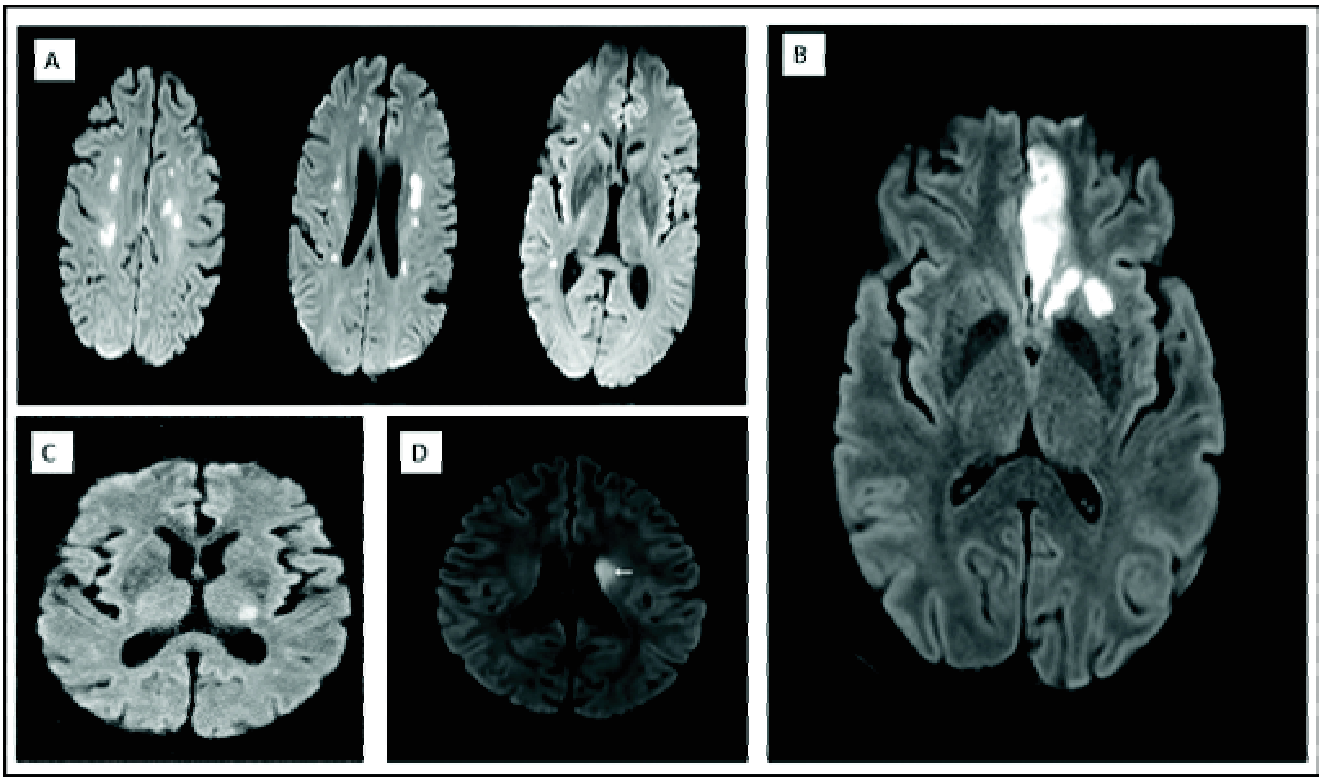

Fig. 2: *A. Multiple Infarcts in cortical and subcortical regions B. Infarcts in Left Basi-frontal and Basal Ganglia region C. Acute Infarct in Posterior Limb of Internal Capsule D. Acute Infarct in left Corona Radiata.*

damage, immunological vessel wall destruction in vasculitis, accelerated atherosclerosis, and finally thrombogenesis, can all play a role in the development of stroke in TBM patients. The same study found a negative correlation between the load of cerebral infarctions, grade of TBM, association with stroke, and overall severity of illness[9]. The most recent systematic review, which was done in 2022, included 71 studies totalling 2194 TBM patients who experienced a stroke. It was discovered that the expected probability of stroke in TBM was 0.30 (95% CI, 0.26 - 0.33). According to their analysis, the pooled proportions of poor outcomes and death were 0.51 and 0.22, respectively[10]. In our study, the subcortical location of infarcts in tubercular zone heralded poorer prognosis, Amongst 20 cases, 13 (43%) were subcortical, 3 out of 20 (15%) were cortical and 4 out of 20 (2%) were brainstem infarcts. Among those 10 out of 20 (50%) were in tubercular zone, 3 out of 20 (15%) were in ischemic zone and 14 out of 20 (70%) were in other areas. In another study on 40 patients with TBM, two-thirds of patients complicated by cerebral infarct had poor outcome despite adjunct dexamethasone therapy. Stroke was associated with poor outcome at 3 months of treatment but not at 6 months. This may be due to the high frequency of basal ganglia lacunar stroke which have better outcome compared to those with larger stroke[11]. In another study, like many other studies, the most frequent locations of infarction were internal capsule and basal ganglia. Stroke, especially the middle cerebral artery territory infarct, was associated with poor prognosis at 6 months. However, outcome of TBM was also found to be associated with many other factors such as cranial nerve and focal neurologic deficits, vision impairment, meningeal enhancement, advanced stage of TBM, low Glasgow coma scale score, baseline modified Rankin scale and high protein content of CSF[12]. In another study significant poor prognostic indicators (for patients who either deteriorated or died) among patients having stroke were presence of cranial nerve deficit, internal capsule infarct, centrum semiovale and brainstem infarcts. Infarcts in posterior cerebral circulation had poor prognosis[13]. There are no specific guidelines but various studies in the existing literature are in favour of antiplatelet agents as repurposing drug in infection-related ischaemia, cancer-related strokes etc. Asian studies also found efficacy and relative safety of antiplatelet agents in TBM related infarctions[14-18]. Efficacy of use of antiplatelet agents for mortality benefit cannot be completely estimated in the present study due to very small sample size, different infarction patterns and coexisting comorbidities. Although hypertension, diabetes mellitus, cardiomyopathies, etc., predispose individuals to ischaemic stroke, it is impossible to rule-out the confounding effect of these co-morbidities in onset of ischaemic cerebrovascular ischaemic events in

patients with TBM. However, this adds to the limitation of the present study.

Conclusion

The pattern of infarction in TBM can assist in forecasting outcomes. The variables which also add-in include immune status of patient (HIV reactive), TBM clinical grade, rapid deterioration, response to antitubercular treatment including resistance patterns as well as CSF findings. Large infarctions as well as multiple infarctions carry a poorer prognosis.

References

1. World Health Organisation (WHO). Global Tuberculosis Report 2022. Geneva, WHO, 2022. www.who.int/publications/i/item/9789240061729.
2. Jilani TN, Avula A, Zafar Gondal A *et al*. Active Tuberculosis. [Updated 2023 Jan 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513246.
3. Rock RB, Olin M, Baker CA *et al*. Central nervous system tuberculosis: pathogenesis and clinical aspects. *Clin Microbiol Rev* 2008; 21 (2): 243-61.
4. Hsieh FY, Chia LG, Shen WC. Locations of cerebral infarctions in tuberculous meningitis. *Neuroradiology* 1992; 34: 197-9.
5. Kaushal H, Goyal G. Revisiting the tubercular zone: A poor prognostic finding on neuroimaging. *J Neurosci Rural Pract* 2024; 15: 370-2.
6. Kaushal H, Goyal G, Bhatt R, Kain R. Neuroimages: Ischaemic Zone and Tubercular Zone- "Two Faces of the Same Coin", Clinical Research and Reports. *Bio Res Scientia Publishers* 2024; 2 (2): 1-2.
7. Misra UK, Kalita J, Maurya PK. Stroke in tuberculous meningitis. *J Neurol Sci* 2011; 303: 22-30.
8. Tai MS, Viswanathan S, Rahmat K *et al*. Cerebral infarction pattern in tuberculous meningitis. *Sci Rep* 2016; 6: 38802.
9. Soni N, Kumar S, Shimle A *et al*. Cerebrovascular complications in tuberculous meningitis-a magnetic resonance imaging study in 90 patients from a tertiary care hospital. *Neuroradiol J* 2020; 33: 3-16.
10. Sy MC, Espiritu AI, Pascual JL. Global frequency and clinical features of stroke in patients with tuberculous meningitis: A systematic review. *JAMA Netw Open* 2022; 5: e2229282.
11. Chan KH, Cheung RT, Lee R. Cerebral infarcts complicating tuberculous meningitis. *Cerebrovasc Dis* 2005; 19 (6): 391-5.
12. Anuradha HK, Garg RK, Agarwal A *et al*. Predictors of stroke in patients of tuberculous meningitis and its effect on the outcome. *QJM* 2010; 103 (9): 671-8.
13. Kalita J, Misra UK, Nair PP. Predictors of stroke and its significance in the outcome of tuberculous meningitis. *J Stroke Cerebrovasc Dis* 2009; 18 (4): 251-8.
14. Patrignani P, Patrono C. Aspirin, platelet inhibition and cancer prevention. *Platelets* 2018; 29: 779-85.
15. Rothwell PM, Price JF, Fowkes FG *et al*. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: Analysis of the time course of risks and benefits in 51 randomised controlled trials. *Lancet* 2012; 379: 1602-12.
16. Ogundeji AO, Pohl CH, Sebolai OM. Repurposing of Aspirin and Ibuprofen as Candidate Anti-Cryptococcus Drugs. *Antimicrob Agents Chemother* 2016; 60: 4799-4808.
17. Lee MR, Lee MC, Chang CH *et al*. Use of Antiplatelet Agents and Survival of Tuberculosis Patients: A Population-Based Cohort Study. *J Clin Med*. 2019; 8 (7): 923.
18. Rohilla R *et al*. Efficacy and safety of aspirin as an adjunctive therapy in tubercular meningitis: A systematic review and meta-analysis. *eClinical Medicine* 2021; 34: 100819.

ORIGINAL ARTICLE

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Assessment of Prognostic Value of SOFA Score and Lactate/Albumin Ratio in Sepsis

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Abstract

Background: Sepsis necessitates prompt identification and management to improve patient outcomes. The SOFA (Sequential Organ Failure Assessment) score is commonly used to assess the severity of sepsis. The Lactate/Albumin Ratio (LAR) has emerged as a significant biomarker for sepsis prognosis. This study aims to evaluate the role of LAR in predicting sepsis outcomes compared to the SOFA score.

Method: A prospective observational study was conducted in the ICU of Hamidia Hospital, Bhopal. One hundred sepsis patients diagnosed based on systemic inflammatory response syndrome (SIRS) criteria were included. Exclusion criteria included patients requiring albumin supplementation, trauma patients, those under 18 years, pregnant ladies, and patients of malignancy. Routine blood investigations were done. Serum lactate levels, albumin levels, LAR, and SOFA scores were noted. Patients were monitored throughout their hospital stay. Data was analysed using IBM SPSS version 20. ROC curve analysis was used to assess the predictive accuracy of LAR and SOFA scores for mortality.

Results: Our study showed that the majority of the patients were male (62%).The mean age of patients was 47.31 ± 17.33 years. ROC analysis showed that SOFA had sensitivity and specificity of 76.8% and 72.7% respectively at a cut-off of 4.50 while LAR had sensitivity and specificity of 91.1% and 75% at a cut-off of 0.650 for mortality prediction.

Conclusion: The lactate/albumin ratio is a significant predictor of sepsis outcomes. While the SOFA score provides detailed organ-specific assessments, LAR offers a quick, biomarker-based tool suitable for emergency and resource-limited settings.

Key words: SOFA score, LAR, SIRS.

Introduction

Sepsis, a life-threatening organ dysfunction caused by a dysregulated host response to infection, requires prompt identification and management to improve patient outcomes[1]. Acute organ dysfunction (more than or equal to two SOFA score points) along with infection-related symptoms is one of the clinical criteria for sepsis. A total of six factors are taken into consideration while calculating the SOFA (Sequential Organ Failure Assessment) score including liver function, cardiovascular system, pulmonary functions, coagulation profile and renal functions[2].

Globally, sepsis is a leading cause of morbidity and death in intensive care units (ICUs). The epidemiology of sepsis is not well understood in India. However, a large multi-centric study involving 135 ICUs found a prevalence of 46.2% and 33.2%, respectively, based on the sepsis-2 and sepsis-3 classification with a mortality rate of 27.6%[3].

In intensive care units (ICUs), a number of prognostic scoring systems, such as SAPS (Simplified Acute Physiology Score I-III), LODS (Logistic Organ Dysfunction System), APACHE II (Acute Physiology and Chronic Health Evaluation II), MODS (Multiple Organ Dysfunction Score), and SOFA, are used to predict patient outcomes. These prognostic systems, although providing extensive organ dysfunction, are often time-consuming and frequently indicate the prognosis quite late[4-7]. Improving outcomes requires early diagnosis and proper care. Sepsis risk classification and prognosis prediction can also be achieved using various other biomarkers such as total leucocyte count, blood glucose, platelet count, serum albumin, serum lactate, and procalcitonin[8,9].

Precise prognostication is essential for directing therapeutic choices and forecasting results in patients with sepsis. One such biomarker that has gained popularity recently is the Lactate/Albumin Ratio (LAR).

SOFA Score: The sub-score measures six organ systems in respiratory, cardiovascular, hepatic, coagulation renal and neurological on a 0 - 4 scale. The cumulative SOFA score may range from 0 to 24 where higher scores suggest organ dysfunction and increased risk of mortality. By assessing

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several organ systems, the SOFA score allows for an appraisal of a patient's condition from a body-wide perspective which is specific and sensitive to sepsis severity.

Chen X *et al* (2021) found that the mean SOFA score for survivors was 7.03 ± 3.95, while it was 8.95 ± 4.42 for non-survivors which was clinically significant[10]. According to Shin J *et al* (2018), non-survivors had the highest maximum SOFA score (maximum score of 10) compared to survivors (maximum score of 7) ($p < 0.05$)[11]. Similarly, according to Shadvar K *et al's* study from 2022, non-survivors' mean SOFA scores were significantly higher than those of survivors (15.59 ± 1.34 *versus* 14.07 ± 1.19; $p < 0.05$)[12]. Hence, SOFA score has been a time proven reliable marker of sepsis.

Serum Lactate/Albumin Ratio: Increased anaerobic glycolysis, tissue hypoperfusion, and cellular dysfunction in sepsis lead to lactic acidosis, making serum lactate a significant predictive factor. According to current recommendations, serum lactate levels should be measured within an hour of any suspected sepsis episode, and if values are more than 2 mmol/L, assessments should be repeated. Elevation of serum lactate levels can also be caused by other factors such as concomitant liver or renal impairment[13]. Critically sick individuals often also have lower serum albumin levels, a vital plasma protein that is involved in many physiological functions. Serum values of albumin less than 3.5 g/dL are linked to higher rates of morbidity and increased risk of mortality[14].

While serum albumin and serum lactate levels can be used to predict death in sepsis patients on their own, research indicates that the lactate-to-albumin ratio (L/A ratio) is a more accurate predictor of high-risk sepsis cases and can avoid mortality[15]. The lactate-to-albumin ratio may also be useful in giving detailed information about the patient's nutritional condition and physiological changes. Since the lactate-to-albumin ratio is computed using regularly measured laboratory values, is straightforward to apply in clinical settings and doesn't come with extra expenditures.

Numerous studies have emphasized the lactate-to-albumin ratio's prognostic significance in sepsis. Erdogan *et al* (2022) demonstrated that among patients with pneumo-sepsis, particularly those who have renal and hepatic failure, can prognosticate mortality independently based on their lactate-to-albumin ratio The APACHE-2, SOFA scores, and lactate levels were also associated with the higher L/A ratio[16]. Nofal *et al* (2021) showed that, when compared to each score separately, the combination of lactate-to-albumin ratio with SOFA and SAPS II scores had the best predictive value for 28-day mortality in patients with septic shock[17].

Cakir and Turan (2021) mentioned lactate-to-albumin ratio was a better predictor of death in septic patients than lactate or albumin alone. However, the lactate-to-albumin ratio had similar prediction accuracy when measured against the SOFA score. Particularly in patients with liver failure, the lactate-to-albumin ratio provided extra prognostic value, although the SOFA score remained a reliable predictor of death[18].

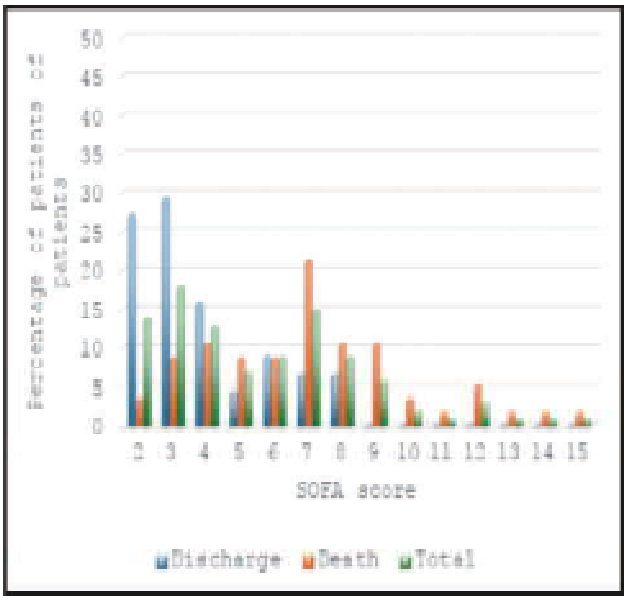

Fig. 1: *Distribution of SOFA Score among survivors and non- survivors.*

The primary aim of this study was to evaluate the role of the lactate/albumin ratio in predicting the outcome of sepsis when compared to SOFA scores. The specific objectives were: to evaluate the prognostic value of the lactate/albumin ratio in sepsis and to evaluate and compare the significance of lactate/albumin ratio with SOFA score ratio in sepsis.

Material and Methods

This prospective observational study was conducted in the ICU of Gandhi Medical College and Hamidia Hospital, Bhopal, from July 2022 to July 2023 after approval from institutional ethics committee. A total of 100 patients admitted with sepsis, as defined by SIRS criteria, were included in the study. Patients requiring albumin supplementation, pregnant ladies, patients of trauma or malignancy and those under 18 years of age were excluded.

Data Collection

Socio-demographic data, clinical presentation, co-morbidities, and laboratory investigations were recorded for all patients including complete blood count, Liver function test, Renal Function test and Arterial Blood gas analysis (ABG). Serum lactate and albumin levels were measured, and the LAR was calculated at the time of admission. SOFA score was also calculated for patients at

the time of admission. Patients were monitored throughout their hospital stay.

Statistical analysis

Data were analysed using IBM SPSS version 20. ROC curve analysis was performed to determine the optimal cut-off for LAR in predicting mortality. A p-value of less than 0.05 was considered statistically significant.

Results

Table I: Socio-demographic data.

S. No.	Variable	N (%)
1.	**Age in years** (Mean ± SD)	47.31 ± 17.33
2.	**Gender**	
	Male (mean age)	62% (46.39 ± 16.29 years)
	Female (mean age)	48% (48.82 ± 19.04 years)
3.	**Source of Infection**	
	Lower respiratory tract infections	46%
	Genito-urinary tract infections	22%
	Gastrointestinal infections	10%
	Skin and soft tissue infections	9%
	Bloodstream infections	7%
	Others	6%
4.	**Septic shock**	
	Present	45%
	Absent	55%
5.	**Co-morbidities**	
	No Co-morbidities	65%
	One Co-morbiditiy	25%
	>One Co-morbidities	10%

Table I demonstrates the socio-demographic data of the 100 patients enrolled. In our study, majority of the patients were male (62 %). Mean age of the patients was 47.31 ± 17.33 years. Co-morbidities were present in 35% of patients. Most common sources of sepsis in our patients were Lower respiratory tract infections (46%) followed by Genitourinary infections 22%. Sepsis was complicated by septic shock in 45% of patients.

In our study, majority of cases with favourable outcome had SOFA score of 3 (29.5%), whereas 27.3% cases had SOFA score of 2 (Fig. 1). However, majority of cases who succumbed to death had SOFA score of 7 (21.4%). None of the discharged cases had SOFA score above 8 whereas 26.9% cases who succumbed to death had SOFA score above 8. Mean SOFA score in all the cases was 5.63 ± 2.75. Mean SOFA score amongst discharged cases was 3.86 ± 1.89 and that among non-survivors was 7.02 ± 3.01. The observed association of SOFA score with outcome was found to be statistically significant ($p < 0.05$).

Mean serum lactate to albumin ratio in cases with sepsis at the time of presentation was 1.49 ± 0.60, which was found to be significantly higher ($p < 0.065$) among non-survivors (2.27 ± 1.79) as compared to survivors (0.52 ± 0.24).

ROC Curve Analysis

In our study, ROC curve analysis revealed SOFA score to be a good predictor of mortality with AUC of 0.816, with sensitivity and specificity of 76.8% and 72.7% respectively at a cut-off of 4.50 (Fig. 2).

ROC curve analysis was also done for LAR. The predictive accuracy of LAR was 0.943; (95% CI - 0.899 - 0.987). At the cut-off of 0.650, the sensitivity and specificty of LAR was 91.1% and 75%, respectively (Fig. 2).

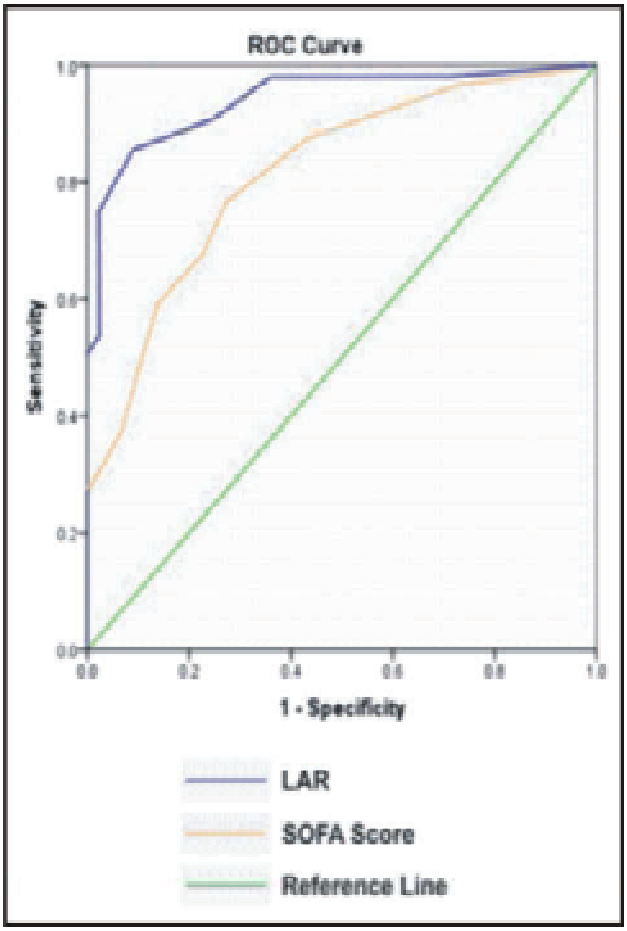

Fig. 2: ROC curve analysis comparing Lactate/Albumin ratio (LAR) and SOFA score with outcome.

Discussion

In recent years, there has been a lot of interest in comparing the Lactate Albumin Ratio (LAR) and Sequential Organ Failure Assessment (SOFA) score as prognostic measures in critical care situations. In our study, the LAR was found to be a highly reliable predictor of mortality by ROC curve analysis. The admission LAR was a good indicator of mortality (0.943; 95% CI - 0.899 - 0.987). The LAR sensitivity and specificity were 91.1% and 75% at the cut-off of 0.650.

On the other hand, the mean SOFA score for patients who were discharged was 3.86 ± 1.89, whereas the score for patients who died was 7.02 ± 3.01. Using a cut-off point of 4.50, ROC curve analysis showed that SOFA score was a good predictor of mortality with an AUC of 0.816 and sensitivity and specificity of 76.8% and 72.7%, respectively at a cut-off value of 4.50.

When reviewing literature and analyzing the comparison of SOFA score and LAR, Mishra *et al* (2018) found that a high LAR is an independent risk factor for sepsis mortality, and the Lac/Alb × age score – which is renowned for its accuracy and simplicity – offers significant clinical utility for prognosis assessment. This makes it a useful tool to use in conjunction with multi-dimensional indices like SOFA for a thorough evaluation of sepsis[19]. A more comprehensive viewpoint was offered by Cakir E *et al* in their analysis of the incorporation of both SOFA and LAR into clinical practice recommendations. They contended that merging these assessment scores may provide a more thorough evaluation of individuals who were in severe sepiss. When combined with metabolic and nutritional information from LAR, the comprehensive organ-specific assessment of the SOFA score may improve prognostic evaluation accuracy[18].

The SOFA score is extensive and provides details regarding specific organ dysfunctions but needs specific clinical data and many laboratory measures, which can be time-consuming and may not always be practical in all healthcare settings. Consistency in SOFA score evaluations may also be affected by interobserver variations at times. LAR, on the other hand, provides a faster insight which may be helpful for early diagnosis and intervention in patients suffering from sepsis, though lacking the specific organ dysfunction details that can be provided by SOFA score. Hence both SOFA score and Lactate/albumin ratio have their strengths and limitations.

Furthermore, depending on the patient demographic and clinical setting, predictive accuracy of these scores may change. For instance, the organ-specific examination of SOFA score may offer more significant prognostic information in trauma patients or those with non-septic/septic critical diseases. Conversely, LAR may be more indicative of the underlying pathophysiological mechanisms in sepsis or situations with metabolic disruptions. Hence the use of lactate-albumin ratio clinical practice may improve the management and prognosis of sepsis patients.

Conclusion

The lactate/albumin ratio is a significant predictor of sepsis outcomes and can be utilised for early risk stratification in ICU settings. Its use in clinical practice may improve the management and prognosis of sepsis patients. The comparison between SOFA score and Lactate Albumin Ratio highlights the complementary nature of these tools in critical care settings. While the SOFA score provides a comprehensive organ-specific assessment crucial for detailed prognostic evaluation, the lactate-to-albumin ratio provides a quick and easy-to-use biomarker-based tool that can be particularly useful in emergency and resource-limited settings. Our study is limited by its single-center design and relatively small sample size. Larger multicentric studies are needed to validate these findings and establish standardised LAR cut-off values for clinical use.

References

1. Singer M, Deutschman CS, Seymour CW *et al*. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016; 315 (8): 801-10.
2. Seymour CW, Liu VX, Iwashyna TJ *et al*. Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016; 315 (8): 762-74.
3. Hammond NE, Kumar A, Kaur P *et al*. Estimates of Sepsis Prevalence and Outcomes in Adult Patients in the ICU in India: A Cross-sectional Study. *Chest* 2022; 161 (6): 1543-54.
4. Tian H, Zhou J, Weng L *et al*. Accuracy of qSOFA for the diagnosis of sepsis-3: a secondary analysis of a population-based cohort study. *J Thorac Dis* 2019; 11 (5): 2034-42.
5. Marshall JC, Cook DJ, Christou NV *et al*. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. *Crit Care Med* 1995; 23 (10): 1638-52.
6. Le Gall JR, Klar J, Lemeshow S *et al*. The Logistic Organ Dysfunction system. A new way to assess organ dysfunction in the intensive care unit. ICU Scoring Group. *JAMA* 1996; 276 (10): 802-10.
7. Sadaka F, Ethmane Abou El, Maali C *et al*. Predicting Mortality of Patients With Sepsis: A Comparison of APACHE II and APACHE III Scoring Systems. *J Clin Med Res* 2017; 9 (11): 907-10.
8. Reinhart K, Bauer M, Riedemann NC, Hartog CS. New approaches to sepsis: molecular diagnostics and biomarkers. *Clin Microbiol Rev* 2012; 25 (4): 609-34.
9. Samraj RS, Zingarelli B, Wong HR. Role of biomarkers in sepsis care. *Shock* 2013; 40 (5): 358-65.
10. Chen X, Zhou X, Zhao H *et al*. Clinical Value of the Lactate/Albumin Ratio and Lactate/Albumin Ratio × Age Score in the Assessment of Prognosis in Patients With Sepsis. *Front Med (Lausanne)* 2021; 8: 732410.

11. Shin J, Hwang SY, Jo IJ *et al*. Prognostic Value of The Lactate/Albumin Ratio for Predicting 28-Day Mortality in Critically ILL Sepsis Patients. *Shock* 2018; 50 (5): 545-50.
12. Shadvar K, Nader-Djalal N, Vahed N *et al*. Comparison of lactate/albumin ratio to lactate and lactate clearance for predicting outcomes in patients with septic shock admitted to intensive care unit: an observational study. *Sci Rep (Internet)* 2022 (cited 2024); 12 (1): 13047.
13. Park IH, Yang JH, Jang WJ *et al*. Clinical significance of lactate clearance in patients with cardiogenic shock: results from the RESCUE registry. *J Intensive Care* 2021; 9 (1): 63.
14. Wiedermann CJ. Controversies Surrounding Albumin Use in Sepsis: Lessons from Cirrhosis. *Int J Mol Sci*. 2023; 24 (24): 17606.
15. Kabra R, Acharya S, Shukla S *et al*. Serum Lactate-Albumin Ratio: Soothsayer for Outcome in Sepsis. *Cureus* 2023; 15 (3): e36816.
16. Erdooan M, Findikli HA. Prognostic value of the lactate/albumin ratio for predicting mortality in patients with pneumosepsis in intensive care units. *Medicine (Baltimore)* 2022; 101 (4): e28748.
17. Nofal WH, Abo Alela SK, Aldeeb MM, Elewa GM. Prognostic Value of Lactate/Albumin Ratio combined with APACHE II, SAPS II and SOFA Score for Predicting Mortality in Critically Ill Patients with Septic Shock. *QJM: An Inter J Med (Internet)* 2021 (cited 2024);114(Supplement_1): hcab086.084.
18. Cakir E, Turan IO. Lactate/albumin ratio is more effective than lactate or albumin alone in predicting clinical outcomes in intensive care patients with sepsis. *Scand J Clin Lab Invest* 2021; 81 (3): 225-9.
19. Mishra LS, Raw BK, Ilahi MT. Serum lactate/albumin ratio as a predictor of organ failure and mortality in severe sepsis and septic shock. *Int J Sci Res* 2018; 7 (11): 72-4.

REVIEW ARTICLE

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Neutrophilic Bronchial Asthma – Diagnostic and Therapeutic Challenge

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Abstract

Asthma is a chronic inflammatory disease, with an arrangement of cells involved in the pathogenesis of the disease. The character of neutrophils in the development of bronchial asthma is found to be complex, as they may trigger activation of immune competent cells and are a significant source of free oxygen radicals and enzymes participating in airway remodeling[1]. Approximately 3.6 - 10% of patients with asthma have severe refractory disease, which is uncontrolled on high doses of inhaled corticosteroids and long-acting β2-agonists. Some of these individuals with severe disease suffer from neutrophilic phenotype. Neutrophilic asthma is a severe and persistent disease, with frequent exacerbations and hospitalisations. Neutrophilic asthma is not responsive to high dose inhaled corticosteroids and to novel monoclonal antibody therapies. There is need for targeted precision biologics and other treatment modalities for patients with neutrophilic asthma, such as long-acting phosphodiesterase-4 inhibitors, macrolide antibiotics and bronchial thermoplasty[2].

Introduction

Asthma is a significant public health problem, affecting more than 358 million individuals globally and its prevalence has been increasing during the last 40 years[3]. It is the most common chronic respiratory disease in children in developed countries and its prevalence is steadily increasing in the developing world. Asthma is a chronic inflammatory airway disease with several distinct phenotypes, characterised by different immuno-pathological pathways, clinical presentation, severity of disease and response to treatment[4].

Phenotypes

The phenotypes of asthma include eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic asthma. Patients with eosinophilic asthma have an eosinophil count ≥3%, whereas patients with neutrophilic asthma have elevated sputum neutrophil count between 61% and 64%, depending on the study[5]. Mixed granulocytic phenotype is characterised by increase in both eosinophils (>3%) and neutrophils (>61% or >64%). Paucigranulocytic phenotype embraces patients with very few eosinophils (<3%) and neutrophils (<61% or <64%) in induced sputum[6]. Approximately 3.6 - 10% of patients with asthma have severe refractory disease, which is not controlled inspite of treatment with high-dose Inhaled Corticosteroids (ICS) and Long-Acting β2-Agonists (LA β2-Agonists (LABA). Neutrophilic asthma is the most common phenotype in adult patients presenting with acute severe asthma[7]. The American Thoracic Society (ATS) guidelines on the definition of severe refractory asthma lists two major and seven minor criteria for making the diagnosis of severe phenotypical asthma . The ATS criteria for established diagnosis of refractory asthma (neutrophilic) include fulfilling one, or both major criteria and at least two minor criteria[8].

Major criteria

1. Treatment with continuous or near continuous (>50% of the year) oral corticosteroids.
2. Need for treatment with high-dose inhaled corticosteroids.

Minor criteria

1. Need for additional daily treatment with controller medication (long-acting β2-agonist, leukotriene receptor antagonist, theophylline).
2. Asthma symptoms needing short-acting β2-agonists use on a daily or near daily basis.
3. Persistent airway obstruction (FEV1 <80% predicted, diurnal peak flow variability <20% predicted).
4. One or more urgent care visit for asthma.
5. Three or more oral steroid bursts per year.

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6. Prompt deterioration with >25% reduction in oral or inhaled corticosteroids.
7. Near fatal asthma event in the past.

Triggers

In general, any of the following factors if present, may trigger development of severe asthma[9] – Pollen, Mold, Mites, Animal dander, Perfumes and Odors, Air pollution, Laughter, Smoke, Dust. However, the factors that triggers the onset of symptoms of Neutrophilic Asthma are different[10] – Overuse of inhaled corticosteroids, Chronic infections, Obesity, Adult onset of symptoms of Asthma.

Challenges

Neutrophilic asthma is associated with more frequent visits to urgent care and hospitals, medication side-effects due to high-doses or long-term use of corticosteroids, reduced quality-of-life and increased risk of acute fatal asthma attack. If one parent has asthma, the child may be 3 - 6 times more likely to develop it than someone whose parents do not have it[11]. Some people with asthma experience most of their symptoms during the night. Over time, this can lead to serious sleep deprivation[12]. Neutrophilia in lung tissue is thought to be responsible for chronic inflammation that is thought to cause scarring of cells lining airways. This makes airway walls thicker, and the air passages narrower than normal[13]. This causes airway obstruction that allows air to get in but not out (air trapping). Because such scarring is permanent, diminished lung function and asthma symptoms are only partially reversible with treatment. This means that some shortness of breath may occur even on good asthma days[14].

Diagnosis

- Adult onset, most cases after 20 years of age.
- Less atopic compared with eosinophilic asthma
- Less severe exacerbations compared with eosinophilic asthma
- Less subepithelial fibrosis as compared to eosinophlic asthma
- Sputum neutrophil count, 40% - 64%;
- Sputum Eosinophils <2% - 3%
- Low FeNO <30 ppb
- Fixed airflow limitation (low FEV1)
- High hydrogen sulfide levels
- Less responsiveness to methacholine challenge tests
- Corticosteroid unresponsiveness

Neutrophilic asthma is characterised by a high neutrophil count in induced sputum ranging from 40% to 76% of sputum cells, or a neutrophil count of 500×10^4/mL. Additionally, patients with neutrophilic asthma have less sputum eosinophil count which has been quoted to be between less than 1.9% and 3% by various authors. Increased neutrophils in sputum has been associated with severe persistent asthma , fixed airway obstruction, with very low Forced Expired Volume in 1 second (FEV1) and post-brochodilator FEV1[15]. Shaw and colleagues, have reported that both patients with eosinophilic asthma and neutrophilic asthma had low pre-bronchodilator FEV1, but only patients with neutrophilic asthma had lowest post-bronchodilator FEV1, indicating persistent airflow limitation[16]. Furthermore, patients with neutrophilic asthma are less atopic and have less responsiveness to methacholine challenges compared with patients with eosinophilic asthma[17]. Patients with neutrophilic asthma are unresponsive to LABA and high-dose ICS and the newly introduced targeted biologics. Furthermore, neutrophilic asthma is typically associated with a worse quality-of-life and has a poor prognosis[18].

Treatment

Treatment of neutrophilic asthma requires novel anti-inflammatory agents and therapeutic strategies targeted against airway smooth muscle hypertrophy and airway remodeling, such as phosphodiesterase-4 inhibitors, macrolide antibiotics and bronchial thermoplasty[19].

Phosphodiesterase-4 inhibitors are appropriate as add-on therapy for patients with neutrophilic asthma, because they suppress immune cell trafficking, activation and degranulation. They also suppress the release of cytokines, chemokines and growth factors which promote subepithelial membrane fibrosis, Airway smooth muscle cell proliferation, airway smooth muscle hypertrophy and airway remodeling . Long-acting selective PDE4-inhibitors, such as roflumilast have been shown to significantly reduce airway hyper responsiveness, which is a key feature of neutrophic asthma. Oral roflumilast 500 mg morning or evening has been shown be beneficial as add-on treatment for fixed airflow limitation[20].

Macrolide Antibiotics-Azithromycin (250 mg daily three times per week) as add-on treatment in patients with nonesonophilic asthma, defined by normal blood eosinophil counts and normal FeNO, resulted in significantly fewer severe exacerbations during 26-week period compared with controls. Azithromycin significantly reduced severe exacerbations and lower respiratory tract infection in non-eosinophilic asthma phenotype by approximately 67% compared to 38% in placebo group. Clarithromycin in

patients severe refractory asthma reduced neutrophil count and sputum IL-8 levels, Neutrophilic asthma is nonresponsive to corticosteroids. Corticosteroid-resistance is associated with airway hyperresposniveness and decreased Histone Deactylase 2 (HDAC2) activity and expression . HDAC2 has been shown to inhibit inflammatory protein coding genes such as granulocyte macrophage colony stimulating factor or cyclooxygenase 2, promoted by IL-1β, TNF-α and NFκB kinase. Macrolides reverse corticosteroid insensitivity by restoring the HDAC activity, via inhibiting Phosphoinositol 3 Kinase (PI3K) pathway and by attenuating TNF-α and IL-17 immune responses[21] .

Bronchial Thermoplasty (BT) is a bronchoscopic treatment for subjects aged 18 years and above with severe persistent asthma not responding to high-dose ICS and LABA. Selection and preparation of patients for BT is very important and the procedure should be performed by experienced pulmonologists or bronchoscopists. Bronchial thermoplasty has a long-term safety profile and may be considered for patients with predominant chronic airflow obstruction and patients who do not respond to anti-IgE, anti-interleukin biologics, or macrolides. Patients with neutrophilic phenotype of asthma are suitable candidates for bronchial thermoplasty because they have excessive airway smooth muscle hypertrophy, hyperplasia and hyper-responsiveness. They are also unresponsive to treatment with high-dose ICS, LABA, LTRA and interleukin antagonists targeted against eosinophilic asthma[22].

References

1. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet* 2008; 372 (9643): 1107-19.
2. Backman H, Jansson S-A, Stridsman C *et al*. Chronic airway obstruction in a population-based adult asthma cohort: Prevalence, incidence and prognostic factors. *Respiratory Medicine* 2018; 138: 115-22.
3. Behbehani NA, Abal A, Syabbalo NC *et al*. Prevalence of asthma, allergic rhinitis, and eczema in 13- to 14-year-old children in Kuwait: an ISAAC study. *Annals of Allergy, Asthma & Immunology* 2000; 85 (1): 58-63.
4. Bonta PI, Chanez P, Annema JT, Shah *et al*. Bronchial thermoplasty in severe asthma: Best practice recommendations from an expert panel. *Respiration; International Review of Thoracic Diseases* 2018; 95 (5): 289-300.
5. Bosnar M, Euie S, Bonjak B *et al*. Azithromycin inhibits macrophage interleukin-1β production through inhibition of activator protein-1 in lipopolysaccharide-induced murine pulmonary neutrophilia. *International Immunopharmacology* 2011; 11 (4): 424-34.
6. Choi J-S, Jang ANS, Park JS *et al*. Role of neutrophils in persistent airway obstruction due to refractory asthma. *Respirology (Carlton, Vic.)* 2012; 17 (2): 322-9.
7. Ciepiela O, Ostafin M, Demkow U. Neutrophils in asthma – A review. *Respiratory Physiology & Neurobiology* 2015; 209: 13-6.
8. Cigana C, Assael BM, Melotti P. Azithromycin selectively reduces tumour necrosis factor alpha levels in cystic fibrosis airway epithelial cells. *Antimicrobial Agents and Chemotherapy* 200; 51 (3): 975-81.
9. Cowburn AS, Condliffe AM, Farahi N *et al*. Advances in neutrophil biology. *Chest* 2008; 134 (3): 606-12.
10. d'Hooghe JNS, Ten Hacken NHT, Weersink EJM *et al*. Emerging understanding of the mechanism of action of Bronchial Thermoplasty in asthma. *Pharmacology & Therapeutics* 2018; 181: 101-7.
11. Holland K. 2014. Adult-onset asthma. Healthline. https://www.healthline.com/health/asthma/differences-childhood-adult-onset-asthma.
12. Li H, Zuo J, Tang W. Phosphodiesterase-4 inhibitors for the treatment of inflammatory diseases. *Frontiers in Pharmacology* 2018; 9.
13. Martin RJ, Cicutto LC, Smith HR *et al*. Airways inflammation in nocturnal asthma. *The American Review of Respiratory Disease* 1991; 143 (2): 351-7.
14. Pavlidis S, Takahashi K, Ng Kee Kwong F *et al*. "T2-high" in severe asthma related to blood eosinophil, exhaled nitric oxide and serum periostin. The *European Respiratory Journal: Official Journal of the European Society for Clinical Respiratory Physiology* 2019; 53 (1): 1800938.
15. Schleich F, Demarche S, Louis R. Biomarkers in the management of difficult asthma. *Current Topics in Medicinal Chemistry* 2018; 16 (14): 1561-73.
16. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: Assessment and identification using induced sputum. *Respirology (Carlton, Vic.)* 2006; 11 (1): 54-61.
17. Svenningsen S, Nair P. Asthma endotypes and an overview of targeted therapy for asthma. *Frontiers in Medicine* 2017; 4.
18. Syabbalo N. Chronobiology and chronopathophysiology of nocturnal asthma. *International Journal of Clinical Practice* 1997; 51 (7).
19. Vrugt B, Wilson S, Bron A *et al*. Bronchial angiogenesis in severe glucocorticoid-dependent asthma. *The European Respiratory Journal: Official Journal of the European Society for Clinical Respiratory Physiology* 2000; 15 (6): 1014.
20. Wenzel SE, Balzar S, Cundall M, Chu HW. Subepithelial basement membrane immunoreactivity for matrix metalloproteinase 9: Association with asthma severity, neutrophilic inflammation, and wound repair. *J Allergy Clin Immunolo* 2003; 111 (6): 1345-52.
21. Wenzel SE, Schwartz LB, Langmack EL *et al*. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *American Journal of Respiratory and Critical Care Medicine* 1999; 160 (3): 1001-08.
22. Yamasaki A, Okazaki R, Harada T. Neutrophils and asthma. *Diagnostics (Basel, Switzerland)* 2022; 12 (5): 1175.

REVIEW ARTICLE

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Demystifying the Cluster Differentiation (CD) System and Clinico-pathological Implications

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Abstract

The cluster of differentiation (CD) is a nomenclature system that identifies and classifies antigens found on the cell surface of various immune and non-immune cells. CD markers are cell surface proteins often used to determine the identity of various cell types. Applications such as flow cytometry and immunohistochemistry can enable phenotypic characterisation of cells based on the expression by unique CD markers. CD markers are also a useful tool for studying the differentiation and maturation of leukocytes and lymphocytes subsets. The HLDA (Human Leukocyte Differentiation Antigens) workshop, which started in 1982, developed the CD nomenclature and has maintained the list of CD Markers ever since. The number of CD markers has grown constantly since its discovery and has expanded to other cell types besides leukocytes. Today, there are more than 370 CD clusters described in humans. CD molecules have varied functions, often act as receptors or ligand and some CD proteins play a role in cell signaling, cell adhesion, cell inhibition and cell activation. CD markers are being increasingly used for diagnosis and follow-up of haematological malignancies, autoimmune diseases, immunodeficiencies, monitoring of cancer immunotherapy and in stem cell biology research. Evaluation of CD markers is not only of diagnostic value at disease onset, but also serve as prognostic and predictive markers to contribute to the treatment of disease and predict its relapse.

Key words: CD, classification, targeted therapy, surface marker.

Introduction

The cell surface is the site of many important biological processes, whichare involved in the interaction between the cell and its environment[1]. Cell membrane proteins comprise approximately 30% of total human proteins; and play a key role in various physiological functions and pathological conditions[2]. The Cluster Differentiation (CD) system is a classification system used to identify and categorise cell surface molecules, known as clusters of differentiation[3]. CD antigens were originally defined as being present on cell surface of leucocytes and recognised by specific antibody molecule, but now also include some intracellular molecules and molecules present on cells other than leucocytes. In some cases, CD antigens are expressed only at certain stages of development or under certain conditions. CD antigens are integral in several immune functions including cell activation, cell adhesion, and immune response regulation. They serve as receptors and ligands, regulate cell signalling and participate in adaptive immunity[4]. While using one CD molecule to define populations is uncommon, combining markers has allowed for cell types with very specific definitions within the immune system. CD markers have proven critical for the identification and isolation of leucocytes, lymphocyte subsets, diagnosis and follow-up of haematological malignancies, autoimmune diseases, immunodeficiencies, monitoring of cancer immunotherapy and in stem cell biology research. However, there are important gaps in our knowledge of CD molecule expression profiles with newer discoveries coming up each day. This review gives a brief outline on CD system and provides a rationale for their usefulness in the current era. The overlay of the review will be as described as under:

- The CD Nomenclature
- The CD classification based on functions
- Methods of Identification of CD makers
- Clinicopathological implications
- Summary

The CD nomenclature

The CD nomenclature system was first introduced and established on the 1st Human Leukocyte Differentiation Antigen (HLDA) Workshop held in Paris in 1982[5,6,7]. CD nomenclature has since been universally adopted by the scientific community and is officially approved by the International Union of Immunological Societies and sanctioned by the World Health Organisation[8]. CD antigens are recognised by antibodies. Monoclonal antibodies that have similar patterns of reactivity with various tissues or cell type are assigned to a cluster group. An antigen well

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recognised by group of antibodies can be assigned a cluster of differentiation number or CD number once two distinct monoclonal antibodies have been demonstrated to bind to that molecule[9]. The HLDA Workshops implemented a standard nomenclature for clusters of antibodies that reacted with a specific antigen, providing consistency and uniformity in manuscripts referring to identical molecules[8,10]. In addition to defining the CD nomenclature, these workshops have been instrumental in identifying and determining the expression and function of cell surface molecules[8]. Over time, the data generated by the 10 HLDA workshops have led to the characterisation and formal designation of more than 370 CD unique clusters and subclusters. The number of CD markers has grown constantly since its discovery and today there are more than 371 CD clusters described in humans[11,12]. Cluster Differentiation (CD) + Number uses the prefix "CD" followed by a number (e.g., CD3, CD20). Each number represents a specific molecule, with some CDs covering a group of closely related family of proteinsor carbohydrates (e.g., CD1a, CD1b, CD1c, and CD1d). A lower case letter following the CD number (e.g., CD1a) indicates several molecules that share a common chain. Other examples are the integrin chains CD11a, CD11b, and CD11c, all of which share CD18 as a common chain to form different dimers[8,12]. In other cases, lower case letters have been used to name different members of the same gene family, as is the case with CD66 (CD66a, CD66b, CD66c, CD66d, CD66e, and CD66f). In the past, an upper case letter was added to some CDs to group related molecules under the same CD number. This was the case for selectins: CD62L (L-selectin), CD62E (E-selectin), and CD62P (P-selectin). Unfortunately, this turned out to be confusing, because sometimes an "L" was added by some researchers to indicate "ligand," such as for CD154, commonly referred to as CD40L. To avoid confusion, the addition of uppercase "L" has been discontinued and should be avoided[8,13]. Provisional indicator "w" is used if the molecule has not been well-characterised, or has only one monoclonal antibody, as in "CDw186"[8]. The CD designations were used to describe the recognised molecules but had to be clarified by attaching the term antigen or molecule to the designation (e.g., CD2 molecule). Currently, "CD2" is generally used to designate the molecule, and "CD2 antibody" is used to designate the antibody that reacts with CD2 antigen[14]. In the context of cell populations, the presence or absence of a CD molecule is often denoted using '+' or '–' symbols. For example, a "CD34+, CD31–" cell denotes a cell expressing CD34 but lacking CD31. Additionally, some cell populations can be classified as hi, mid, or low (alternatively bright, mid, or dim), indicating varying levels of CD expression[15]. Monitoring the presence, absence and expression profiles of different CD antigens can identify, isolate, and immunophenotype cells in immune processes. Fig. 1 illustrates an example of CD antigen on surface and its corresponding antibody reacting with the specific CD antigen.

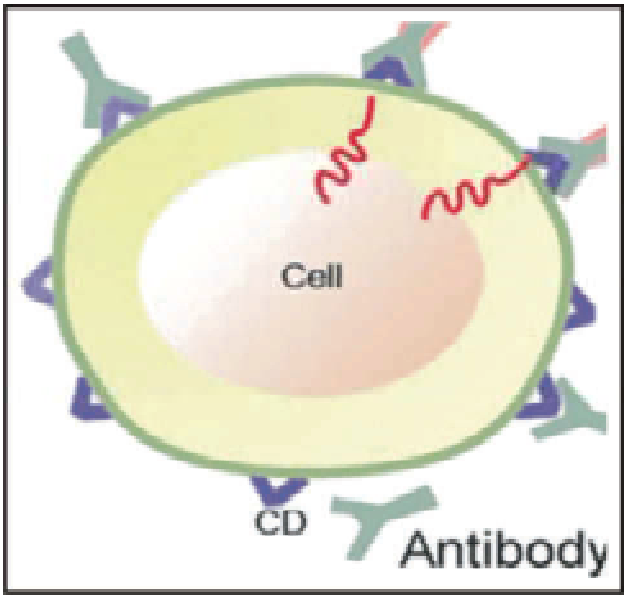

Fig. 1: *Graphical representation of CD antigen on the surface and reactivity by corresponding antibody.*

The CD Classification based on functions

The classification of CD clusters and subclusters is based on the distinct markers on cells that serve as unique identification tags and can readily be distinguished by determining such combination of molecules on their membranes. CD markers have specific functions and can be differently expressed in response to environmental conditions and intracellular genetic changes[16]. Not only the presence of these CD markers but also the absence of expression of CD makers can give a clue to the correct diagnosis. Following descriptions classify CD markers based on their varied functions.

a. Role of CD Markers as cell surface receptors

CD markers can be recognised by specific monoclonal antibodies, generated against the epitopes on the cell surface which serve as cell receptors, facilitating cell recognition and interaction, thereby influencing immune responses, and shaping the intricate dynamics of the immune system. CD markers are found in various immune cell populations like B cells, T-cells, dendritic cells, NK cells, monocytes, macrophages, endothelial cells, epithelial cells, red blood cells, granulocytes, platelets and stem cells[17]. As lymphocytes mature, they express different protein receptors on the cell surface, which can aid in determining the type and maturation stage of the cells being examined.

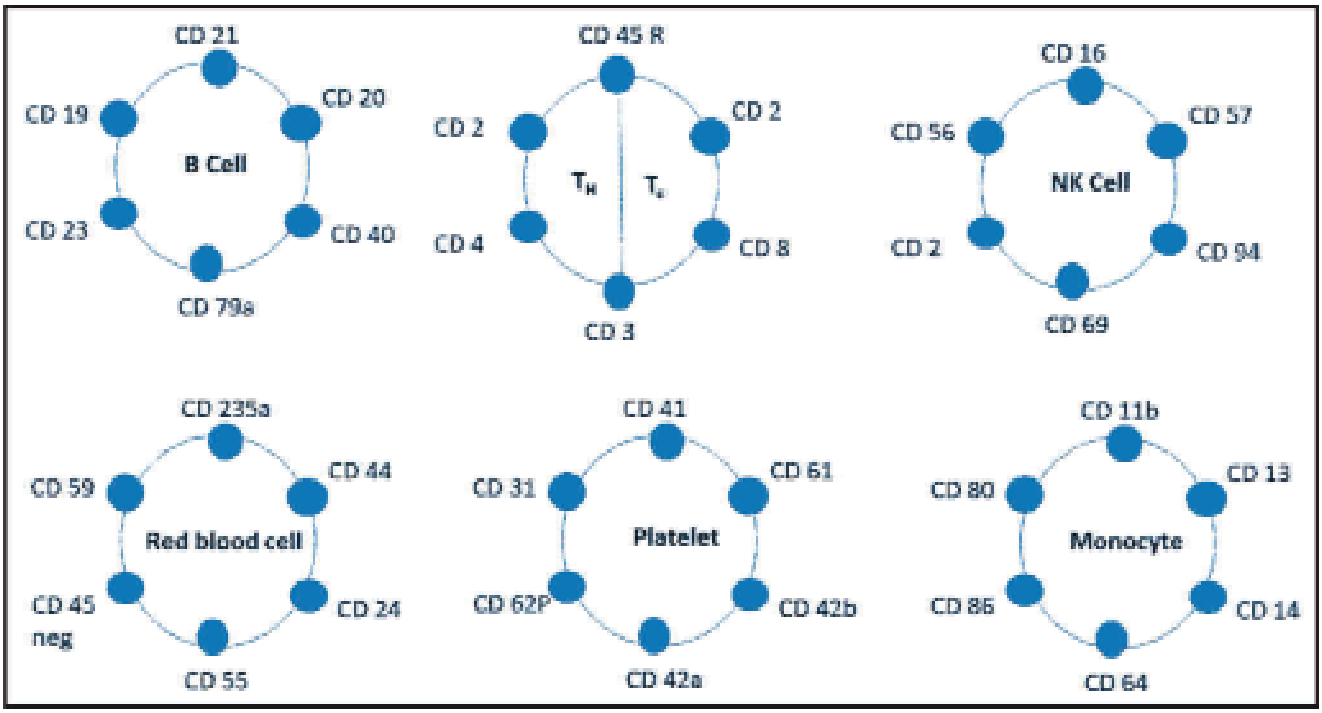

Fig. 2: *Diagrammatic representation of CD antigen distribution on various immune cells.*

CD antigen distribution on various immune cells is illustrated graphically in Fig. 2.

b. Role of CD Markers in other functions

The CD markers play a significant role in signal transduction, cell adhesion, cell migration, cell activation, cell to cell interaction, cell inhibition and in adaptive immunity[18]. Depending on the functional properties, various CD markers have been identified which play a varied role in physiological functions such as:

CD antigens in signal transduction: When a CD antigen activates its receptor, the signal is carried into the cell by means of a second messenger. Example, the marker CD47 is found to have anti-phagocytic signals to macrophages and inhibit natural killer (NK) cells. This enabled researchers to apply CD47 as a potential target to attenuate immune rejection[19,20].

CD antigens in cell adhesion, migration, activation, cell to cell interaction and cell inhibition: Some CD antigens act as cell-cell or cell-matrix adhesion molecules, by which cells form contacts with each other or with their substratum through specialised protein complexes. This intricate mechanism plays a pivotal role in shaping tissue structure during morphogenesis and maintaining tissue cohesion in post-developmental life. CD antigens play a significant role in cell migration and guide immune cells to specific locations in the body. For instance, CD44 aids lymphocyte homing to lymph nodes, and selectins interact with CD15 and CD62L, facilitating leukocyte recruitment to inflammatory sites[21]. CD antigens are also critical to mediating cell interactions. These surface molecules on immune cells, like T-cells and antigen-presenting cells, enable recognition and communication between cells. For example, CD4 and CD8 interact with T-cell receptor (TCR) and major histocompatibility complexes (MHC), playing a crucial role in T-cell activation[22,23]. CD antigens also play a key role in regulating cell inhibition. For instance, programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are CD antigens that, when bound by their ligands, inhibit T-cell activation[24]. CD antigens are used to identify the maturation or activation stage of various cells, including T-cells, B-cells, and other immune cells. For example, in dendritic cells, the most important CD markers of activation are CD80, CD86 and CD83[25].

CD markers in adaptive immunity: The adaptive immune or specific immune response consists of antibody responses and cell-mediated responses, which are carried out by different lymphocyte cells, B-cells, T-cells, NK cells, dendritic cells and monocytes[26]. CD molecules are essential markers for the identification and isolation of immune cells. As lymphocytes mature, they express different protein receptors on the cell surface which helps in determining the type and maturation stage of lymphocytes[18], e.g., CD 45 is the pan leucocyte marker, while CD3 surface antigens

form part of the T-cell receptor complex for antigens. CD3 is expressed exclusively by mature lymphocytes of the T-cell lineage. CD4 and CD8 are subtypes of CD3+ T lymphoid cells and used as markers for the helper and cytotoxic T lymphoid cells, respectively. CD 19 and CD20 are seen exclusively on lymphocytes of the B-cell lineage. In addition, CD 25 expression on T lymphoid cells can serve as markers of activation.

Table I highlights few common CD antigens related to various functions and their expression on various cells.

Methods of identification of CD markers

CD antigens are widely used as cell markers in immunophenotyping thereby allowing the identification of the presence or absence of cell markers as well as to quantify proportions of specific cell populations and lymphocyte subsets. Immunophenotyping is a powerful tool that uses assays like flow cytometry (FCM) or immunohistochemistry (IHC) to gain insights into the composition and dynamics of cell populations and can be performed to detect and quantify CD markers. These techniques involve identifying cell types in heterogeneous cell populations by using different antibodies that target various CD markers. Different combinations of antibodies can be used to identify different groups and sub-groups of cells. By applying CD markers in immunophenotyping techniques, scientists and clinicians can discern specific cell types within complex immune responses, facilitating disease diagnosis, treatment development, and immunological research. The following immuno-phenotyping techniques are commonly used for the identification of CD markers:

Flow cytometry is a common technique to analyse the expression of CD markers on single cells in fluidic medium[27]. FCM involves obtaining a sample of cells, such as blood, bone marrow, or tissues, and incubating them with a panel of fluorescently labelled antibodies that recognise specific cell surface markers[27]. The antibodies can target various CD markers, the labelled cells are then passed through a flow cytometer, which detects and measures the fluorescence emitted by each individual cell, providing quantitative data about the expression levels of different markers. FCM is a key technique for the immunophenotypic diagnosis of acute leukaemia's and chronic lymphoproliferative disorders, with the ability to provide data on simultaneous evaluation of multiple proteins in hundred thousand to millions of single cells[28]. Most CD antigens are expressed at varying levels by many different cell types. Rather than the exclusive expression of a single CD antigen with a particular cell type, it is the peculiar constellation of surface antigens expressed by a given cell that helps assign it to a particular lineage or sub lineage of cells. The resolution of cell subpopulations usually requires two or more colour FCM analysis. Advantage of FCM is that it allows for the simultaneous detection of several markers on a single cell at the same time. A modern 8 - 10 colour FCM can simultaneously measure the expression of 8 - 10 CD markers on single cell, most advanced FCM can analyse upto 18 CD markers also[29]. For example, CD3 can be used as a general marker for T-cells followed by other CD markers to identify regulatory T-cells (CD4 and CD25), T helper cells (CD4), and cytotoxic T-cells (CD8). In case of lymphoid neoplasms, for example if abnormal lymphoid cells are CD 5 positive and CD23 negative on FCM, the diagnosis favours mantle cell lymphoma and not chronic lymphocytic leukaemia

Table I: Showing few common CD antigens related to various functions and their expression on different cells.

Function of CD markers	B Lymphocyte	T lymphocyte	Dendritic cell	Stem cells	Monocyte	Granulocytes	Endothelial cells	Epithelial cell
Signal Transduction	CD79a, CD73, CD53, CD122, CD77, CD69	CD4, CD21, CD5, CD8, CD38, CD55	CD18, CD86, CD33, CD37, CD40, CD150	CD19, CD21, CD55, CD200, CD22, CD135	CD14, CD206	–	–	–
Cell Adhesion	CD22, CD11c, CD35, CD39, CD44, CD50	CD4,CD6, CD31, CD47, CD99, CD84	CD18, CD48, CD33, CD53, CD191, CD106	CD9, CD81, CD22, CD99, CD324, CD48	CD9, CD11c, CD54, CD22, CD36, CD62L	CD43, CD66b, CD99, CD33, CD58, CD35	CD9, CD50, CD34, CD111, CD239, CD225	CD26, CD40, CD44, CD118, CD54, CD113
Cell Migration	CD11a, CD18, CD31, CD44,CD53,CD97	CD9, CD44, CD54, CD97,CD177,CD304	CD1c, CD5, CD11c, CD14,CD47	–	CD44, CD53, CD99, CD302, CD209, CD321	CD16b, CD29, CD53, CD44, CD97, CD99	CD54,CD106,CD225, CD209, CD228, CD309	CD66a, CD66c, CD228, CD318, CD332, CD362
Cell Interaction	CD138, CD22, CD167b, CD326, CD360	CD1d, CD4, CD7, CD28, CD27, CD278	CD37, CD40, CD47, CD80, CD170, CD146	–	CD62L, CD11c, CD18, HLADR	–	–	–
Cell Activation	CD18, CD33,CD36, CD5, CD30, CD46	CD2, CD9, CD4, CD6, CD28, CD43	CD11c, CD19, CD23, CD37, CD38, CD80	–	CD11c, CD18	CD43, CD55, CD66a, CD99, CD261, CD270	–	–
Cell Inhibition	CD46, CD80, CD86, CD264, CD279	CD1d, CD28, CD59, CD161, CD225, CD273	CD2, CD28, CD40, CD137, CD152	–	CD33, CD118, CD172a, CD300f	–	–	–
Adaptive Immunity	CD19, CD20, CD22, CD35, CD95, CD102	CD4, CD8, CD3, CD55, CD59, CD95	CD22, CD33, CD170, CD320	–	CD5, CD35, CD54, CD64, CD95	CD16a, CD35, CD59, CD281, CD305, CD178	–	–

(CLL), though by morphological diagnosis, they have similar appearance. Not only the presence or absence but the intensity of expression of CD markers can also play a significant role leading to correct diagnosis in certain conditions like loss or reduced expression of CD10 can be a dysplastic event in neutrophils in myelodys plastic syndromes. In a case with morphological diagnosis as leukaemia, by FCM when the blast population express myeloid antigens like CD 13, CD33, CD117, the diagnosis favours acute myeloid leukaemia (AML). Reactivity of the most common antibodies (CD Markers) used in FCM of haematolymphoiddis ordersis as depicted in Table II.

Table II: Depicting CD markers used in flow cytometry for Haematolymphoid disorders.

Cell type	CD marker
All leucocytes	CD45+
T lymphocyte	CD45+,CD2+, CD3+, CD4+, CD8+, CD5+,CD7+
T Helper lymphocyte	CD45+, CD3+, CD4+, CD8-
T cytotoxic lymphocyte	CD45+, CD3+, CD8+, CD4-
Regulatory T-cells (Treg)	CD4, CD25, FOXP3 (a transcription factor)
B Lymphocyte	CD45+, CD19+, CD20+, CD24+, CD38, CD22
NK cells	CD16+, CD56+, CD3-, CD31, CD30, CD38
Red blood cells	CD235,
Monocytes	CD4, CD45+, CD14+, CD114+, CD11a, CD11b, CD16+
PNH markers	CD 55, CD59, CD157 , CD64 ,CD24, CD15 ,CD16
Granulocytes	CD45+, CD11b, CD15+, CD24+, CD114+, CD182+
Myeloid Cells	CD13+, CD33+
Stem cells	CD34 +, CD31-, CD117+
Plasma cells	CD38+,CD138+,CD81+,CD27+

Immunohistochemistry (IHC) is a powerful technique that exploits the specific binding between an antibody and antigen to detect and localise specific antigens in cells and tissue[30]. Many lesions show overlapping features on morphological appearance and is not always sufficient to subcategorise the cancer. IHC by identifying various CD antigens in solid tissues can play an important role in the differential diagnosis of the diagnostically challenging lesions on morphology. IHC can be performed conveniently on formalin fixed paraffin embedded (FFPE) tissue[31,32] and by automated methods for high volume processing with reproducibility[33]. IHC is frequently utilised to determine the CD antigens in solid tissues and bone marrow biopsy sections thereby assisting in the diagnosis and classification of neoplasms, determining a metastatic tumour's site of origin and detection of tiny foci of tumour cells inconspicuous on routine haematoxylin and eosin (H&E) staining[34]. Furthermore, it is increasingly being used to provide predictive and prognostic information as well[35].Interpretation of CD antigens in tissues is based on cellular distribution of antibody staining (i.e., membranous, cytoplasmic, nuclear), proportion of positively stained cells, staining intensity and cut-off levels. The use of IHC for determining CD antigens has recently further expanded to assess predictive and prognostic biomarkers in many malignancies including those of the breast, gastrointestinal tract, lung, haematolymphoid and central nervous systems[36]. Table III depicts CD markers in various solid tumours.

Table III: Highlights utility of CD markers as diagnostic and prognostic markers in solid cancers.

Type of cancer	Commonly identified CD Markers	Prognostic CD markers
Breast	CD44, CD24, CD133, CD14, CD200, CD4, CD8, CD4	CD44, CD133, CD14
Colorectal	CD66, CD110, CD133, CD44, CD2, CD89, CD200	CD110, CD133, CD44, CD200
Lung	CD117, CD176, CD166, CD88, CD103, CD66	CD88, CD103
Liver	CD133, CD44, CD90, CD105, CD34, CD151, CD206, CD68	CD151, CD68, CD206

Clinico-Pathologic Implications of CD markers

1. Cancer Diagnosis and Prognosis:

Haematological Malignancies: CD markers are crucial for diagnosing and classifying leukaemias and lymphomas as a specific set of CD markers are expressed on these cells depending on the stage and pathway of differentiation[16]. Abnormal expression of CD markers in bone marrow and peripheral blood is used as the first diagnostic strategy and is also followed to monitor the clinical course of leukaemia/ lymphomas[37]. For example, CD19 and CD20 are markers for B-cells and are used to diagnose B-cell lymphomas/leukaemia. CD34 serves as a marker for hematopoietic stem cells (HSC) and is frequently used to identify immature or undifferentiated cells in leukaemia, particularly acute leukaemia's. Thus, positivity of CD34 differentiates between blasts in leukaemiavs mature lymphomas. Depending upon the expression of various B cell markers, distinguishing being various lymphomas is also possible Similarly, T markers expression like CD2, CD3, CD4, CD8 on leukaemia/lymphoma cells can determine the T-cell origin. In cases of diagnostic dilemma between similar looking blasts on morphological examination, expression of myeloid markers like CD13, CD33, CD117 can determine AML versus acute lymphoblastic leukaemia (ALL). CD15 and CD30 are associated with Reed-Sternberg cells in Hodgkin lymphoma (HL), thereby leading to correct tissue

diagnosis. CD138 (Syndecan-1) serves as a marker for plasma cells and is used in the diagnosis of multiple myeloma, a cancer of plasma cells.

Prognostic Indicators: CD markersare widely used as both prognostic and predictive markers in immunology and oncology/haematology[38] and provide information about the aggressiveness of the disease and predict patient outcomes. CD38 expression is typically associated with increasing proliferation and survival of malignant B-cells and has been recognised as a poor prognostic marker in CLL[39,40]. CD38 positivity in CLL is associated with aaggressive clinical course compared to CD 38 negative cases. Detection of the changes in CD markers' expression in leukaemia also contributes to the prognosis of these disorders. CD3, CD4, and CD8 are markers used to evaluate the presence and composition of T lymphocytes in the tumour microenvironment, which can have prognostic significance in various cancers[41]. CD163 and CD68 are markers of macrophages and are indicated as a prognostic marker in classical HL with the highest tumour associated macrophages having reduced disease-free survival and overall survival[42,43].

Solid Malignancies: Expression ofCD markers in solid tumours can be studied by IHC on tissue sections or as circulating tumor cells in blood sample by utilising FCM. Identification of CD markers on a specific tumor leads to early detection and also serves as a prognostic factor for monitoring the progression of solid tumors[44]. More recently, CD markers have also been used in detecting potential cancer stem cells[45]. CD44 is linked to cancer stem cells in multiple cancer types, including breast, colon, and pancreatic cancer[46] while CD326 (EpCAM) is employed as a marker in the detection and characterisation of certain epithelial cancers, including breast, colorectal, and ovarian cancers[47]. CD99 is expressed in various types of tumours, including Ewing's sarcoma, small round cell tumors, and some soft tissue sarcomas. CD133, as a putative stem cell marker, is associated with more advanced stages of Wilms and neuroblastoma (NB) tumors; therefore, this molecule can be a potential clinical prognostic marker in children suffering from NB or Wilms tumour[48]. Monitoring of tumor progression through CDs markers expressed on circulating tumour cells could be a new diagnostic and prognostic factor in the future. Table III depicts few CD markers identified in solid tumours serving as diagnostic and prognostic markers.

2. Immunotherapy and Targeted Therapies

CD markers are used to develop targeted therapies. Advances in genetic technology have led to a growing number of approved immunotherapeutic agents. As opposed to older generation chemotherapy which targets fast-replicating cells which can be both cancerous and healthy, these newer generation drugs target only those cells with a specific CD "tag." Immunotherapeutic agents can be monoclonal antibodies which are tagged to drugs or radiation-emitting substances that have the ability to kill cancerous cells expressing the specific CD marker on their surface[49]. For example, CAR-T-cell therapy involves modifying T-cells to express chimeric antigen receptors (CARs) that target specific CD markers on cancer cells, such as CD19 in B-cell malignancies[50]. The predictable expression of CD molecules on various hematologic malignancies has allowed for therapeutic targeting of the malignancy with monoclonal antibodies (MAbs). The most advanced targeted therapy inrecent times is rituximab which has received Food and Drug Administration (FDA) approval and is in wide spread clinical application[51]. Rituximab targets CD20, which is expressed on the majority of B-cells and it has shown great activity in treating CD20-positive B-cell leukemias and lymphomas[52]. CD24, also known as Heat Stable Antigen (HSA), has been extensively studied in the field of immunotherapy in various solid malignancies, and as a novel molecule for targeted drug delivery and imaging[53]. Among the drugs currently approved by FDA for use in immunotherapy is depicted in Table IV.

3. Autoimmune Diseases:

CD markers are routinely used to study the phenotype and functionality of immune cell populations in autoimmunity. FCM is the most useful technique to identify and quantitate CD markers in autoimmunity. CD4+/CD8+ (helper/suppressor) T lymphocyte ratio assessment along with autoantibody detection and HLA-DR+ T lymphocyte measurements have been well studied in autoimmune disorders. Other useful CD biomakers such as TCRα/β, CD-, CD8- double negative T-cells are elevated in autoimmune lymphoproliferative syndromes (ALPS) syndrome. FOXP3+CD25+CD4+ Treg (regulatory T) population is found to be reduced in various autoimmune disorders[54]. Decreased absolute numbers of both CD27+ and CD27– B-cells as well as decreased proportions of IgD+CD27+ memory B-cells are noted in SLE. Thus, combined with other clinical parameters B-cell profiling can help identify potential biomarkers relevant to lupus disease[55]. Cell surface markers such as CD74 can be monitored in multiple sclerosis by FCM not only to assess disease activity and progression, but also to evaluate the clinical efficacy of treatment[56]. In addition, CD19 and CD20 counts are also used as markers to evaluate treatment efficacy of rituximab, a monoclonal antibody directed at CD20+ B-cells.

Table IV: Depicting CD marker as therapeutic targets according to the clinical application of specific monoclonal antibodies.

CD Marker	Disease	Example of theuraptic antibody against CD marker
CD 3	Autoimmune disease, transplant rejection	OKT3 (muromonab), otelixizumab, catumaxomab
CD19	Lymphomas, Acute lymphoblastic leukaemia	Blinatumomab, taplitumomab
CD20	Lymphomas, autoimmune diseases, Immune thrombocytopenia purpura, Chronic lymphocytic leukaemia (CLL)	Rituximab, afutuzumab, ibritumomabtiuxetan, ocaratuzumab, ocrelizumab, ofatumumab, tositumomab
CD30	Hodgkin's lymphoma and anaplastic large cell lymphoma	Brentuximabvedotin
CD33	Acute myeloid leukaemia	Gemtuzumabozogamicin
CD52	Acute leukaemia, SLL/CLL/Peripheral T-cell lymphoma	Campath (Alemtuzumab)
CD75, CD38	Multiple myeloma	Milatuzumab, Daratumumab
CD4	Psoriasis, HIV, autoimmune diseases	Ibalizumab, cedelizumab, clenoliximab, priliximab
CD6	Autoimmune diseases, Sjögren's syndrome	Itolizumab
CD11a	Psoriasis, autoimmune diseases	Efalizumab
CD125	Asthma	Benralizumab
CD340	HER2+ Breast cancer	Herceptin® (trastuzumab)

4. Stem cell enumeration, Transplantation and Graft Monitoring

CD34, is a marker of HSC in bone marrow and blood. Collection and infusion of CD34+ HSC following chemotherapy is critical in bone marrow transplantation[57]. Evaluation of CD34 + HSC harvest adequacy is achieved by CD34 cell counting using FCM as the number of viable CD45+/CD34+ cells will determine the quality of the harvested specimen for bone marrow transplantation. CD markers are also used to monitor and assess graft-versus-host disease (GVHD) in organ transplantation. Certain CD markers help in evaluating the extent of immune response against the transplanted organ. Analysis of peripheral blood CD 8 +T lymphocytes may help to indicate early rejection.

5. Immunodeficiency diseases

Primary immune deficiency disorders (PIDDs) are a group of inherited disorders affecting single or multiple components of the immune system, resulting in increased predisposition to infections and immune dysregulation. A preliminary lymphocyte subset analysis with CD19, CD20 (B-cells), CD 3, CD4, CD8, CD7 (T-cells), NK cells (CD16, CD56) by FCM is the first-line investigation for PIDD. Further subset analysis of B cells and T-cells can be done by utilising CD markers to determine naïve, memory B and T lymphocytes in various immunodeficiency disorders[58].

6. Infectious Diseases

HIV Monitoring:

Many studies have established the utility of CD4+ T-cell count as a critical marker for monitoring HIV infection and progression. The decline in CD4+ T-cells correlates with disease progression and immune system compromise in HIV patients. CD 4 T lymphocyte count is also being used for initial assessment of *in vivo* antiretroviral drug activity and is utilised in determining antiretroviral therapy eligibility and time to initiate therapy[59].

Sepsis: There have been a number of studies looking at the ability of CD64 expression on neutrophils to detect the presence of infection and/or the presence of sepsis. Even quantitative CD64 expression has been shown to correlate with the progression of sepsis to severe sepsis in few studies on critically ill patients[60]. The expression of the integrin CD11b, which enhances the ability of neutrophils to adhere to the endothelium in sites of inflammation, is also increased in bacterial infection, and some have proposed the use of both CD64 and CD11b together to diagnose sepsis[61].

7. CD markers in neuroscience research

CD markers have proven invaluable for the study on neuronal and glial cells thereby providing insights into their functions and interactions[62]. Such identification of specific CD markers is pivotal in investigations related to neural development, neurodegenerative disorders, and brain tumorssuch as glioma. For example, CD56 (NCAM) is associated with neuroendocrine tumours and neural development disorders like autism and schizophrenia[62]. CD133 (Prominin-1) is linked to brain tumour stem cells in glioblastoma, breast cancers and other brain cancers[63]. CD31 (PECAM-1) relates to endothelial cells in the blood-brain barrier and is pertinent in neuroinflammatory conditions.

CD184 (CXCR4) is implicated in HIV-associated neurocognitive disorders (HAND) due to its role in viral entry into the central nervous system.

Summary

Cluster of Differentiation (CD) markers serve as a vital classification system for cell surface molecules not only on immune cells but also on a variety of other cell types. Each CD marker is assigned a unique number that corresponds to a specific cell surface protein or antigen. CD markers have multifaceted roles in immunology, haematology, oncology, neurosciences, stem cell research and immunotherapy. As comprehension of the immune system and cell biology advances, researchers continue to uncover and elucidate the pivotal role of CD markers not only as diagnostic marker but also prognostic and predictive markers in various fields.

References

1. Zola H. Human leukocyte differentiation antigens as therapeutic targets: the CD molecules and CD antibodies. *Expert Opin Biol Ther* 2001; 1 (3): 375-83.
2. Várady G, Cserepes J, Németh A *et al*. Cell surface membrane proteins as personalised biomarkers: where we stand and where we are headed. *Biomark Med* 2013; 7 (5): 803-19.
3. Tian HF, Xing J, Tang XQ *et al*. Cluster of differentiation antigens: essential roles in the identification of teleost fish T lymphocytes. *Mar Life Sci Technol* 2022; 4 (3): 303-16.
4. Cruse JM, Lewis RE, Wang H *et al*. Cluster of Differentiation (CD) Antigens. In: Cruse JM, Lewis RE, editors. *Immunology Guidebook*. Amsterdam: Elsevier, 2004; 47-124.
5. Clark G, Stockinger H, Balderas R *et al*. Nomenclature of CD molecules from the Tenth Human Leucocyte Differentiation Antigen Workshop. *Clin Transl Immunology* 2016; 5 (1).
6. Bernard A, Boumsell L. Human leukocyte differentiation antigens. *Presse Med* 1984; 13 (38): 2311-6.
7. Fiebig H, Behn I, Gruhn R *et al*. Characterisation of a series of monoclonal antibodies against human T cells. *AllergImmunol (Leipz)* 1984; 30 (4): 242-50.
8. Engel P, Boumsell L, Balderas R *et al*. CD Nomenclature 2015: Human Leukocyte Differentiation Antigen Workshops as a Driving Force in Immunology. *J Immunol* 2015; 195 (10): 4555-63.
9. Kumar S, Lütteke T, Schwartz-Albiez R. GlycoCD: a repository for carbohydrate-related CD antigens. *Bioinformatics* 2012; 28: 2553-55.
10. HCDM responsible for HLDA workshop and CD molecules. Human Cell Differentiation Molecules Council. Available from: http://www.hcdm.org/MoleculeInformation/tabid/54/Default.aspx. Accessed 21 Apr 2016.
11. Zola H, Swart B, Banham A *et al*. CD molecules 2006 – human cell differentiation molecules. *J Immunol Methods* 2007; 319 (1-2): 1-5.
12. Zola H, Swart B, Nicholson I *et al*. CD molecules 2005: human cell differentiation molecules. *Blood* 2005; 106 (9): 3123-6.
13. Beare A, Stockinger H, Zola H, Nicholson I. Monoclonal antibodies to human cell surface antigens. *Curr Protoc Immunol* 2008; 80 (1): 4A.1-A.4A.73.
14. Ho IC, Tai TS, Pai SY. GATA3 and the T-cell lineage: essential functions before and after T-helper-2-cell differentiation. *Nat Rev Immunol* 2009; 9 (2): 125-35.
15. Shahrabi S, Ghanavat M, Behzad MM *et al*. CD markers polymorphisms as prognostic biomarkers in hematological malignancies. *Oncol Rev* 2020; 14 (2): 466.
16. Kalina T, Fišer K, Pérez-Andrés M *et al*. CD Maps-Dynamic Profiling of CD1-CD100 Surface Expression on Human Leukocyte and Lymphocyte Subsets. *Front Immunol* 2019; 10: 2434.
17. Deuse T, Hu X. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat Biotechnol* 2019; 37 (3): 252-8.
18. Jalkanen S, Saari S, Kalimo H *et al*. Lymphocyte migration into the skin: the role of lymphocyte homing receptor (CD44) and endothelial cell antigen (HECA-452). *J Invest Dermatol* 1990; 94 (6): 786-92.
19. Li QJ, Dinner AR, Qi S *et al*. CD4 enhances T-cell sensitivity to antigen by coordinating Lck accumulation at the immunological synapse. *Nat Immunol* 2004; 5: 791-9.
20. Holler PD, Kranz DM. Quantitative analysis of the contribution of TCR/pepMHC affinity and CD8 to T-cell activation. *Immunity* 2003; 18: 255-64.
21. Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. *Am J Clin Oncol* 2016; 39 (1): 98-106.
22. Schmidt SV, Nino-Castro AC, Schultze JL. Regulatory dendritic cells: there is more than just immune activation. *Front Immunol* 2012; 3: 274.
23. Chaplin DD. Overview of the immune response. *J Allergy ClinImmunol* 2010; 125 (2 Suppl 2).
24. McKinnon KM. Flow Cytometry: An Overview. *Curr ProtocImmunol* 2018; 120: 5.1.1-5.1.11.
25. Pedreira CE, Costa ES, Lecrevisse Q *et al*. Overview of clinical flow cytometry data analysis: Recent advances and future challenges. *Trends Biotechnol* 2013; 31: 415-25.
26. O'Donnell EA, Ernst DN, Hingorani R. Multiparameter flow cytometry: advances in high resolution analysis. *Immune Netw* 2013; 13 (2): 43-54.
27. Bento LC, Correia RP, Mangueira CLP *et al*. The use of flow cytometry in myelodysplastic syndromes: A review. *Front Oncol* 2017; 7: 270.
28. Taylor CR, Shi SR, Barr NJ. Techniques of immunohistochemistry: principles, pitfalls, and standardisation. In: Dabbs DJ, editor. Diagnostic immunohistochemistry: *Theranostic and Genomic Applications*. 3rd ed. Philadelphia: Saunders; 2010.
29. Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* 1991; 39: 741-8.
30. Magaki S, Hojat SA, Wei B *et al*. An introduction to the performance of immunohistochemistry. *Methods Mol Biol* 2019; 1897: 289-98.
31. Wolff AC, Hammond ME, Hicks DG *et al*. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch Pathol Lab Med* 2014; 138 (2): 241-56.
32. Rizk EM, Gartrell RD, Barker LW *et al*. Prognostic and predictive

immunohistochemistry-based biomarkers in cancer and immunotherapy. *Hematol Oncol Clin North Am* 2019; 33 (2): 291-9.

33. Karai B, Habok M, Remenyi G *et al*. A novel flow cytometric method for enhancing acute promyelocyticleukaemia screening by multidimensional dot-plots. *Ann Hematol* 2019; 98 (7): 1413-20.
34. Parker TL, Strout MP. Chronic lymphocytic leukaemia: prognostic factors and impact on treatment. *Discov Med* 2011; 11 (57): 115-23.
35. Deaglio S, Vaisitti T, Serra S *et al*. CD38 in chronic lymphocytic leukaemia: from bench to bedside? *Mini Rev Med Chem* 2011; 11 (6): 503-7.
36. Deaglio S, Vaisitti T, Zucchetto A *et al*. CD38 as a molecular compass guiding topographical decisions of chronic lymphocytic leukaemia cells. *Semin Cancer Biol* 2010; 20 (6): 416-23.
37. Palomero J, Panisello C, Lozano-Rabella M *et al*. Biomarkers of tumour-reactive CD4+ and CD8+ TILs associate with improved prognosis in endometrial cancer. *J Immunother Cancer* 2022; 10 (12).
38. Werner L, Dreyer JH, Hartmann D *et al*. Tumour-associated macrophages in classical Hodgkin lymphoma: hormetic relationship to outcome. *Sci Rep* 2020; 10 (1): 9410.
39. Xie Y, Yang H, Yang C *et al*. Role and mechanisms of tumour-associated macrophages in hematological malignancies. *Front Oncol* 2022; 12: 933666.
40. Rezaeeyan H, Shahrabi S, McKee TD, Saki N. The expression of CD markers in solid tumours: significance in metastasis and prognostic value. *Histol Histopathol* 2018; 33 (10): 1005-12.
41. Keysar SB, Jimeno A. More than markers: biological significance of cancer stem cell-defining molecules. *Mol Cancer Ther* 2010; 9 (9): 2450-7.
42. Dzobo K, Sinkala M. Cancer stem cell marker CD44 plays multiple key roles in human cancers: immune suppression/evasion, drug resistance, epithelial-mesenchymal transition, and metastasis. *OMICS* 2021; 25 (5): 313-32.
43. Baeuerle PA, Gires O. EpCAM (CD326) finding its role in cancer. *Br J Cancer* 2007; 96 (3): 417-23.
44. Mehrazma M, Madjd Z, Kalantari E *et al*. Expression of stem cell markers CD133 and CD44 in paediatric solid tumours: a study using tissue microarray. *Fetal Paediatr Pathol* 2013; 32 (3): 192-204.
45. Zahavi D, Weiner L. Monoclonal antibodies in cancer therapy. *Antibodies (Basel)* 2020; 9 (3): 34.
46. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J* 2021; 11 (1): 69.
47. Davis T, Gore-Langton RE, Cheson BD. Current clinical trials of the anti-CD20 monoclonal antibody rituximab. *Oncology (Williston Park)* 2000; 14: 1440-7.
48. Foon KA. Monoclonal antibody therapies for lymphomas. *Cancer J* 2000; 6 (5): 273-8.
49. Yang Y, Zhu G, Yang L, Yang Y. Targeting CD24 as a novel immunotherapy for solid cancers. *Cell Commun Signal* 2023; 21 (1): 312.
50. Cabral-Marques O, Schimke LF, de Oliveira EB Jr *et al*. Flow cytometry contributions for the diagnosis and immunopathological characterisation of primary immunodeficiency diseases with immune dysregulation. *Front Immunol* 2019; 10: 2742.
51. Nagafuchi Y, Shoda H, Fujio K. Immune profiling and precision medicine in systemic lupus erythematosus. *Cells* 2019; 8 (2): 140.
52. Benedek G, Meza-Romero R, Bourdette D, Vandenbark AA. The use of flow cytometry to assess a novel drug efficacy in multiple sclerosis. *Metab Brain Dis* 2015; 30 (4): 877-84.
53. Ashihara E, Shimazaki C, Okano A *et al*. Infusion of a high number of CD34+ cells provides a rapid hematopoietic recovery and cost savings in autologous peripheral blood stem cell transplantation. *Jpn J Clin Oncol* 2002; 32 (4): 135-9.
54. Rawat A, Arora K, Shandilya J *et al*. Flow cytometry for diagnosis of primary immune deficiencies: a tertiary center experience from North India. *Front Immunol* 2019; 10: 2111.
55. Kagan JM, Sanchez AM, Landay A, Denny TN. A brief chronicle of CD4 as a biomarker for HIV/AIDS: a tribute to the memory of John L. Fahey. *For Immunopathol Dis Therap* 2015; 6 (1-2): 55-64.
56. Wang X, Li ZY, Zeng L *et al*. Neutrophil CD64 expression as a diagnostic marker for sepsis in adult patients: a meta-analysis. *Crit Care* 2015; 19 (1): 245.
57. Genel F, Atlihan F, Gulez N *et al*. Evaluation of adhesion molecules CD64, CD11b, and CD62L in neutrophils and monocytes of peripheral blood for early diagnosis of neonatal infection. *World J Pediatr* 2012; 8 (1): 72-5.
58. Robinson JP, Ostafe R, Iyengar SN *et al*. Flow cytometry: the next revolution. *Cells* 2023; 12 (14): 1875.
59. Nagwa AE, Ahmed MM. Significance of CD133 expression in invasive ductal carcinoma of the breast. *Asian Pac J Cancer Biol* 2022; 7 (1): 9-14.
60. Wang X, Li ZY, Zeng L *et al*. Neutrophil CD64 expression as a diagnostic marker for sepsis in adult patients: a meta-analysis. *Crit Care* 2015; 19 (1): 245.
61. Genel F, Atlihan F, Gulez N *et al*. Evaluation of adhesion molecules CD64, CD11b, and CD62L in neutrophils and monocytes of peripheral blood for early diagnosis of neonatal infection. *World J Pediatr* 2012; 8 (1): 72-5.
62. Robinson JP, Ostafe R, Iyengar SN *et al*. Flow cytometry: the next revolution. *Cells* 2023; 12 (14): 1875.
63. Abd El-Sadek N, Mohammed AMH. Significance of CD133 expression in invasive ductal carcinoma of the breast. *Asian Pac J Cancer Biol* 2022; 7 (1): 9-14.

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Auditory Testing – Interpretation for Physicians

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Hearing is a special sense that allows us to identify objects in the world based on the sound they produce and this makes communication using sound possible. Sound is derived from objects that vibrate producing pressure variations in a sound-transmitting medium, such as air. Thus, a sound wave propagating outward from a vibrating object can reach the tympanic membrane (eardrum) of a listener causing it to vibrate and initiate the process of hearing. The external ear collects sound vibrations from the air and focuses these sounds onto the tympanic membrane. Vibrations are transmitted through the middle ear by the ossicular chain (malleus, incus, and stapes). The stapes transmits these vibrations to the cochlear fluids through the oval window producing motion. The hair cells in the cochlea convert the physical vibrations into action potentials transmitted via the auditory nerve to the brainstem for further processing. Deafness may occur due to interruption at any point along this pathway. Sound can also be transmitted through the bones of the skull directly to the cochlea.

According to WHO, a person is said to have hearing loss if he is not able to hear as well as someone with normal hearing (meaning hearing thresholds of 25 dB or better in both ears). It can be mild, moderate, moderately severe, severe or profound, and can affect one or both ears (Table I). The prevalence of hearing loss varies with age; at least 25 per cent of patients between 51 and 65 years of age, and more than 50 per cent of patients older than 80 years, have objective evidence of hearing loss[1]. The common causes of hearing loss include congenital or early onset childhood hearing loss, chronic middle ear infections, noise-induced hearing loss, age-related hearing loss, and ototoxic drugs that damage the inner ear.

Broadly there are three types of hearing loss: conductive hearing loss (CHL), sensorineural hearing loss (SNHL)and mixed hearing loss. A conductive hearing loss happens when pathology lies in outer and middle ear such as wax in the ear canal, tympanic membrane perforation, middle ear ossicular discontinuity/fixation. Damage to inner ear or nerve pathways from inner ear to brain causes SNHL such as presbycusis (age related hearing loss), oto-toxicity (drug induced hearing loss), noise induced hearing loss, tumours involving/compressing auditory nerve. When there are components of both CHL and SNHL, it is called mixed hearing loss.

It is important for all physicians to pick up hearing loss in their patients due to various systemic diseases and ototoxic drugs. This can effectively be done by hearing tests.

This article will discuss few screening tests which can be used by General Practitioners for hearing assessment. These include Whisper voice test, Finger rub test, Watch ticking test and Scratch test. These screening tests are less reliable and patients can be referred for definitive hearing tests on suspicion of hearing loss. The present article will focus on two such tests: Tuning fork tests and Pure tone audiometry.

Tests for screening of hearing loss (for general practitioners)

1. Whisper voice test

In most Western countries, National Health Guidelines encourage general practitioners to screen elderly people for hearing loss using whispered voice test. Because the tuning fork test evaluates hearing at a single low frequency, it is not appropriate for most elderly patients with presbycusis, who typically have lost the ability to hear high frequencies[2].

Procedure: The examiner stands behind the patient, about an arm's length away, and whispers a series of letters and numbers into one ear while the patient covers the other ear. The patient then repeats what they heard. The test is considered passed if the patient correctly repeats at least three out of the six letters and numbers. To maintain uniformity, the test should be performed after a slow, complete, silent exhalation. The number-letter combination should be different for each ear.

Several studies also examined the reliability or reproducibility of the whispered voice test. Uhlmann *et al*[3] compared the results of an otolaryngologist and an audiologist for 63% of the patients and found a correlation of 0.67 and Macphee *et al*[4] found concordance between a geriatrician and an otolaryngologist of 0.88.

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Limitations of Whisper voice test

1. One area of concern is the reproducibility of the whispered voice test. The results of the studies that measured reliability indicate that the test can be reliable if a standard procedure is used.
2. The most appropriate letters, numbers, or words for testing also needs further investigation. In the elderly population, where presbycusis is the most common type of hearing loss, difficulty in hearing sounds in the higher frequencies is common. As the consonants of speech are usually higher frequency sounds than the vowels[5] using different consonants and vowels in testing could alter the results of the test considerably.
3. The greatest difficulty in standardising the test is the loudness of the whisper. Only few studies mentioned that the whispered sequence occurred after a full expiration. This seems to be an important determinant of the loudness of the whisper.
4. With the test sensitivity much lower in children than adults, it might be argued that the test is of limited value in children[6].

2) Finger rub test

This is a crude and easy test for screening for hearing loss. In the finger rub test, the examiner rubs his or her fingers together near the patient's ear and asks the patient whether they have heard the sound. CALFRAST is calibrated finger rub auditory screening test.

Procedure[7]

With the hand comfortably dry, the CALFRAST sound was produced by briskly rubbing the thumb across the distal fingers. The subject was encouraged to listen carefully so as not to miss the faintest sound. With the subject's eyes closed, the examiner stood nose to nose, 6 to 10 inches in front of the subject, and extended both arms straight laterally so that the moving fingers would be equidistant from the examiner's and subject's ears, a distance of approximately 70 cm. First, a strong finger rub (CALFRAST–Strong 70), as strong as one can perform without snapping the fingers, was presented to each side separately and was repeated three times. If the subject reported accurately, the next test was the faint finger rub (CALFRAST–Faint 70), the softest rub that the examiner could hear with arms fully extended. If the participant heard CALFRAST–Faint 70 bilaterally, the testing was complete.

If the participant did not hear CALFRAST–Strong 70 with either ear, still louder stimuli were produced by bringing the strong rub closer to the tested ear at standard intervals of approximately 35, 10, and 2 cm. Halving the stimulus distance to approximately 35 cm was conveniently estimated by flexing the elbow to 90 degrees (CALFRAST–Strong 35). One hand breadth was conveniently used to mark the 10 cm distance (CALFRAST–Strong 10). The 2 cm distance stimulation was presented as close to the tragus as possible, without touching the earlobe (CALFRAST–Strong 2). The CALFRAST level for each ear was the weakest stimulus perceived.

3) Clock ticking test

During this test, an examiner places a watch next to the patient's ear and ask them to note when they can no longer hear the ticking.

4) Scratch test

This test was decribed as a means of diagnosing an acute post-operative dead ear, alternative to the Weber test[8], when the tuning fork is not available.

Procedure: In the post-operative period, the bandage was scratched in the midline and patient was asked whether he can hear the sound[9].

Tuning fork tests

Tuning forks (Fig. 1) are used as a simple test in the OPD to establish the probable presence or absence of a significant conductive element to hearing loss. The sound is produced by setting the tuning fork into vibration. They are typically used to provide early diagnostic information when audiometry is not available or possible[10]. The most preferred tuning fork is a 512Hz tuning fork. At this frequency, in comparison to the 256Hz and 1024 Hz tuning forks, the tone does not fade too quickly, produces limited overtones and is not vibrotactile[11]. The practitioner shall hold the tuning fork by its stem and strike one side of the tines, two thirds of the way along the tine from the base, on a padded surface or the practitioner's elbow or ball of hand. Do not strike on a hard surface as this will introduce harmonic overtones and may damage the tuning fork. The test should be undertaken in a quiet room.

Air conduction and bone conduction

Before we discuss the types of tuning fork tests, let us understand two important terms called as air conduction and bone conduction testing. In tuning fork tests, we test for air conduction as well as bone conduction. Air conduction means when sound is given through the External auditory canal. It is transmitted through tympanic membrane, middle ear ossicles and then reaches cochlea. It tests both conductive and sensorineural pathways. For air conduction testing, after striking, the tuning fork is held in front of the ear canal. Bone conduction testing is done by

placing the tuning fork over the mastoid process after striking. Here, the sound is transmitted to cochlea through skull bones. It directly measures cochlear function. Air conduction is always better than bone conduction in normal individuals.

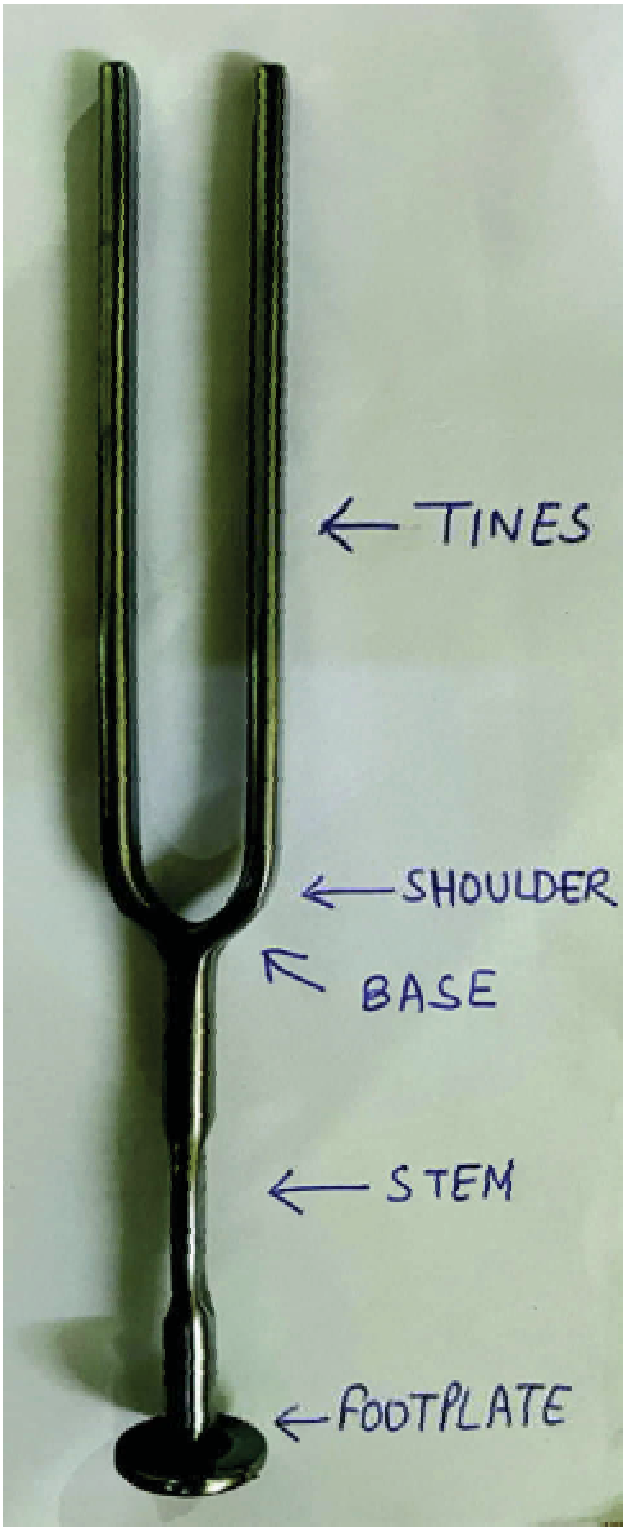

Fig. 1: *Tuning Fork.*

The main tuning fork tests are Rinne, Weber and Absolute bone conduction (ABC). The practitioner shall instruct the patient on each of the tests. Tuning fork tests are particularly subjective and response bias must be accounted for when determining their validity as diagnostic tools. Clear and concise instructions will limit misinterpretation by the patient. Tuning fork tests have been the mainstay of otologic examination in OPD. The Weber test has been mainly used to establish a diagnosis in patients with unilateral hearing loss to distinguish between conductive and sensorineural hearing loss[12].

The Weber Test

The Weber test is a test of lateralisation of the sound and is more commonly preferred in asymmetric hearing loss.

Procedure[13]: Strike the tuning fork (512 Hz) and place it on the midline, typically on the patient's forehead but it can also be placed on the vertex, bridge of the nose or chin. Stabilise the patient's head using other hand. Hold the tuning fork in place for up to 4 seconds (Fig. 2). After giving the patient listening time, ask them where the tone is heard: is it equal in both ears (central), or towards the left or right ear. The patient is asked to which ear the sound feels lateralised.

Interpretation

1. With symmetrical hearing or a symmetrical hearing loss the sound should be central.
2. With an asymmetrical sensorineural loss, the sound should be heard in the better ear.

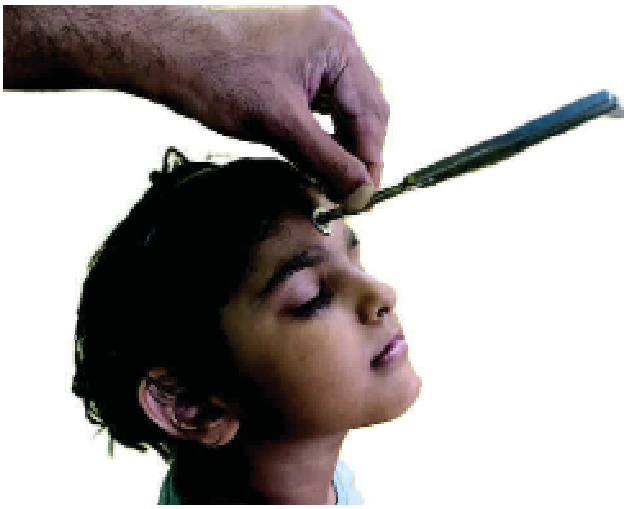

Fig. 2: *Weber Test.*

3. With an asymmetrical conductive hearing loss the sound should be heard in the poorer ear.

Principle: In normal individuals (without hearing loss), the inner ear is more sensitive to sound via air conduction than bone conduction (in other words, air conduction is better than bone conduction).

In the presence of a purely unilateral conductive hearing loss, there is a relative improvement in the ability to hear a bone-conducted sound. This can be explained by the following:

- ***Masking effect:*** The sound heard via the affected ear has less environmental noise reaching the cochlea via air conduction compared to the unaffected ear, which receives sounds from both bone conduction and air conduction. Therefore, the affected ear is more sensitive to bone-conducted sound[14].
- ***Occlusion effect:*** Most of the sound transmitted via bone conduction travels to the cochlea. However, some of the low-frequency sounds dissipate out of the canal. A conductive hearing loss will prevent external dissipation of these frequencies and lead to increased cochlear stimulation and loudness in the affected ear[15].

In the presence of sensorineural hearing loss, the sound will be perceived louder in the unaffected ear, which has a better cochlea. Thiagarajan and Arjunan[16] reported that the Weber test can determine a difference of 5 decibels between two ears in terms of bone conduction thresholds.

The Rinne Test

This test is commonly done by loudness comparison method. We compare loudness of air conduction testing with bone conduction.

Procedure[13]: The practitioner should start with the ear to which the Weber has lateralised to. Place the vibrating tuning fork approximately 25 mm from the ear canal entrance with its tines parallel for about 2 seconds. Now without any interruption and without touching the tines, press the footplate of tuning fork firmly against the mastoid (without any hair getting between the footplate and the mastoid). The tuning fork is held in place for another 2 seconds (Fig. 3). After giving the patient listening time, ask them whether the tone is louder next to the ear or behind the ear. The patient should respond verbally.

Interpretation

1. If air conduction (next to the ear canal) is louder, it is interpreted as Rinne positive result indicating either normal hearing or a Sensorineural hearing loss (AC >BC).
2. If bone conduction (held on mastoid) is louder, it is a Rinne negative result, indicating a Conductive Hearing loss (BC >AC).

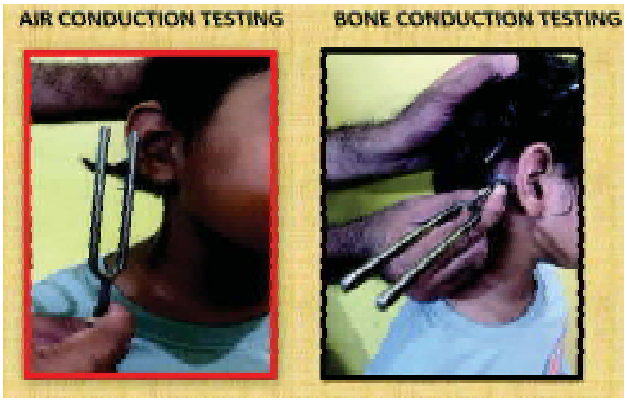

Fig. 3: *Rinne Test.*

Principle

Rinne Positive: The patient is positive on that side (the ossicular chain is doing what it should be doing, acting as an amplifier).

Rinne Negative: If the bone conduction through the mastoid process is heard louder than through the air, the patient is Rinne negative. This is seen in CHL. This is because air vibrations are not transmitted across the external auditory canal, the tympanic membrane, the ossicular chain, or the oval window.

Rinne False Negative: This occurs with a severe sensorineural loss predominantly on the test side. This occurs when the bone conduction transmits through the skull to the opposite ear and is detected through cross hearing by the better cochlea (in the non-test ear). It can be distinguished by considering if the Weber test result is contradictory and asking the patient which ear the bone conduction part of the test was heard in.

Absolute Bone Conduction Test (ABC)

This is a test of cochlear function.

Procedure

In this test, bone conduction is tested by placing the vibrating tuning fork over the mastoid region with occlusion of external auditory canal (EAC) to prevent air conduction sounds interfering with bone conduction. The patient is asked to inform when he stops hearing the sound. Then the same vibrating tuning fork is placed over the mastoid bone of examiner himself. The examiner then hears the sound.

Interpretation

1. If the examiner is also not able to hear any sound then it is interpreted as 'Same as Examiner' and indicates normal cochlear function.
2. If examiner is still able to hear sound the sound of tuning fork when patient has stopped hearing it is interpreted as 'Shortened' and indicates SNHL.

Table I: Classification of hearing loss[20].

Grade Of Impairment	Pure Tone Audiometric Threshold[a,B]
Normal Hearing	0 - 25 dB
Mild Hearing Loss	26 - 40 dB
Moderate Hearing Loss	41 - 60 dB
Severe Hearing Loss	60 - 80 dB
Profound Hearing Loss	>80 dB

[a]In the better ear; [B]Average of 500, 1000, 2000 and 4000 Hz.

Table II: Summary of Tuning Fork Tests.

	Rinne	Weber	ABC
Normal	Positive	Central	Same as examiner
CHL	Negative	Lateralised to deaf ear	Same as examiner
SNHL	Positive	Lateralised to normal ear	Shortened (as compared to examiner)

Pure Tone Audiometry

The tuning fork tests give us an idea of presence or absence of hearing loss and type of hearing loss. But to find the degree of hearing loss, Pure tone Audiometry (PTA) is performed. It is done with the use of an audiometer. The graph thus plotted is called an audiogram. The advantage of an audiogram is that it provides information about the type and degree of hearing loss. Also, it provides a permanent record which can be included in the patient's file for future reference and comparison and is essential for documentation especially for medicolegal cases. Audiometry is more challenging in patients younger than five years.

Pure tone testing is the measurement of an individual's hearing sensitivity to pure tones at different frequencies. A pure tone audiology threshold at a specific frequency is the decibel level at which a sound is perceived 50% of the time. The decibel scale used in pure tone audiometry is *dB Hearing Level (dB HL)*. The dB HL intensity scale is based on normal human hearing with 0 dB HL representing the median threshold for otologically normal young adults[17]. The basic audiological assessment focuses on pure tone air conduction thresholds in the frequency range 0.25 - 8 KHz.

Pure tone thresholds at each frequency are plotted on a graph called an *audiogram*, which depicts the type, degree and configuration of the hearing loss(Fig. 4). The x-axis denotes the frequency being tested and the y-axis denotes the decibel level at which the patient perceives the pure tone sound. Using specific symbols, a mark is placed on the graph at the decibel level where the patient perceives the particular frequency of sound being tested. This is done separately for both ears.

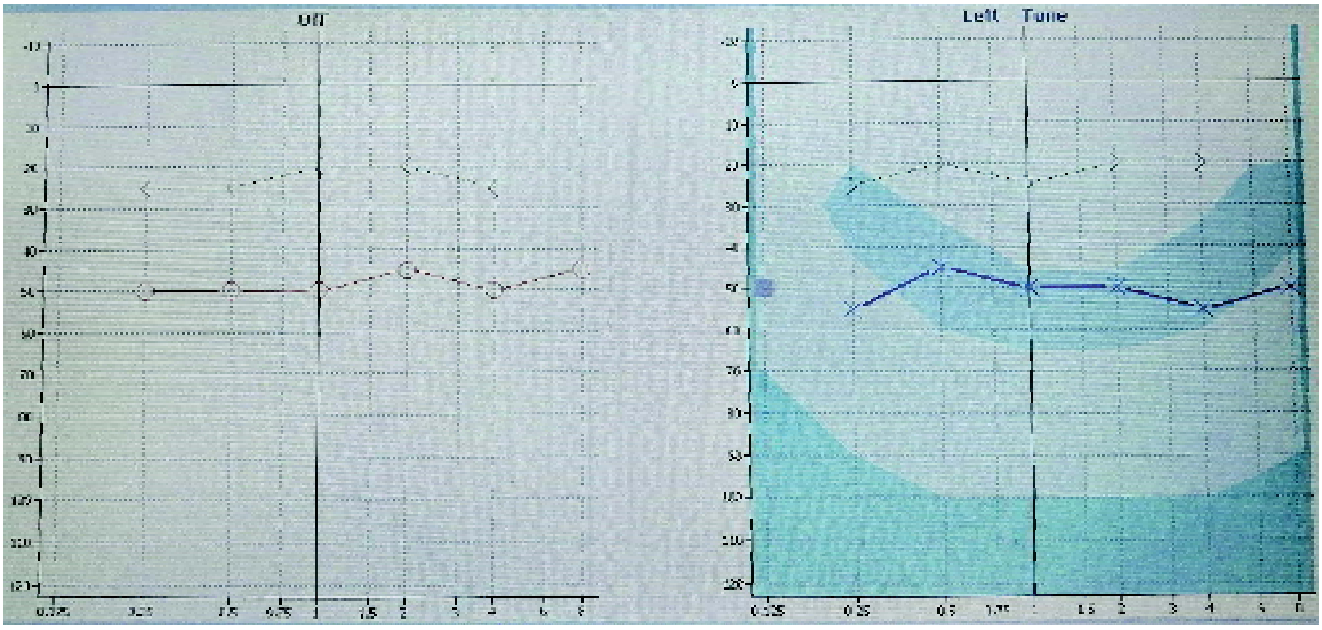

Fig. 4: *Audiogram.*

For air conduction testing, sound is given using head phones. Whereas for bone conduction testing, sound is given by a bone oscillator placed over the mastoid bone. Testing is done separately for both ears. There are specific symbols to record air conduction and bone conduction thresholds on an audiogram for both ears as shown below.

	Umasked Air	Unmasked Bone	Masked Air	Masked Bone
Right	0	<	△	[
Left	X	>	□	]

Usually, the symbols for right ear are drawn in red colour and left ear are in blue colour on an audiogram.

Masking

If the pure-tone threshold difference or asymmetry between ears at any frequency is equal to or greater than 40 dB, the sound energy from the test ear can stimulate the nontest ear, causing the nontest ear to respond to the stimulus. To prevent this crossover of sound from one ear to the other, narrow band noise is presented to the nontest ear and thresholds are recorded as masked. This procedure in audiology is called as Masking.

Interpretation

The values of the thresholds are inserted into the audiogram blank sheet. The tone frequency expressed in Hertz is recorded on the horizontal axis, while the vertical axis shows the tone intensity expressed in Decibels[18].

A normal audiogram displays air and bone conduction lines with thresholds of ≥25 dB HL at each of the tested frequencies in both ears. An air-bone gap is noted on the audiogram, which is present when the difference between air conduction and bone conduction thresholds at a specific frequency is greater than 15 dB HL and is indicative of CHL. With a pure CHL, the bone conduction thresholds are normal, but the air conduction thresholds indicate hearing loss. On an audiogram, pure SNHL is indicated by overlapping of the lines representing air conduction and bone conduction without the presence of any air-bone gaps >10 dB[19] (Fig. 5).

The audiogram gives a fundamental description of auditory sensitivity. According to the international classification of hearing loss, to calculate the degree of hearing loss, it is necessary to summarise the four values, i.e., the lowest audible sound intensity using the frequencies of 500, 1,000, 2,000 Hz, and 4,000 Hz and then to divide the sum by 4 to get the arithmetic average using the following formula:

I(500) + I(1000) + I(2000) + I(4000)/4

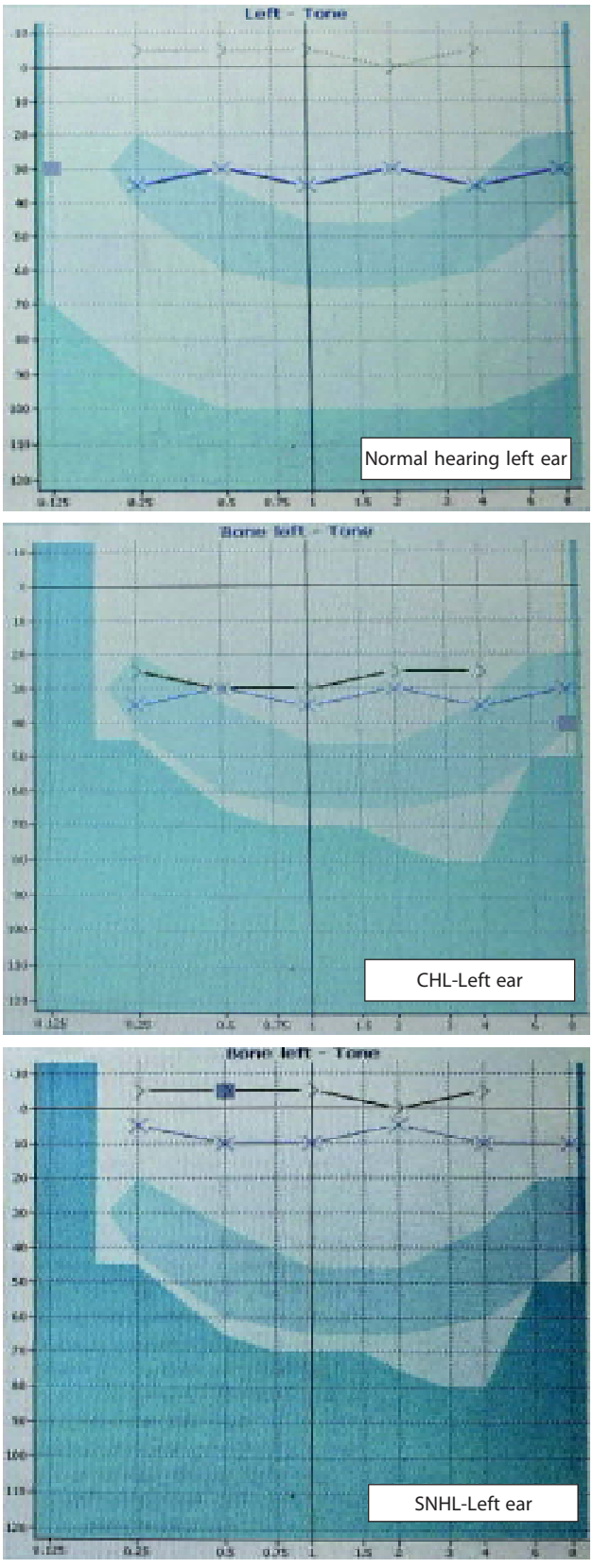

Fig. 5: *Various Types of Audiogram.*

References

1. Walker JJ, Cleveland LM, Davis JL *et al*. Audiometry Screening and Interpretation. *American Family Physician* 2013; 87: 41-6.
2. Pirozzo S, Papinczak T, Glasziou P. Whispered voice test for screening for hearing impairment in adults and children: systematic review. *BMJ* 2003; 327 (7421): 967.
3. Uhlmann RF, Rees TS, Psaty BM. Validity and reliability of auditory screening tests in demented and non-demented older adults. *J Gen Intern Med* 1989; 4: 90-6.
4. Macphee GJ, Crowther JA, McAlpine CH. A simple screening test for hearing impairment in elderly patients. *Age Ageing* 1988; 17: 347-51.
5. Fleming KC, Evans JM, Weber DC. Practical functional assessment of elderly persons: a primary-care approach. *Mayo Clin Proc* 1995; 70: 890-910.
6. Pirozzo S, Papinczak T, Glasziou P. Whispered voice test for screening for hearing impairment in adults and children: systematic review. *BMJ* 2003; 327 (7421): 967.
7. Torres-Russotto D, Landau WM, Harding GW *et al*. Calibrated finger rub auditory screening test (CALFRAST). *Neurology* 2009; 72 (18): 1595-600.
8 Buckland JR, Geyer M, Maleki N. The use of the weber tuning fork test and 'scratch test' in post-operative tympanomastoid surgery. *Clinical Otolaryngology* 2006; 31 (6): 581.
9. Iacovidou A, Giblett N, Doshi J. How Reliable Is the "Scratch Test" Versus the Weber Test After Tympanomastoid Surgery. *Otology and Neurotology* 2014.
10. Turton L, Batty S. Recommended procedure rinne and weber tuning fork tests. *British Society of Audiology* 2022.
11. Khanna S, Tonndorf J, Queller J. Mechanical parameters of hearing by bone conduction. *J Acoustic Soci Amer* 1976; 60 (1): 139-54.
12. Blakley BW, Siddique S. A qualitative explanation of the Weber test. *Otolaryngol Head Neck Surg* 1999; 120 (1): 1-4.
13. Recommended procedure rinne and weber tuning fork tests BSA 2022.
14. Surendran S, Stenfelt S. The Outer Ear Pathway During Hearing By Bone Conduction. *Hear Res* 2022; 421: 108388.
15. Goldstein DP, Hayes CS. The occlusion effect in bone conduction hearing. *J Speech Hear Res* 1965; 8: 137-48.
16. Thiagarajan B, Arjunan K. Tuning fork tests. *Webmedcentral Otorhinolaryngol* 2012; 3: WMC003279.
17. Recommended procedure Rinne and weber tuning fork tests BSA 2022.
18. Soer M. Open access guide to audiology and hearing aids for otolaryngologists. The Open Access Atlas of Otolaryngology, Head & Neck Operative Surgery.
19. Pure-tone Audiometer. Kapul AA, Zubova EI, Torgaev SN. IOP Conf. Series: *Journal of Physics*: Conf. Series 881, 2017, 012010.
20. Olusanya BO, Davis AC, Hoffman HJ. Hearing loss grades and the International Classification of functioning, disability and he health. *Bull World Health Organ* 2019; 97 (10): 725-8.

CASE REPORT

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Slowly Progressive Familial Amyotrophic Lateral Sclerosis: G93C Variant of Superoxide Dismutase 1 Mutation

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Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease with an unknown aetiology. In the literature, 5 - 10% of ALS cases are familial. There can be various mutations that cause ALS; the most common are the C9orf72 or SOD1 mutations. In the Asian population, SOD1 mutation is mostly described. We present an unusual variant of the SOD1 mutation, which is characterised by distal motor neuron disease without any bulbar affection and very slow progression. This is the second genetically proven familial ALS among all case reports published and the first reported G93C/G94C variant SOD1 mutation from India.

Key word: Familial ALS, slow ALS , rare ALS, SOD1 mutation, G93C variant.

Introduction

Amyotrophic lateral sclerosis is a disorder of progressive weakness, wasting, and spasticity because of anterior motor horn cell affection. Sporadic ALS aetiology is often unknown, but 5 - 10% of ALS are familial. There is a low incidence of ALS in India as compared to the US[1]. Familial ALS in India is reported to be <5%. The mutation common in the Asian cohort is the SOD mutation, while in the Caucasian cohort, the most common mutation is C9orf72, accounting for 40% of familial ALS[1]. In India, there has been only one case report on familial ALS by Devi *et al*. It showed an L84F mutation in exon 4 of SOD1 with the novel nucleotide variation c255G77 inherited in four members of the family with autosomal dominant inherited ALS[2]. Till now, only 3 case reports from India, have showed a familial pattern, and only one was genetically proven. All cases had a decreased life expectancy, and familial ALS was reported to have more prominent bulbar symptoms than sporadic cases[3].

Case report

A 37-year-old lady presented with gradually progressive asymmetric quadriparesis with wasting of both upper and lower limbs of 5-year duration. The patient first noticed weakness at 32 years of age when she noticed difficulty climbing stairs, standing for long hours, or walking long distances. The symptoms were initially noted in her right leg but involved her left lower limb after 6 months. Gradually, over the next year, she had difficulty getting up in the toilet or from the ground. She started noticing around this time that her legs were thinning out; there was no history of any distal weakness in the form of difficulty wearing a slipper or buckling of the knee or ankle. There were no sensory symptoms. For the past 2 years, she noticed weakness in the distal muscles of her left hand; she had difficulty peeling vegetables, holding her phone, or typing with her left hand. This had progressed and was now affecting the right hand too. For the past year, she has had difficulty buttoning, breaking chapati (Indian bread), or holding a spoon. So far, there was no history of difficulty turning in bed or requiring support for getting out of bed; there was no dysphagia or dysarthria. The patient was able to carry-out her daily activities with support, and her ALS functional score was 35 (Table I). On examination, she had no fasciculations or fibrillations in the tongue; she had fasciculations over both biceps and triceps and quadriceps muscle; there was distal wasting of both upper and lower limbs and polyminimyoclonus in both upper limbs; the power was 4 in both upper limbs in distal muscles; 4/5 in proximal muscles; similarly, 3 in both lower limbs in antigravity muscles; and 4 in other muscles. She had absent reflexes in both lower limbs and bilateral plantars that were extensor; no sensory or cerebellar signs were present.

Table I: ALS functional rating scale-revised in this patient (ALSFRS-R)[14].

Bulbar		Fine motor		Gross Motor		Respiratory	
Speech	4	Handwriting	3	Turn in bed	2	Dyspnoea	3
Salivation	4	Cutting Food	2	Walking	2	Orthopnoea	4
Swallowing	4	Dressing	2	Climbing stairs	1	Respiratory Insufficiency	4

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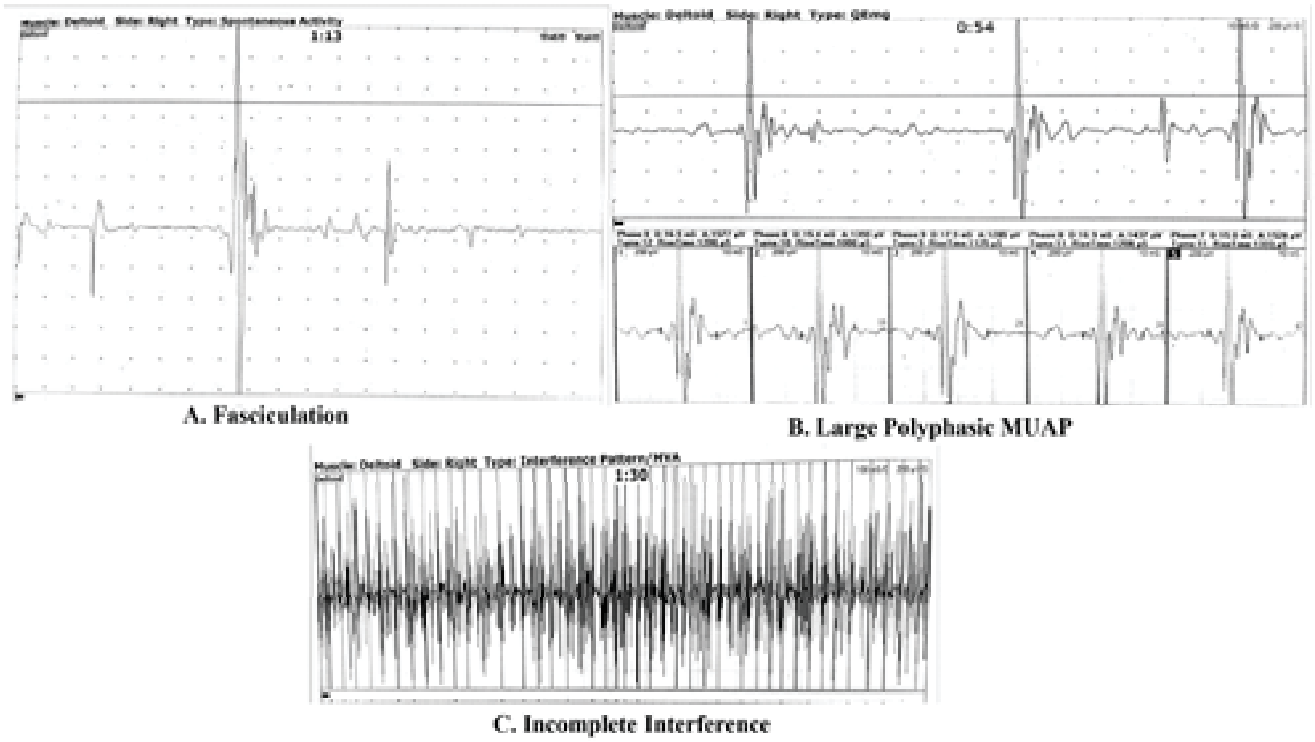

Fig. 1: *Electromyographic pattern of the patient.*

Her NCV was suggestive of motor axonal affection in both upper and lower limbs, there was no affection of sensory nerves, CPK level was normal, and EMG was suggestive of a neurogenic pattern (presence of fibrillations, positive sharp waves and fasciculations along with large polyphasic motor unit action potential with increased duration and incomplete interference) (Fig. 1) in three contiguous segments. An electrophysiological diagnosis of possible ALS was kept, according to modified Awaji criteria[4].

She underwent blood investigations and PET scan to determine the mimics of Motor Neuron Disease. Her whole exome sequence was sent because of a strong familial history of similar illness in family members (see pedigree below in Fig. 2), which was suggestive of the 'c.280 G >T (p.Gly94Cys)' variant of the SOD1 gene in exon 4.

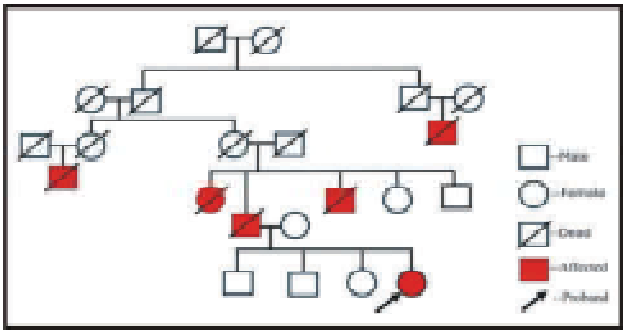

Fig. 2: *Pedigree chart of the patient.*

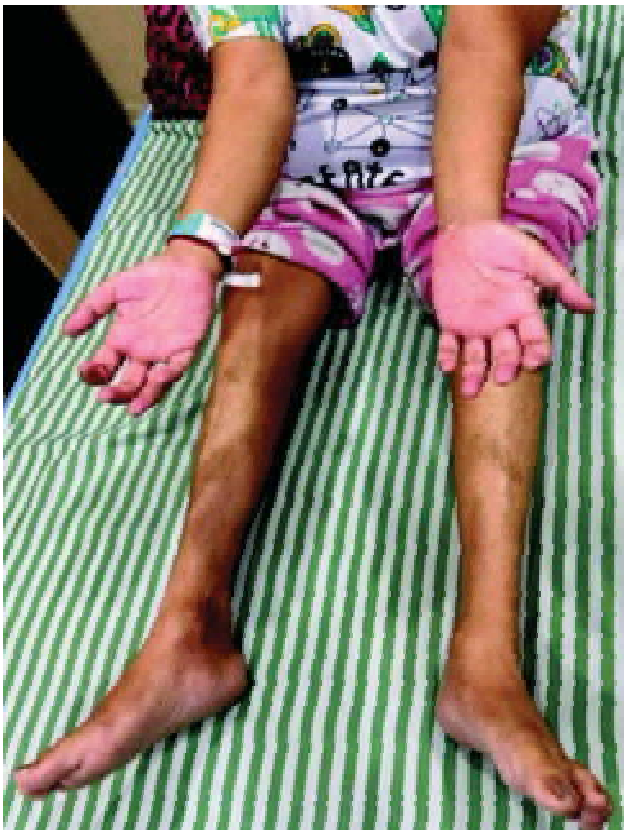

Fig. 3: *Wasting of distal muscles of Upper and Lower limbs.*

Discussion

Amyotrophic lateral sclerosis is characterised by the presence of upper and lower motor neuron features, bulbar involvement, and a poor prognosis. So far, no effective treatment is available for the disease. 5 - 10% of cases may have familial ALS with a predominant autosomal dominant mutation. The frequency of mutations in common ALS-causing genes varies by geographic location; the most common genetic mutation in the Caucasian population is C9orf72, which accounts for >40% of familial and 5 - 20% of sporadic ALS[1-3]. Approximately 20% of familial ALS in the Asian population has mutations in the Cu/Zn superoxide dismutase (SOD1) gene. At least 46 different SOD1 mutations are known to exist, but because the mutations are dispersed throughout the SOD1 structure, their exact molecular mechanism has not been determined[5]. The principle biochemical action of SOD1 is to convert potentially toxic superoxide radicals into hydrogen peroxide, but a toxic gain of mutation for mutant SOD1 causes impaired free radical scavenging[6]. The symptoms in familial ALS are similar to those in sporadic cases involving muscle weakness and atrophy, speech difficulties, swallowing, and respiratory dysfunction due to the progressive degeneration of upper and lower motor neurons[7]. Although familial ALS is generally associated with an earlier age of onset and commonly manifests symptoms in the lower limb, there can be variable disease duration, life expectancy, and clinical progression according to genetic subtypes[3]. The SOD1 mutation generally tends to affect people around 50 years of age[9,10], and there is a tendency to progress with more prominent bulbar symptoms in familial ALS than in sporadic cases.

There are very sparse case reports of familial ALS from India. Only two reports of the SOD1 mutation have been reported till now[11,12]. All cases had an age of onset of around 50 years and an autosomal dominant pattern; there was rapid progression, and the life expectancy was a maximum of 2 years. The median survival in Chinese and Indian studies suggests longer periods of survival compared to Caucasians and an earlier age of onset[9]. Migration studies have also observed similar patterns. In one study of migrants from the Indian subcontinent to the UK, a younger age of onset of patients with lower mortality rates was observed[14].

We have a very unusual presentation of ALS, with the patient relatively preserved even after 5 years of the onset of the illness. The average survival of patients with limb-onset ALS in Asian as well as Caucasian cohorts is 2 years, with 114.8 months of survival reported in an Indian cohort by Devi *et al*[11]. Our patient had survived for more than 5 years and had no bulbar symptoms. Her ALS functional score was 35 out of 40.

Many authors report pathological differences between familial and sporadic ALS, with prominent involvement of the posterior column, spinocerebellar tract, and Clarke's column in familial ALS[7,13]. Our patient had no such complaint involving sensory or cerebellar structures. She had a slower progression of disease as compared to other cases. The patient's father had died at the age of 55 years within 4 years of the onset of disease; her paternal uncle and aunt, suffering from similar illnesses, also died within a few years of the onset of disease. The patient's gradual progression can be explained by the G93C variant which is associated with a less severe disease phenotype, while among other variants, L144S is associated with the least severe form and G41S is associated with the most aggressive form of ALS[7,13]. The G93C mutation is associated with a purely lower motor neuron clinical phenotype and the absence of bulbar involvement. A hint to possible mechanisms in this diversity of phenotypes was provided in a recent report on the selective association of mutant SOD1 with mitochondria of affected tissues in transgenic mouse models of ALS[16].

Conclusion

We hereby present an unusual case of ALS, with autosomal dominant inheritance, asymmetric quadriparesis, and a gradual onset and progression. She had the disease for more than 5 years, but her functional scale was still 35/40, which makes it a unique case with prolonged survival, among all cases reported till now. The case shows a rare variant of Superoxide Dismutase 1 mutation affecting the G93C variant; this variant is commonly associated with slow progression and good survival.

References

1. Shahrizaila N, Sobue G, Kuwabara S *et al*. Amyotrophic Lateral sclerosis and motor neuron syndromes in Asia. *J Neurol Neurosurg Psychiatry* 2016; 87: 821-30.
2. Gouri Devi M, Rao V N, Prakashi R *et al*. Neuroepidemiological study in semi urban and rural areas in South India: pattern of neurological disorders including motor neurone disease. In: Gauri Devi M ed. *Motor Neurone Disease: Global Clinical Patterns and International Research*. New Delhi: Oxford and IBH, 1987; 11-21.
3. Nalini A, Thennarasu K, Gourie-Devi M *et al*. Clinical characteristics and survival pattern of 1,153 patients with amyotrophic lateral sclerosis: experience over 30 years from India. *J Neurol Sci* 2008; 272: 60-70.
4. Costa J, Swash M, de Carvalho M. Awaji Criteria for the Diagnosis of Amyotrophic Lateral Sclerosis: A Systematic Review. *Arch Neurol* 2012; 69 (11): 1410-6.
5. Ogaki K, Li Y, Atsuta N *et al*. Analysis of C9 or f72 repeat expansion in 563 Japanese patients with amyotrophic lateral sclerosis. *Neurobiol Aging* 2012; 33: 2527.e11-6.
6. Shaw CE, Enayat E, Powell JF *et al*. Familial amyotrophic lateal sclerosis: Molecular pathology of a patient with a SOD 1 mutation. *Neurology* 1997; 49: 1612-6.

7. Chen L, Zhang B, Chen R *et al*. Natural history and clinical features of sporadic amyotrophic lateral sclerosis in China. *J Neurol Neurosurg Psychiatry* 2015; 86: 1075-81.
8. Bae JS, Hong YH, Baek W *et al*. Current status of the diagnosis and management of amyotrophic lateral sclerosis in Korea: a multi-center cross-sectional study. *J Clin Neurol* 2012; 8: 293-300.
9. Paulo Victor Sgobbi de Souza *et al*. Clinical and genetic basis of familial amyotrophic lateral sclerosis. *Arq Neuropsiquiatr* 2015; 73 (12): 1026-37.
10. Berdyfiski M, Miszta P, Safranow K *et al.* SOD1 mutations associated with amyotrophic lateral sclerosis analysis of variant severity. *Sci Rep* 2022; 12: 103.
11. Vats A, Gourie-Devi M, Verma M *et al*. Identification of L84F mutation with a novel nucleotide change c.255G > T in the superoxide dismutase gene in a North Indian family with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener* 2015; 1-7.
12. Kulshreshtha D, Singh AK, Maurya PK, Thacker AK. Familial amyotrophic lateral sclerosis from north India: case report and brief review. *Neurol India* 2014; 62 (2): 210-1.
13. Shamshiri H, Fatehi F, Davoudi F *et al*. Amyotrophic lateral sclerosis progression: Iran-ALS clinical registry, a multicentre study. *Amyotroph Lateral Scler Frontotemporal Degener* 2015; 16: 506-11.
14. Elian M, Dean G. Motor Neuron disease and multiple sclerosis among immigrants to England from the Indian subcontinent, the Caribbean, and east and west Africa. *J Neurol Neurosurg Psychiatry* 1993; 14: 116-23.
15. Cedarbaum JM, Stambler N, Malta E *et al*. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. *J Neurol Sci* 1999; 169 (1-2): 13-21.
16. Liu J, Lillo C, Jonsson PA *et al.* Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. *Neuron* 2004; 435-17.

CASE REPORT

JIACM 2024; 25 (4): 215-17

Human Herpes Virus-6 Meningoencephalitis in an Immunocompetent Peripartum Lady

Sonali Bhattu, SH Talib**, Abdulla Ibji***, Amjad Syed Ali*****

Abstract

We describe a case of Human herpesvirus-6 (HHV-6) meningoencephalitis in an immunocompetent peripartum lady who presented to the emergency department with febrile illness, disorientation, myalgia and altered levels of consciousness. Encephalitis occurs in immunocompromised people and is the most feared complication of HHV-6 disease. Two strains of this virus are A and B. HHV-6B is the predominant strain. This virus is characterised by lifelong latency in peripheral blood mononuclear cells and brain tissues. Hence, latent infection/reactivation occurs in immunocompromised patients. The literature is scanty about CNS involvement in immunocompetent adult patients. Meningoencephalitis developing in an immunocompetent peripartum case is unreported in Asian literature. HHV-6 infection should be kept in mind in any immunocompetent patient with meningoencephalitis of uncertain aetiology.

Key words: HHV-6, meningoencephalitis, immunocompetent, peripartum, acyclovir.

Introduction

HHV-6 is the sixth herpes virus discovered infection in humans and is nearly ubiquitous in the first two years of life with sero-prevalence rate of 95% in most studies[1]. The virus was isolated in 1986 among patients of lymphoproliferative diseases. Encephalitis occurs in immunocompromised patients and is the most feared complication of HHV-6 disease. Infection by this Beta – Herpes virus is characterised by two strains A and B. HHV-6B is the predominant strain of the virus. HHV-6A disease has been documented to cause illness in only immunosuppressed host[2]. The clinical difference is not well documented, so for the purposes of management, they are treated the same. HHV-6 is also characterised by lifelong latency in peripheral blood mononuclear cells and brain tissues following primary infection[3]. The latent infection or reactivation often occurs in immunocompromised patients and may cause fever, pneumonia, rashes, hepatitis and meningoencephalitis. Literature is scanty for central nervous system involvement in adult immunocompetent patients suffering from meningoencephalitis, especially in the peripartum period.

Case report

We describe a case of HHV-6 meningoencephalitis in an immunocompetent 25-year-old lady, who had recently delivered a baby. She had a history of pregnancy induced hypertension and was on oral Nifedipine 10 mg every 12 hourly. The records indicated her pregnancy was otherwise uneventful. She had a normal delivery of a female baby weighing 2,900 g. She was discharged the next day, in an apparently healthy condition with normal vitals.

Two days post-delivery she developed febrile illness and altered levels of consciousness. She also offered history of headache and myalgia. Physical examination revealed healthy episiotomy wound with normal gynaecological examination. Her Pulse was 120 beats per minute, regular with good volume, blood pressure 140/100 mmHg (on Nifedipine 10 mg BD). Her respiratory rate was 20 breaths per minute with a temperature of 101° F. Cyanosis was absent. She had neck stiffness, right lateral rectus palsy and normal pupils. Owing to thick throat secretions and respiratory discomfort she was intubated for 4 days. Deep tendon reflexes were brisk with bilateral extensor plantar response. Muscle strength and tone were however normal. Other systemic examination was unrevealing. As she was febrile and showed signs of meningism, a diagnosis of meningoencephalitis was clinically entertained. The details of investigations on haemogram, renal function tests, liver function tests, thyroid function tests, urine and cerebrospinal fluid (CSF) examination done are shown in Table I. The neurotropic virus panel in CSF done by real time PCR (RTPCR) is shown in Table II.

She was started on empirical parenteral Ceftriaxone, Acyclovir and Dexamethasone. Cranial magnetic resonance imaging (MRI) with contrast was performed on the second day of present hospitalisation. Neurophysician opined

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hyperintensity in left temporal lobe, rest features were normal. Antibiotics were discontinued on 4th day of hospitalisation and treatment with acyclovir and dexamethasone continued for next 7 days. Her lateral rectus palsy, fever and plantar response receded after 7 days of acyclovir and dexamethasone therapy. Patient's general condition improved with normal mental status. She recovered from the disease without any morbidity and was discharged in healthy condition on 10th day of hospitalisation.

Table I: Showing investigations done on the patient on admission and discharge.

Investigations	On Admission (Day 1)	On Discharge (Day 10)	Reference Range
Haemogram			
Haemoglobin (gm%)	11.7	10.5	13 - 16.2
Total Leucocyte Count (c/cmm)	19,050	9,630	4,000 - 10,000
Platelet Count (million/cmm)	326	231	150 - 410
Packed Cell Volume (%)	36.1	32.9	40 - 50
Blood Sugar Random (BSR) (mg%)	111	83	
Renal Function Tests			
Blood Urea (mg/dL)	14	20	19 - 43
Serum Creatinine (mg/dL)	0.5	0.6	0.8 - 1.5
Serum Sodium (mEq/L)	141	137	135 - 148
Serum Potassium (mEq/L)	4.0	3.7	3.5 - 5.5
Liver Function Tests (LFT)			
Total Bilirubin (mg/dL)	0.6	Not done	0.2 - 1.3
Direct Bilirubin (mg/dL)	0.4		0 - 0.4
Indirect Bilirubin (mg/dL)	0.2		0 - 0.8
SGOT (U/L)	33		17 - 59
SGPT (U/L)	22		21 - 72
Alkaline Phosphatase (U/L)	156		38 - 125
Total Proteins	6.5	Not done	
Serum Albumin (g/dL)	3.3	Not done	
Prothrombin Time	11.8 Seconds	Not done	
C Reactive Protein (mg/dL)	0.8	Not done	0.3 - 1
Widal Test	Negative	-	
Dengue Ns1 Antigen	Non Reactive	-	
HIV 1/ HIV2	Non Reactive	-	
HBsAg	Non Reactive	-	
Hepatitis C Virus Antigen	Negative	-	
TSH/FT3/FT4	0.88/1.97/1.20	-	
Peripheral Blood Smear	Normocytic	Normocytic	
	Normochromic	Normochromic	
	Polymorphs 86%	Polymorphs 72%	
	Lymphocytes 14%	Lymphocytes 28%	
	with Adequate Platelets	with Adequate Platelets	
Urine Albumin Dipstick	Trace	Nil	Nil
Sugar	Nil	Nil	Nil
PUS Cells	1-2/HPF	Nil	Nil
Cerebrospinal Fluid			
Volume ExaminedColour	2 MLColourless and Clear		
Coagulum	Absent		
Cobweb	Absent		
Cells	20 Cells/HPF		
Polymorphs	30%		
Lymphocytes	70%		
RBCs	Nil		
Proteins	92 mg/dL		
Sugar	63 mg/dL		

Table II: Neurotropic virus panel on Cerebrospinal Fluid (CSF).

Method – Real Time Polymerase Chain Reaction (RTPCR)	
Name of the Virus	**Result**
Human Herpes Virus 6 (HHV6)	Detected
Herpes Simplex Virus (HSV1)	Not Detected
Herpes Simplex Virus (HSV2)	Not Detected
Parvovirus B19	Not Detected
Epstein Barr Virus (EBV)	Not Detected
Varicella Zoster Virus (VZV)	Not Detected
Adenovirus	Not Detected
Enterovirus	Not Detected
Parechovirus	Not Detected
Varicella Zoster Virus (VZV)	Not Detected
Cytomegalovirus (CMV)	Not Detected
Human Herpes Virus 7 (HHV7)	Not Detected

Discussion

HHV-6 infection has been associated with complications of varying severity in haematopoietic stem cell transplant (HSCT) recipients and to a lesser degree among solid organ transplant recipients. Mayo clinic data reported an incidence of HHV-6 encephalitis in 1.7% (9/571). While incidence was low the mortality rate in these patients was 50% and those who survived had high rates of persistent neurologic disease deficits[4]. Management of such patients described is controversial and ill defined, although, the treatment in immunocompromised patients with HHV-6 encephalitis, treatment is more rewarding with Acycolvir, Ganciclovir or valganciclovir[5-8]. HHV-6 viral infection is able to persist in the latent form in central nervous system or sometimes in cervical canal which may reactivate with resultant neurological diseases[9]. Yilmaz *et al* described 17 cases of HHV-6 meningoencephalitis in immunocompetent adults,

including their own case. Exact figures on mortality were not shown as database literature was inconclusive and did not describe this information completely[10].

HHV-6 infection in immunocompetent adult individuals may manifest as a mononucleosis – like illness presenting with fever, lymphandenopathy and hepatitis. HHV-6 also has the ability to be chromosomally integrated (CiHHV-6), occurring in <1% of total population who newly acquire the infection and pass on the disease via vertical transmission. Yao *et al*[9] examined for evidence of HHV-6 infection in CSF of encephalitis patients using PCR and HHV-6 antibody reactivity. HHV-6 DNA was detected in 40% encephalitis patients while the controlled group remained negative. Integrated chromosomal DNA complicated the result as among 1 - 2% of the general population CiHHV-6 is inherited from their parents, as demonstrated by some authorities[2]. In immunocompromised patients PCR of HHV-6 DNA is often used for work-up with cell free CSF sample. This is considered as PCR technique of choice for the purpose. We did not use cell free CSF for the test. However, HHV-6 DNA was strongly positive suggestive of HHV-6 related CNS disease.

HHV-6 infection of CNS is reported with variable degrees of involvement in the pons, cerebral cortex, thalamus and medullary cord. HHV-6 exculsively involves medial temporal lobes (hippocampus and amygdala). Frontal lobes and cerebral lobes can also be involved. The MRI may show high signal T2W with mild enhancement[10]. The changes resolve as the condition improves. In our case the changes were noted in temporal lobe showing hyperintensities in the left side of temporal lobe that resolved post-therapy.

Infectious Disease Society of America recommends foscarnet or ganciclovir as first-line therapy for HHV-6 encephalitis; however, acyclovir who used in other cases with good outcome[11]. The present case received acyclovir therapy with steroids for 10 days. Patient was reviewed two weeks later with normal vitals and without neurological deficits.

Conclusion

HHV-6 is a well known infection commonly seen in immunosuppressed patients. Central nervous system infection resulting from HHV-6 in an immunocompetent host in the peripartum period with meningoencephalitis is rare. Data on this immunocompetent peripartum patients is unreported in the literature though limited cases of encephalitis are described among pregnant women. HHV-6 should be kept in mind when patients present with meningoencephalitis even among apparently immunocompetent people.

References

1. Yamanishi K, Okuno T, Shiraki K *et al*. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988; 1: 1065-7.
2. Endo A, Watanabe K, Ohye T *et al*. Molecular and virological evidence of viral activation from chromosomally integrated human herpesvirus 6A in a patient with X-linked severe combined immunodeficiency. *Clin Infect Dis* 2014; 59: 545-8.
3. Campadelli-Fiume G, Mirandola P, Menotti L. Human herpesvirus 6: an emerging pathogen. *Emerg Infect Dis* 1999; 5: 353-66.
4. Fida M, Hamdi AM, Bryson A *et al*. Long-term Outcomes of Patients With Human Herpesvirus 6 Encephalitis. *Open Forum Infect Dis* 2019; 6: ofz269.
5. Fotheringham J, Akhyani N, Vortmeyer A *et al*. Detection of active human herpesvirus-6 infection in the brain: correlation with polymerase chain reaction detection in cerebrospinal fluid. *J Infect Dis* 2007; 195: 450-4.
6. Soldan SS, Berti R, Salem N *et al*. Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. *Nat Med* 1997; 3: 1394-7.
7. Donati D, Akhyani N, Fogdell-Hahn A *et al*. Detection of human herpesvirus-6 in mesial temporal lobe epilepsy surgical brain resections. *Neurology* 2003; 61: 1405-11.
8. Birnbaum T, Padovan CS, Sporer B *et al*. Severe meningoencephalitis caused by human herpesvirus 6 type B in an immunocompetent woman treated with ganciclovir. *Clin Infect Dis* 2005; 40: 887-9.
9. Yao K, Honarmand S, Espinosa A *et al*. Detection of human herpesvirus-6 in cerebrospinal fluid of patients with encephalitis. *Ann Neurol* 2009; 65: 257-67.
10. Yilmaz M, Yasar C, Aydin S *et al*. Detection of human herpesvirus-6 in cerebrospinal fluid of patients with encephalitis. *Clin Neurol Neurosurg* 2018; 171: 106-8.
11. Tunkel AR, Glaser CA, Bloch KC *et al*. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2008; 1: 303-27.

CASE REPORT

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Acromegaly with Retinitis Pigmentosa

Saurabh Gupta, SK Verma**, Pranav Jivani***, Mehtab Kapoor****, Parteek Setia****, Ashish Chauhan****, Ashwini Mishra*****

Abstract

Acromegaly or gigantism is a disorder of excess growth hormone. The most common aetiology of acromegaly is Pituitary Adenoma which is easily detected on MRI. Visual disturbances are common in a macroadenoma due to compression of the optic chiasma. Unusual ocular findings like Rubeosus Iridis and Retinitis Pigmentosa are rarely described in this disorder. We hereby present a similar case with these findings in a patient with Pituitary Neuroendocrine tumour (Pit NET).

Key words: Pituitary neuroendocrine tumour (Pit NET), retinitis pigmentosa, acromegaly

Introduction

Pituitary Neuroendocrine tumours (Pit Nets) constitute around 12 % of all intracranial tumours[1]. Visual symptoms often accompany pituitary tumours due to chiasmal compression. Common symptoms are bitemporal hemianopia and Ophthalmoplegia[2]. Retinitis Pigmentosa is a hereditary degenerative disorder of the retina leading to vision loss and tubular vision. Association with functional pituitary tumours is rare with unclear aetiopathogenesis. Lawrence -Moon- Beidl Syndrome also has a similar association with Retinitis Pigmentosa and endocrine disorders such as obesity and hypogonadism. The literature is sparse regarding association of growth hormone secreting Pit NET and Retinitis Pigmentosa. Here we present a case with classical symptoms of pituitary macroadenoma with severe vision loss and retinal pigmentary changes.

Case Report

A 47-year-old male patient, resident of district Ratlam in Central India, presented to our institute with complaints of chronic headache, chronic cough and diminished visual acuity. On initial examination his vitals were within normal limits. Physical features favouring gigantism including frontal bossing, macroglossia, enlarged extremities and prognathism were obvious (Fig. 1 and 2). No prior history of diabetes, hypertension or any cardiac ailment was found.

Endocrine work-up revealed normal thyroid and gonadotrophin levels and surprisingly normal serum prolactin levels too. Growth hormone (GH) levels were significantly raised along with suppressed serum cortisol levels (Table I). Other laboratory parameters were within normal limits except HbA1c in prediabetes range. MRI revealed dumbbell shaped pituitary macroadenoma with dimensions 23 mm (SI) x 20 mm (AP) x 18 mm (TR) involving the sellar, suprasellar and left parasellar region. Superiorly the mass was casuing indentation of the optic chiasma and bilateral Internal carotids laterally (Fig. 3).

Ocular examination revealed bilateral inferolateral lenticular displacement along with Rubeosus Iridis. Fundus examination revealed primary pigmentary changes of Retinitis Pigmentosa (RP) (Fig. 4). Left eye had no vision and right eye had only tubular vision on visual field charting (Fig. 5).

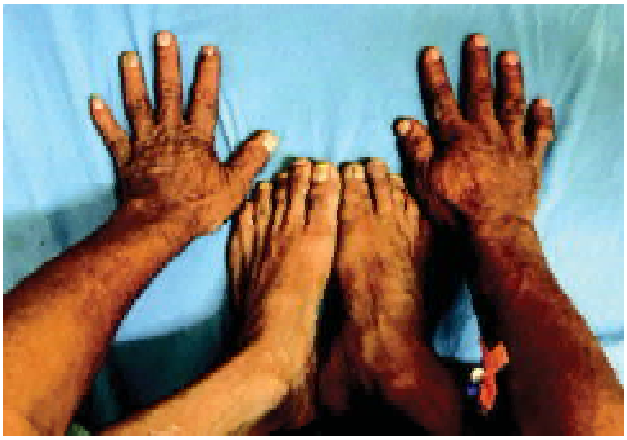

Fig. 1: *Coarse acral features of gigantism.*

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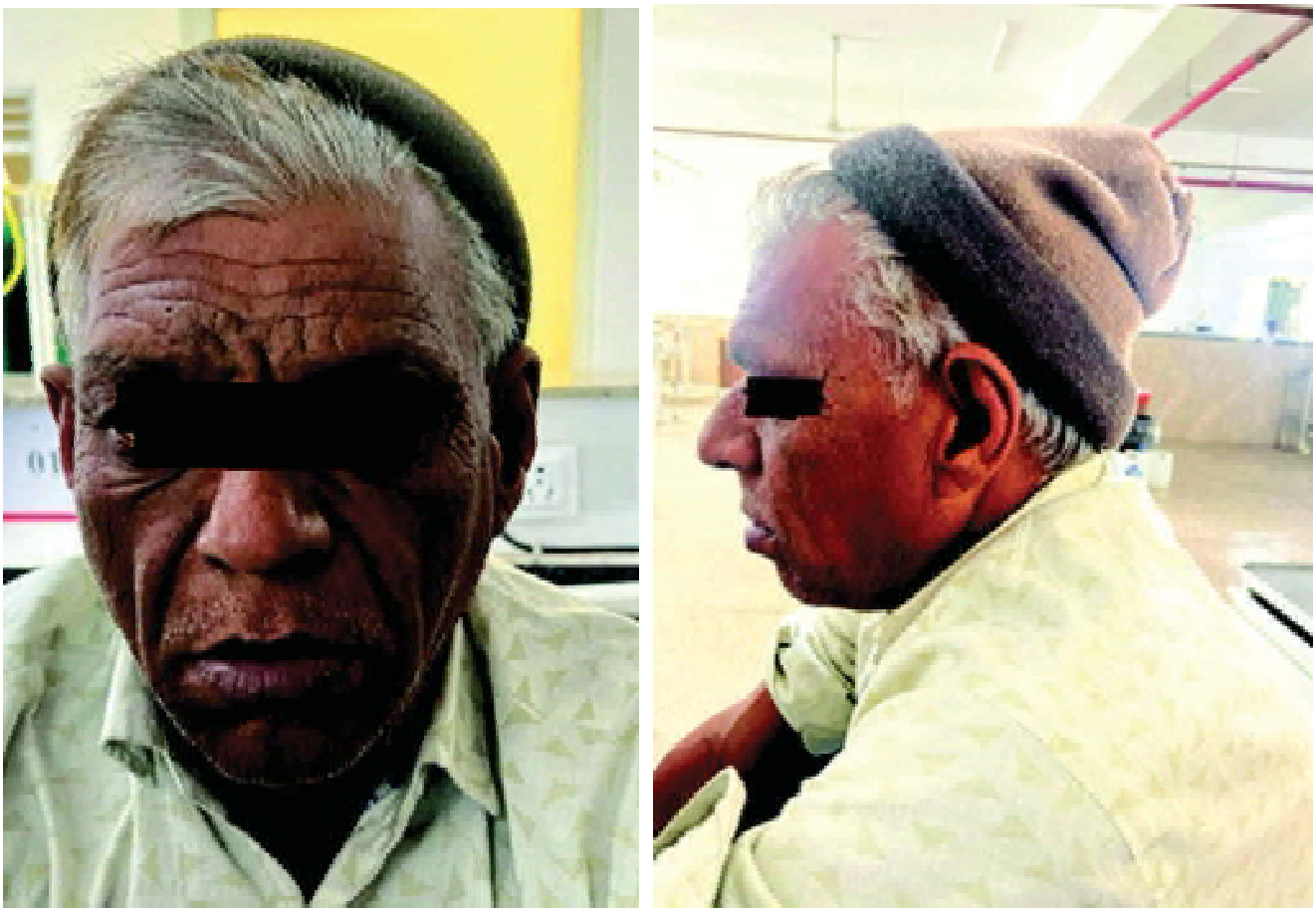

Fig. 2: *Facial features suggestive of Growth Hormone (GH) mediated soft tissue enlargement.*

Table I: Hormonal work-up of patient before and 1 month after surgery.

Parameter with Normal Reference Range	Pre-Operative	Post-Operative Day 30
Haemoglobin (>12 g/dL)	12.9	14.4
Total Leucocyte Count (4,000 - 11,000 cells/cumm)	4,500	10,730
Platelet Count (1.5 - 4 lac cells/cumm)	2.17	2.90
Serum Cortisol (140 - 690 nmol/L)	51.2 ↓	241
Growth Hormone (0.05 - 3 ng/mL)	24.7 ↑	13.3
Serum Prolactin (<20 ng/mL)	11 ↓	8.77
Testosterone (3.6 - 13.9 ng/mL)	1.88 ↓	Not performed
Estradiol (10 - 50 pg/mL)	5.2 ↓	Not performed
TSH (0.5 - 5 mIU/L)	0.58	0.71
T3 (0.8 - 1.8 ng/mL)	0.7	1.34
T4 (5 - 12 ug/dL)	8.18	9.75

He was advised for immediate neurosurgical intervention in view of imminent vision loss and referred to a specialist centre for the same. He underwent transsphenoidal pituitary adenoma resection soon afterwards and the tumour histopathology revealed a Pituitary Neuroendocrine tumour (Pit NET). Immunophenotyping and proliferative index could not be performed on the tissue sample. Post-operative hormonal levels on day 30 revealed significant reduction in GH levels and improvement in vision and symptoms (Table I). Fundoscopic findings were unchanged. However, patient was lost to follow-up and further assessment could not be done.

Discussion

Pituitary adenomas are among the commonest intracranial tumours. Often detected incidentally on routine MRI or CT imaging done for any non-pituitary indication or detected on autopsy. The peak incidence is from the fourth to the

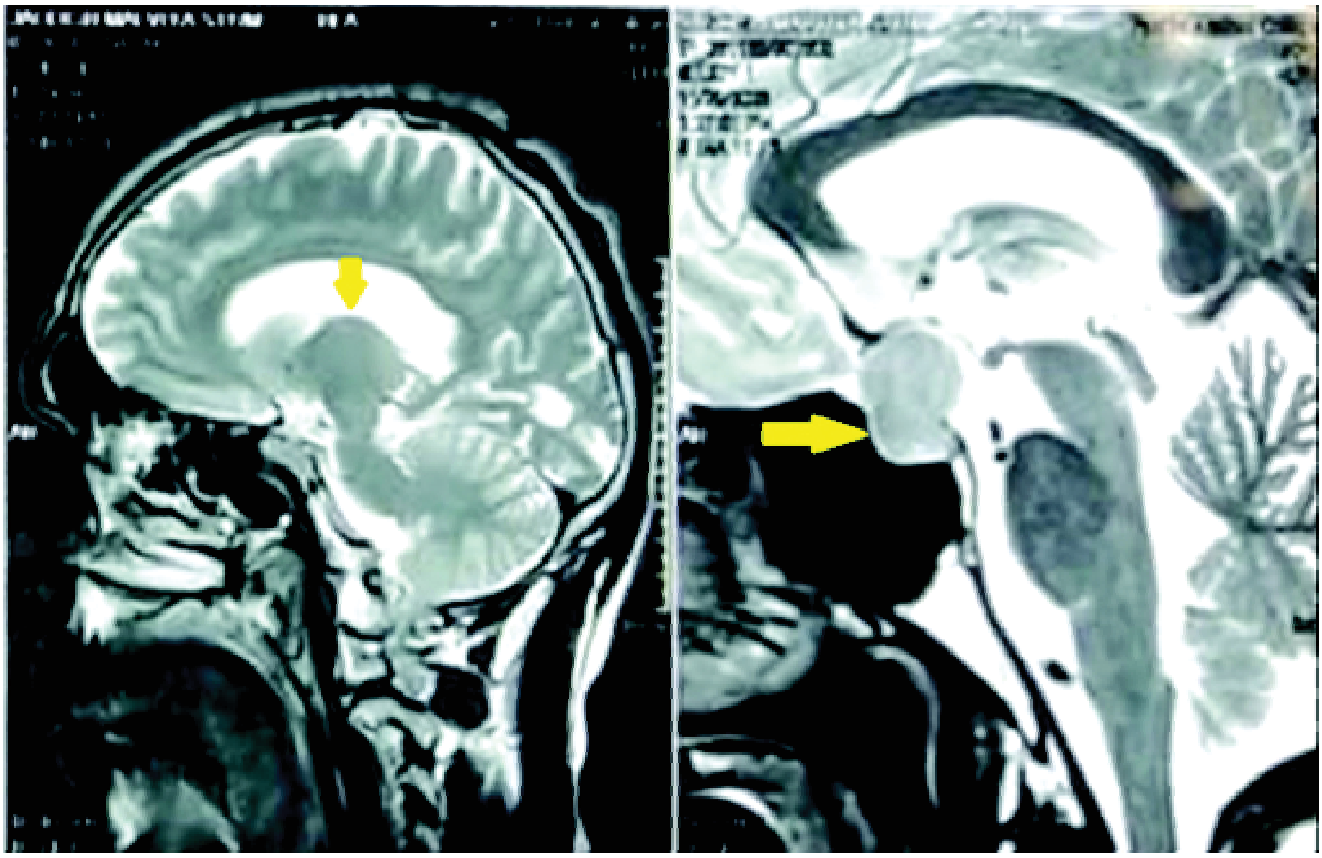

Fig. 3: *MRI pituitary showing Macroadenoma with suprasellar and parasellar extension and compression of Optic chiasma.*

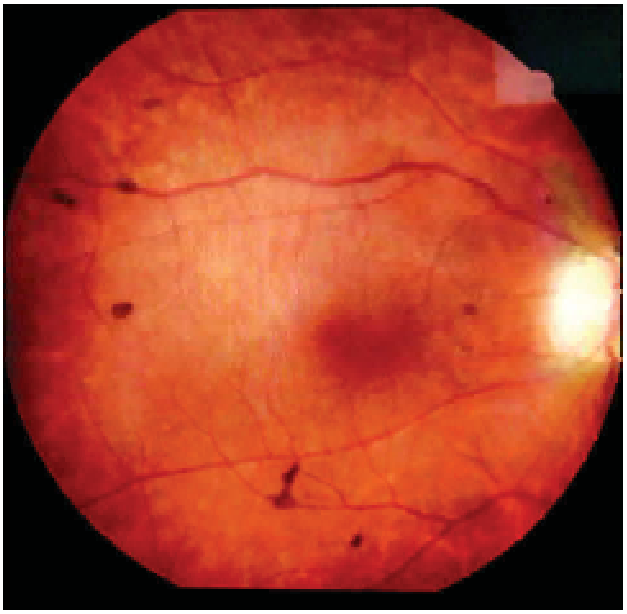

Fig. 4: *Fundus photograph with retinal degenerative and pigmentary changes.*

sixth decade of life with no specific gender predeliction[3]. A high index of suspicion should be kept for co-existent headache and visual symptoms as in our patient. These neurological symptoms are not characteristic of RP, thus point towards an intra-cranial pathology. However, there also were clear clues regarding excess GH in the form of acral and soft tissue enlargement as shown in Fig. 1 and 2. What was unusual in the case was the presence of retinal pigmentary changes in both the eyes along with visual field defects most likely due to chiasmal compression by the tumour.

Pituitary neuroendocrine tumour (Pit NET) is the third most commonly diagnosed intracranial tumour in the world[4]. In 2017, the International Pituitary Pathology Club proposed the use of the term "neuroendocrine tumour" rather than "adenoma" for adenohypophyseal tumours[5]. World Health Organisation (WHO) in 2022 revised the classification of pituitary tumours and a major nomenclature change was introduced by which pituitary adenomas were referred to as Pit NETs[6], Further, Pit NETs were classified into functioning or nonfunctioning tumours on the basis of hormone over secretion leading to conditions such as acromegaly, prolactinoma or Cushing's disease. 30% of all Pit NETs still remain nonfunctioning (nfPit NETs) which are the commonest and called as macroadenomas[3,7]. Somatotroph adenomas are classified as PIT1 lineage on histopathology

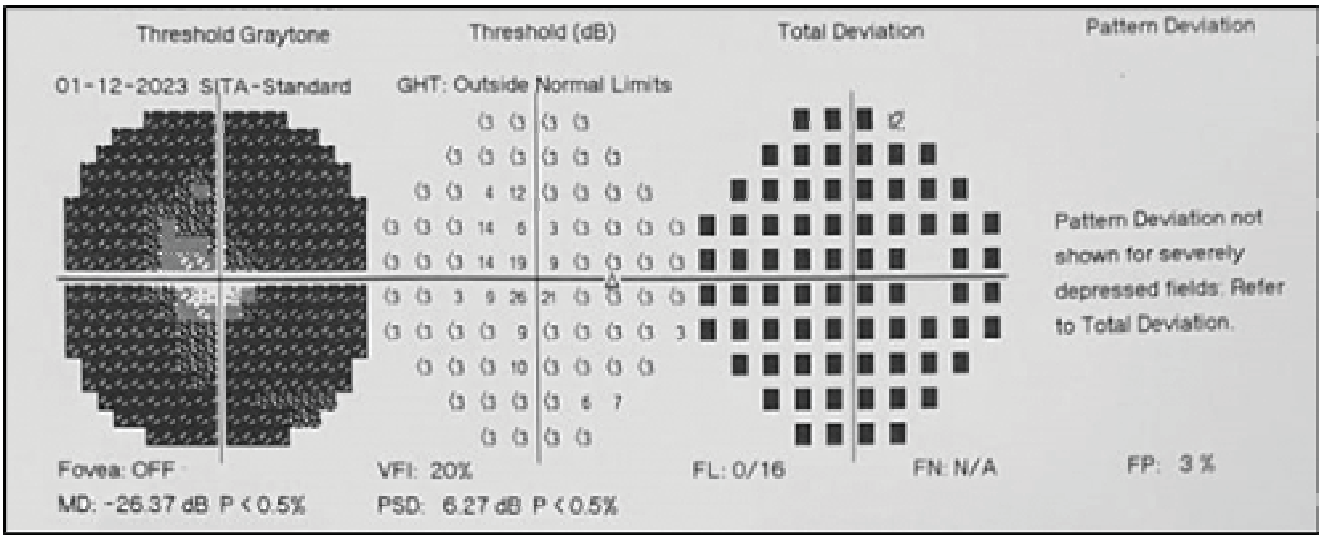

Fig. 5: *Perimetry showing restricted tubular vision in Right eye.*

and molecular biology (Fig. 3). Immunostaining for hormones including adrenocorticotropic hormone (ACTH), growth hormone (GH), prolactin (PRL), β-thyroid-stimulating hormone (β-TSH), β-follicle-stimulating hormone (β-FSH), and -luteinizing hormone (β-LH) can specifically determine the lineage of the tumour.

PIT 1 lineage

- Somatotroph tumour
- Lactotroph tumour
- Mammosomatotroph tumour
- Thyrotroph tumour
- Mature plurihormonal PIT 1 lineage tumour
- Immature PIT 1 lineage tumour
- Acidophil stem cell tumour
- Mixed somatotroph and lactotroph tumour

TPIT lineage

- Corticotroph tumour

SF 1 lineage

- Gonadotroph tumour

Tumours with no distinct cell lineage

- Plurihormonal tumour
- Null cell tumour

Fig. 6: *WHO 2022 classification of Pituitary Neuroendocrine tumours (PitNETs).*

Somatotroph (GH) adenomas/PitNETs are rare amongst all pituitary tumours[8]. This tumour typically arises from the adenohypophysis and is biochemically active, leading to acromegaly and gigantism. Due to excessive IGF-1 and IGFBP-3 levels, neovascularisation is often seen in various body tissues[9]. Similar changes are also seen in the eye as severe proliferative retinopathy and Iris neovascularisation[10]. It is a well-known clinical entity and was also seen in our patient as Rubeosus iridis. Retinal pigmentary changes have been described as an isolated entity and alongside many other endocrine disturbances as early as 1972 by JM Smail[11].

Retinitis pigmentosa (RP) is a hereditary retinal disorders with a worldwide prevalence of 1 in every 3,000 - 5,000 persons[12]. RP is a constellation of progressive visual dysfunction, restricted peripheral vision (tunnel vision) and loss of central vision in the elderly population[13].

What is not well described in existing literature are retinal changes in the form of pigmentary degeneration along with pituitary tumours. There are some scattered case reports of the same in patients with Acromegaly and Chromophobe adenoma[14]. Although tubular vision is a hallmark of RP, compressive effect of pituitary tumour may also lead to similar findings in visual field charting. Thus, fundoscopy plays an important role in ruling-out retinal involvement as a cause of vision loss and should be performed upfront.

We thus found this unusual association in our patient and felt the need for reporting it. It might also be an association by chance. Further, immunohistology studies and proliferative markers are needed to isolate the exact tumour phenotype which might answer some queries. These could not be performed in this subject due to financial constraints. Being a rare association, we must not subject every patient of RP to extensive imaging to rule-out Pituitary adenoma as it is not cost effective. However, every patient of PitNet must undergo a fundoscopic examination. Diminished visual acuity in this patient can be attributed both to the retinal changes and direct chiasmal compression. However, repeat perimetry

in post-operative follow-up is required to assess the same.

Conclusion

Pituitary macroadenoma causing Acromegaly and Retinal Pigmentary changes is an unusual finding. GH secreting pituitary tumours and retinal changes may be an association by chance or involvement of MSH secreting cells in pituitary. Detailed histologic examination of the pituitary tumour mass is may provide answers.

References

1. Asa SL, Mete O, Cusimano MD *et al*. Pituitary neuroendocrine tumours: a model for neuroendocrine tumour classification. *Mod Pathol* 2021; 34 (9): 1634-50.
2. Azmeh A. Neuro-ophthalmology findings in pituitary disease (review of literature). *Pituitary* 2018.
3. Ntali G, Wass JA. Epidemiology, clinical presentation and diagnosis of non-functioning pituitary adenomas. *Pituitary* 2018; 21: 111-8.
4. Chen Y, Wang CD, Su ZP *et al*. Natural history of post-operative nonfunctioning pituitary adenomas: a systematic review and meta-analysis. *Neuroendocrinol* 2012; 96: 333-42.
5. Asa SL, Casar-Borota O, Chanson P *et al*. From pituitary adenoma to pituitary neuroendocrine tumour (PitNET): An International Pituitary Pathology Club proposal. *Endocr Relat Cancer* 2017; 24: C5-C8.
6. Asa SL, Mete O, Perry A, Osamura RY. Overview of the 2022 WHO classification of pituitary tumours. *Endocr Pathol* 2022; 33: 6-26.
7. Whyte E, Nezu M, Chik C, Tateno T. Update on Current Evidence for the Diagnosis and Management of Nonfunctioning Pituitary Neuroendocrine Tumours. *Endocrinology and Metabolism* 2023; 38 (6): 631-54.
8. Araujo-Castro M, Biagetti B, Torre EM *et al*. Pegvisomant and pasireotide in PRL and GH co-secreting vs GH-secreting Pit-NETs. *Endocr Relat Cancer* 2024; 31 (7).
9. Caicedo D, Díaz O, Devesa P, Devesa J. Growth hormone (GH) and cardiovascular system. *Int J Mol Sci* 2018; 19 (1): 290.
10. Silha JV, Krsek M, Hana V *et al*. The effects of growth hormone status on circulating levels of vascular growth factors. *Clinical Endocrinol* 2005; 63 (1): 79-86.
11. Smail JM. Primary pigmentary degeneration of the retina and acromegaly in a case of pituitary adenoma. *Br J Ophthalmol* 1972; 56 (1): 25.
12. Nguyen XT, Moekotte L, Plomp AS *et al*. Retinitis pigmentosa: current clinical management and emerging therapies. *Int J Mol Sci* 2023; 24 (8): 7481.
13. Liu W, Liu S, Li P, Yao K. Retinitis pigmentosa: progress in molecular pathology and biotherapeutical strategies. *Int J Mol Sci* 2022; 23 (9): 4883.
14. Cosemans I, Demaerel P, Wets B *et al*. Retinitis pigmentosa in association with acromegaly: a case report. *Documenta Ophthalmologica* 1999; 98: 175-81.

CASE REPORT

JIACM 2024; 25 (4): 223-26

Post-Traumatic Loculated Haemothorax

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Abstract

Haemothorax is a collection of blood in the pleural cavity usually from traumatic injury. A chest X-ray has historically been the imaging modality of choice upon arrival to the hospital. If the size or severity of a haemothorax warrants intervention, tube thoracostomy has been and still remains the treatment of choice. Most cases of haemothorax will resolve with tube thoracostomy. If residual blood remains within the pleural cavity after tube thoracostomy, it is then considered to be a retained haemothorax (RH), with significant risks for developing late complications such as empyaema and fibrothorax. Once late complications occur, the only definitive treatment is surgery. In order to avoid surgery, research has been focused on removing an RH before it progresses pathologically. The most promising therapy consists of fibrinolytics, which are infused into the pleural space, disrupting the haemothorax, allowing for further drainage. If medical therapy and early procedures fail to resolve the RH, surgery is usually indicated. Surgery historically consisted solely of thoracotomy but has been largely replaced in non-emergent situations by video-assisted thoracoscopy.

Key words: Retained Haemothorax, VATS, intrapleural fibrinolytics, intra-fissure loculation.

Introduction

Blood in the pleural space is referred to as a haemothorax. The differential diagnosis for a patient who has not experienced substantial thoracic trauma is becoming more challenging due to an increase in medical problems and iatrogenic consequences[1].

Blood can enter the pleural space from a variety of vascular structures with consequences dependent upon arterial or venous source, the size of vascular injury, and localisation within lung, chest wall, mediastinum, diaphragm, or retroperitoneum. Fortunately, an invasive diagnosis to define the bleeding vessel is not required in all patients with haemothorax[2]. Yet that fact complicates efforts to define any cohort of specific diagnoses. A further complicating factor to any study of the anatomical causes of haemothorax is the tissue planes through which blood may pass before rupture into the pleural space.

Although the mesothelial cell has a fibrinolytic potential to convert blood clot to a liquid haemothorax rich in fibrin degradation products, transient clotting or lack of fibrinolysis following mesothelial cell injury may occur[1]. These blood clots are difficult to drain through even large-bore chest tubes.

Small amounts of blood in otherwise serous pleural effusions can cause red discoloration and prompt an incorrect diagnosis. For that reason, most clinicians define haemothorax as a pleural fluid haematocrit greater than or equal to 50% of the serum haematocrit[2].

There are 2 different physiological stages of haemothorax resolution: early and late[3]. Some have speculated that an early defibrinating of the haemothorax may occur with an increased pleural fluid protein concentration and a corresponding increase in intrapleural hyperosmotic pressure. This promotes the development of a pleural effusion[4-5]. As a haemothorax remains within the pleural cavity, it will typically complete spontaneous reabsorb within several weeks, especially if the volume is under 300 mL[6].

If it does not reabsorb, it will become a retained haemothorax (RH). RH has been defined as blood occupying at least one-third of the pleural space that cannot be drained by thoracostomy after 72 hours or as clots of at least 500 mL volume. RH can begin to form as early as 24 hours after chest tube placement[6].

Case history

A 44-year-old male patient with a history of chronic smoking presented to our department with complaints of gradually increasing right-sided chest pain which increased on inspiration, and occasional non-productive cough for the past 2 months. Through a comprehensive history it was

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established that patient the patient had suffered a road traffic accident 2 months ago for which an ICD (inter costal drainage) was placed because of haemopneumothorax. Patient's ICD was subsequently removed after resolution of the underlying condition and was discharged.

On examination, vitals were in range, bilateral chest movements were equal on inspection with dull note on percussion in the right infrascapular and axillary regions. Auscultation revealed decreased breath sounds on the right infrascapular and axillary regions. CXR (PA) done initially suggested a homogenous opacity in the right lower zone. Subsequent CECT chest was done and a loculated collection of size 8.7 x 8.6 x .8.0 cm with an approximate volume of 275 mL was seen with an air focus within the right posterior pleural cavity with adjacent atelectasis of right lower lobe

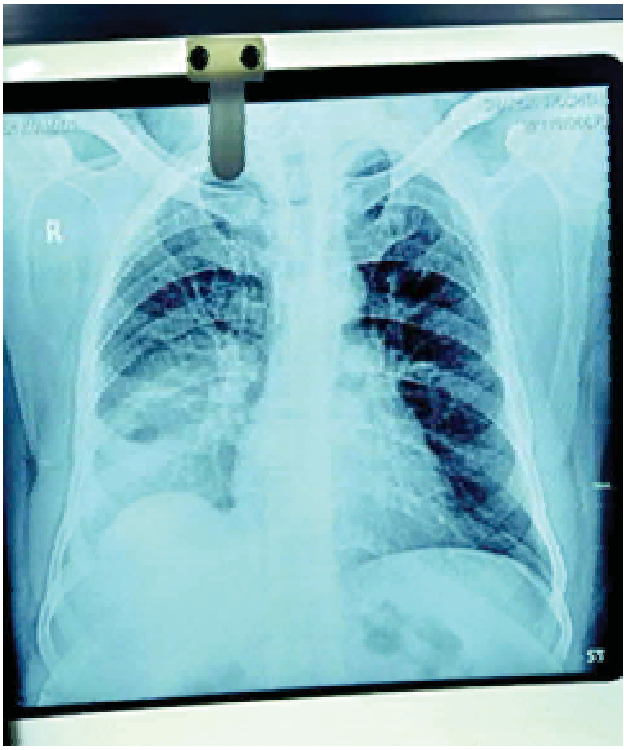

Fig. 1: *Pre-procedural X-ray.*

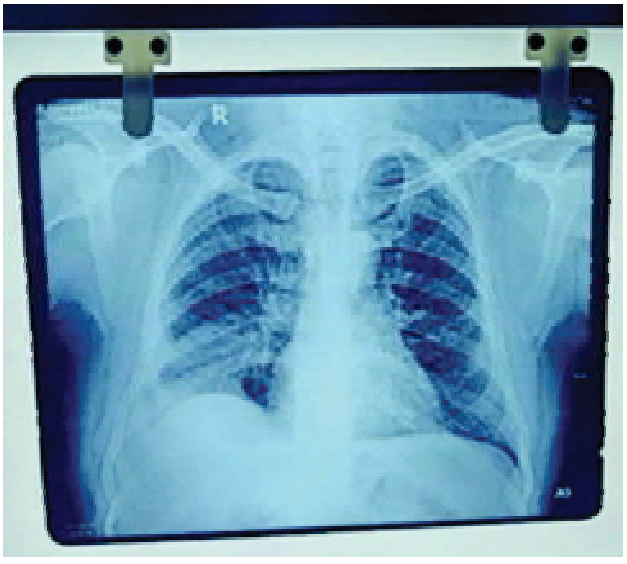

Fig. 2: *Post-procedural X-ray after ICD removal.*

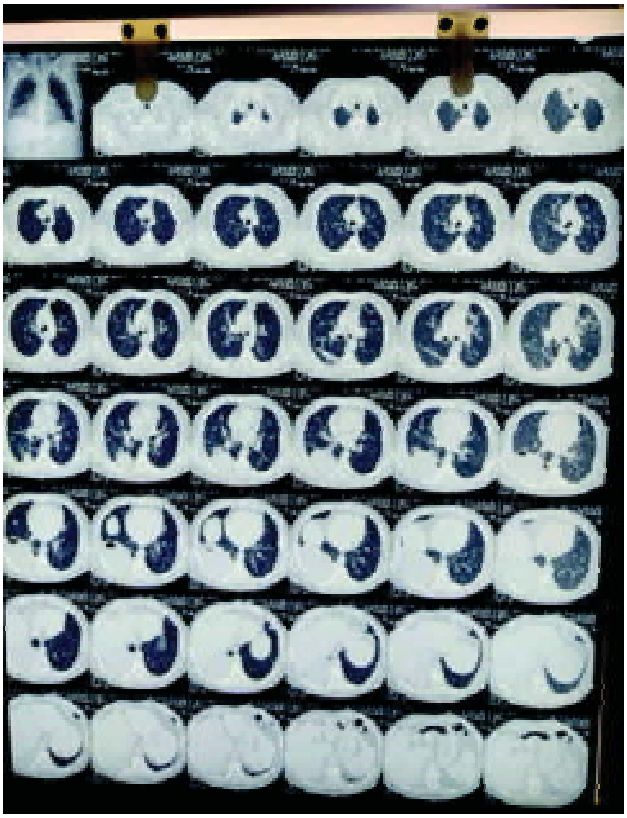

Fig. 3: *Pre-procedural CT scan.*

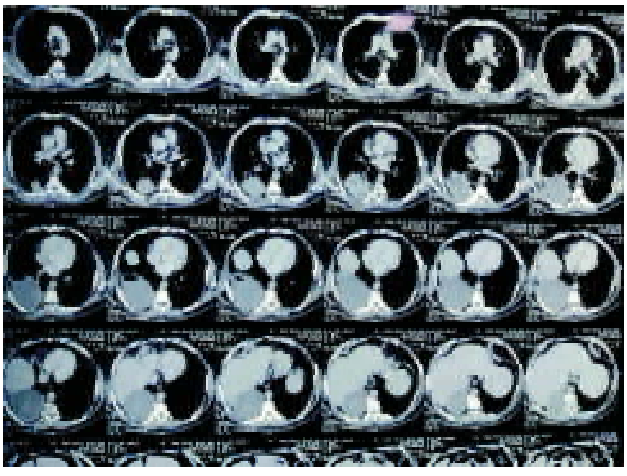

Fig. 4: *Pre-procedural CT scan localising the haemothorax.*

suggestive of right-sided hydropneumothorax.

USG guided aspiration was done which was able to retrieve around 5 mL of pleural fluid, which was sent for work up, but no NAAT based test could be done as the fluid received was haemorrhagic in nature. Patient was further planned for thoracoscopy with the intention of retrieving tissue for biopsy.

During the thoracoscopy, it was noted that there was a loculated haemorthorax within the the right oblique fissure. This loculation and surrounding fluid was evacuated and ICD was placed. At this point the patient's initial fluid reports were made available and had ruled-out tuberculosis. An HPE sample was taken and send for testing which too was in conclusive.

Patient had ICD in situ for subsequent three days, with a total fluid collection of 850 mL after which the ICD was taken out and he was discharged and asked to follow-up.

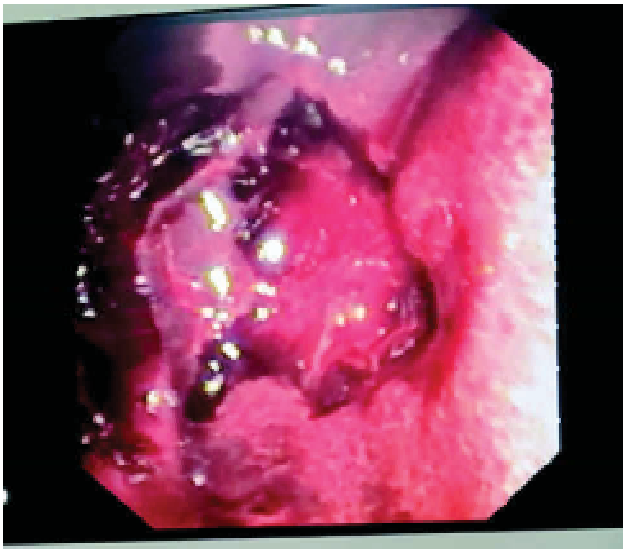

Fig. 5: *Loculated collection of blood in the right oblique fissure.*

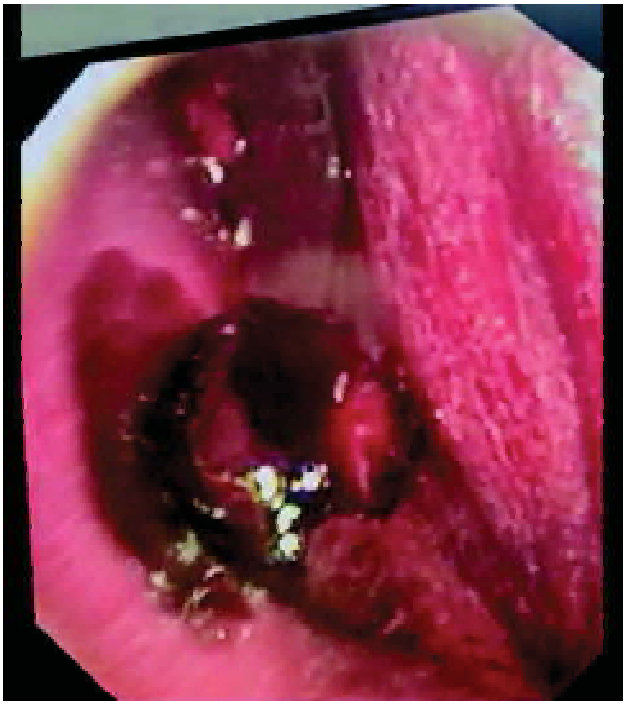

Fig. 6: *Post-evacuation and drainage of the loculated collection.*

Discussion

Haemothorax can be grouped into traumatic and spontaneous haemothorax, the former contributing to majority of cases. Within the spontaneous group, it can further be classified into vascular, coagulopathy, neoplastic and rarest of all are infectious. The differential diagnosis of a loculated haemopneumothorax occurring in a fissure should include an infected bulla; cavitary lung carcinoma, emphysema with congestive heart failure and tuberculosis[7].

RH can undergo progressive organisation over several days to become an empyaema or fibrothorax. Failure to evacuate the haemothorax may be due to malposition or poor drainage of the chest tubes, which can be influenced by the experience of the clinician. Empyaema can occur as a complication of RH due to primary or secondary bacterial contamination and can originate from broncho tracheal lesions, esophageal injuries, penetrating injuries, long-standing clotted thoracostomy tube, and postsurgical exposure[6].

The need for surgical drainage has been considered an important end-point in previous studies on pleuro-fibrinolysis; the latest meta-analyses concluded that fibrinolysis alone may prevent the requirement for surgical intervention[8].

Studies involving non-trauma patients have demonstrated the efficacy of intrapleural fibrinolysis in the management of a variety of complex pleural processes. In his study Carmen *et al*, divided patients in two groups – Group I: alteplase 20 mg in 20 mL saline every 24 h (or alteplase 10 mg in 20 mL saline every 24 hr) and Group II: urokinase 100,000 IU in 20 mL saline every 24 hr; showed that a maximum dose of 10,000 IU urokinase was far superior than alteplase[8].

Intrapleural fibrinolytic therapy with streptokinase has been used in clotted haemothorax with a success rate of 91% to 93%[9-10]. The early initiation of fibrinolytic therapy, before the development of severe pleural adhesions, may lead to a more effective pleural drainage as has been demonstrated in an experimental study[11] and in a study by Boures *et al*[12].

Unfortunately, there is no agreement on where and how intrapleural fibrinolysis should be incorporated into a treatment plan, and not all studies support its effectiveness in treating clotted haemothoraces. For the treatment of post-traumatic retained haemothorax, Ozgur *et al*[13], compared VATS with intraplerual fibrinolysis which was unable to show any clear dominance of VATS except possible shorter hospital stay.

However, Stile *et al* have shown intrapleural tPA is both safe and effective and should be included in the physicians' armamentarium for treating traumatic retained haemothoraces and recommend using intrapleural tPA in patients who present late, or in patients with low physiologic reserves.

Conclusion

Loculated haemothoraces may not always be because of trauma or malignancy, a differential for tubercular infection should always be kept in mind for such patients. A RH should also be dealt with through thoracoscopic means to avoid further fibrosis and empyaema formation.

Appropriate use of fibrinolytics and proper framework for the same has to be established and implemented where VATS is not readily available.

References

1. Charlie strange. Hemothorax. *Seminars in Respiratory and Critical Medicine* 1995; 16: 4.
2. Patrini D, Panagiotopoulos N, Pararajasingham J *et al*. Aetiology and management of spontaneous haemothorax. *J Thorac Dis* 2015; 7: 520-26.
3. Mahoozi H, Volmerig J, Hecker E. Modern management of traumatic hemothorax. *J Trauma Treat* 2016; 5: 1-5.
4. Cohen NS, Braig Z, Collins JN. Prevalence and management of post-traumatic retained hemothorax in a level 1 trauma center. *Am Surg* 2018; 84: e369-e371.
5. Inci I, Ozçelik C, Nizam O *et al*. Thoracic trauma in the elderly. *Eur J Emerg Med* 1998; 5: 445-50.
6. Zeiler J, Idell S, Norwood S, Cook A. Hemothorax: A Review of the Literature. *Clin Pulm Med* 2020; 27: 1-12.
7. Marin M, Kofler. Loculated Hemopneumothorax of a Major Fissure in Patients with COPD and Associated Pleural Disease. *Chest* 1990; 97: 87.1-76.
8. Nie W, Liu Y, Ye J *et al*. Efficacy of intrapleural instillation of fibrinolytics for treating pleural empyema and parapneumonic effusions: a meta-analysis of randomised control trials. *Clin Respir J* 2014; 8: 281-91.
9. Inci I, Ozcelik C, Ulkii R *et al*. Intrapleural fibrinolytic treatment of traumatic clotted hemothorax. *Chest* 1998; 1: 160-5.
10. Jerjes-Sa´nchez C, Ramirez-Rivera A, Elizalde JJ *et al*. Intrapleural fibrinolysis with streptokinase as an adjunctive treatment in hemothorax and empyema: a multicenter trial. *Chest* 1996; 109: 1514-9.
11. Kimbrell BJ, Yamzon J, Petrone P *et al*. Intrapleural thrombolysis for the management of undrained traumatic hemothorax: a prospective observational study. *J Trauma* 2007; 62: 1175-8.
12. Strange C, Baumann MH, Sahn SA, Idell S. Effects of intrapleural heparin or urokinase on the extent of tetracycline induced pleural disease. *Am J Respir Crit Care Med* 1995; 151: 508-15.
13. Samancilar O, Akçam I, Kaya SO, Ozturk O. The Efficacy of VATS and Intrapleural Fibrinolytic Therapy in Parapneumonic Empyema Treatment. *Ann Thorac Cardiovasc Surg* 2018; 24: 19-24.
14. Stiles, Drake, Helmer *et al*. Evaluation of chest tube administration of tissue plasminogen activator to treat retained hemothorax. *The Amer J Surg* 207 (6): 960-3.

CASE REPORT

JIACM 2024; 25 (4): 227-29

An Unusual Case of Renal Extramedullary Haematopoiesis

Santosh Sadafule, Rupi Jamwal**, Jyoti Gupta****

Abstract

Background: Extramedullary haematopoiesis, the proliferation of haematopoietic cells is an entity occurring in conditions with insufficient haematopoiesis.

Case Presentation: We report a rare case of renal extramedullary haematopoiesis (EMH) presenting as mildly bulky kidneys and a renal hilar and pelvic mass mimicking urothelial malignancy in a patient with pancytopaenia on haemogram. There was no osteosclerosis, paravertebral masses, or massive splenomegaly, the usual radiological features of a myeloproliferative disorder on imaging.

Conclusion: The possibility of renal EMH should be considered, in the presence of bulky kidneys or a renal pelvic mass not only in the background of the classical imaging clues, but even without the telltale signs of a myeloproliferative disorder on imaging if the blood picture suggests so.

Key words: Renal mass, extramedullary, haematopoiesis.

Introduction

Extramedullary haematopoiesis (EMH), rarely encountered in general radiologic practice, is a common feature of chronic myeloproliferative disorders. EMH occurs in response to inadequate erythropoiesis in the bone marrow. If red marrow reconversion is unable to meet the body's demand for blood cells, haematopoiesis will shift outside of the bone marrow, in most cases occurring in the paravertebral region or the liver and spleen, the site of physiological EMH during fetal life[1,2]. Rarely, other organs can be involved. Renal involvement by EMH is relatively uncommon and can present in various forms like parenchymal, pelvic, or perirenal.

Herein we report a rare case of renal EMH in a patient with pancytopaenia with the absence of osteosclerosis and other radiological signs of a myeloproliferative disorder on imaging.

Case Report

A 50-year-old male presented with complaints of chronic fatigue and weakness. There was no history of fever or haematuria. A haemogram revealed pancytopaenia. Ultrasonography (USG) of the abdomen showed mild hepatosplenomegaly, mildly bulky kidneys and a hyperechoic mass in the left renal sinus conferring a faceless appearance to the left kidney.

Multiphasic Computed tomography (CT) of the abdomen revealed a rounded contour of both kidneys with a homogenous, hypo-attenuating, and minimally enhancing soft tissue density mass replacing the left renal sinus and extending into the left renal hilum, obscuring the renal vessels (Figs. 1a, 1b, 2a). The soft tissue density was seen to fill and expand the renal pelvis and proximal ureter (Fig. 1a, 2b). Few tiny (5 to 7 mm) round, focal hypoenhancing lesions were seen scattered in the bilateral renal parenchyma.

There were no osteosclerotic or paravertebral soft tissue masses. Primary urothelial mass and lymphoma were considered as possibilities and a USG-guided Fine Needle Aspiration Cytology (FNAC) was carried. The bone marrow

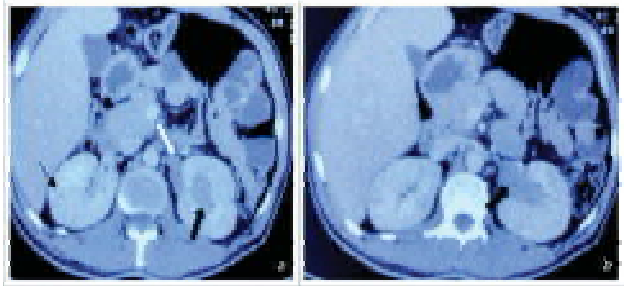

Fig. 1a: *Axial CECT image shows rounded contour of both kidneys with multiple small, round, focal hypoenhancing lesions (thin black arrow) scattered in bilateral renal parenchyma. A soft tissue density mass is seen replacing the fat in the left renal sinus, (black arrow) and encasing and filling the left renal pelvis (white arrow).*

Fig. 1b: *Axial CECT image shows expansile hypoenhancing soft tissue density mass (black arrow) in left renal hilum.*

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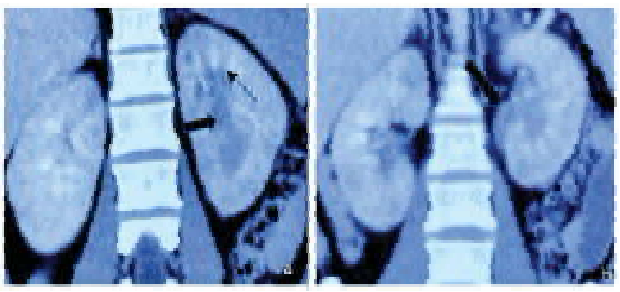

Fig. 2a: *Coronal CECT image shows shows hypoenhancing soft tissue density mass (thick black arrow) in the left hilum stretching the calyces (thin black arrow).*

Fig. 2b: *Coronal CECT (3 mm MIP) image shows hypoenhancing soft tissue density mass (black arrow) in the left renal hilum filling and expanding the left renal pelvis and proximal ureter.*

biopsy, given pancytopaenia, revealed marrow fibrosis with a paucity of normal haemopoietic elements consistent with myelofibrosis. Fine needle Aspiration cytology (FNAC) from the left renal pelvic mass revealed a polymorphous infiltrate composed of immature erythroid cells, myeloid cells, fat cells, and lymphocytes (Fig. 3), consistent with extramedullary haematopoiesis.

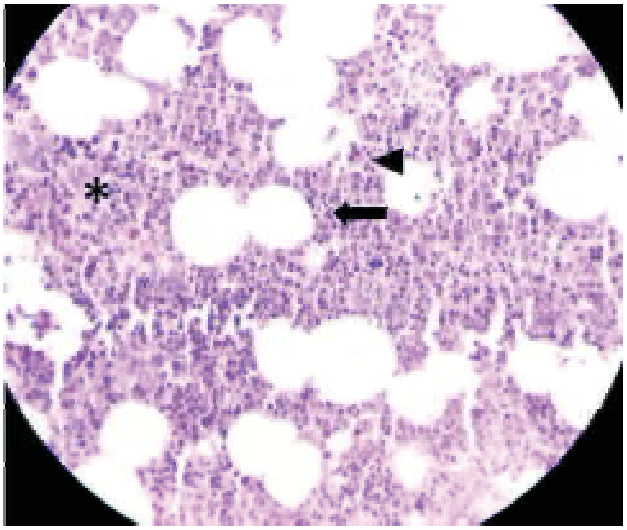

Fig. 3: *40 X view of haematoxylin and eosin (H & amp; E) stained section showing aggregates of haematopoeitic precursor cells including erythroblasts (arrowhead), myeloid cells (arrow), fat cells (thin arrow) megakaryocytes* within the parenchyma.*

Discussion

Extramedullary haematopoiesis (EMH) is a compensatory mechanism for inadequate haemopoiesis. It refers to deposits of erythroid precursors in sites other than the bone marrow.

The haematopoietic and stromal cell lines occur in bone marrow and organs like the spleen and liver which were haematopoietic in the fetus. In conditions such as myelofibrosis with myeloid metaplasia, control of stem cell differentiation is lost, and peripheral blood cytopaenia occurs due to haematopoietic elements of the marrow being replaced by fibrosis.

Neoplastic stem cells can circulate and migrate to secondary haematopoietic organs giving rise to EMH, particularly in the spleen, liver, and lymph nodes that are part of the reticuloendothelial system. Even though the reticuloendothelial system is the main site of EMH, other organs such as the lungs, gastrointestinal tract, breast, skin, kidneys, and adrenals can be recruited for haemopoiesis either due to presence of haematopoietic precursors or due to the circulating stem cells being deposited in these organs[1,2,3].

The differential diagnosis of a renal pelvic mass on CT includes lymphoma, urothelial mass, and renal sinus lipomatosis[3]. Lipomatosis typically has very low attenuation and does not enhance on CT. Renal lymphoma may present as either a mildly enhancing perirenal mass encasing the entire a kidney or renal hilum and pelvicalyceal system[4]. Intrapelvic transitional cell carcinoma typically manifests as a hypoenhancing intrapelvic mass with expansile growth where the shape of the kidney remains intact.

Renal involvement by EMH can present as a hypoattenuating and hypoenhancing mass in the perirenal, parenchymal, intra or para-pelvic location. In parenchymal type of involvement, the kidneys may either be enlarged diffusely or have small focal lesions. In perirenal type of involvement, a hypoattenuating, hypoenhancing uniform mass or nodules are seen engulfing the kidneys without distorting their shape. Pelvic involvement could be an extension of parenchymal lesions, but can also be isolated. In our case there was a urothelial mass involving left renal pelvis and proximal ureter extending to the renal sinus encasing the pelvicalyces.

Additionaly, co-existing parenchymal involvement of both kidneys was seen on imaging as rounded contour of kidneys with small focal hypoenhancing lesions on CT. Bulky kidneys can also be seen in lymphoproliferative disorders.

Clinically, renal EMH can be asymptomatic or can present with symptoms ranging from abdominal discomfort to renal failure due to either ureteral obstruction or extensive parenchymal involvement resulting in interstitial nephritis[5].

Most reports have mentioned renal extramedullary haematopoiesis presenting on imaging as a homogeneous, hypoattenuating, soft tissue mass showing minimal enhancement on CT as in our case. In most of these reports, bilateral renal masses encasing the pelvicalyces were present[3,5,6]. EMH presented as solitary renal parenchymal

mass in the reports by Ahuja *et al*[8] and Mubeen *et al*[9]. Peri renal mass engulfing renal parenchyma was seen in the case report by Ricci *et al* and Imai *et al*[7,10].

Kurien *et al* reported a case of EMH presenting as acute renal injury due to parenchymal involvement causing interstitial nephritis[5]. In our case there was parenchymal involvement causing bulky kidneys and tiny lesions in bilateral renal parenchyma. All these case reports have shown radiological features of an underlying myeloproliferative disorder like massive splenomegaly and osteosclerosis on imaging.

In the right clinical setting, CT can indicate a diagnosis of EMH. Our case was unique as there were no osteosclerosis, thoracic paravertebral masses, or substantial splenomegaly to suspect EMH in the presence of myelofibrosis. Our case was further challenging as there was a unilateral mass in the renal pelves with features overlapping with a transitional carcinoma involving the pelvis and proximal ureter.

Conclusion

Despite many reports, little attention has been paid to the overall spectrum of imaging findings of renal extramedullary haematopoiesis. EMH should not only be included in the differential diagnosis of a non-specific renal mass in a confirmed case of myelofibrosis but should also be considered in an incidental renal mass in cases with peripheral pancytopaenia lacking radiological features of an underlying myeloproliferative disorder.

Acknowledgement: We thank the department of pathology of our institution for providing us with the histopathology image of the case.

References

1. Ginzel AW, Kransdorf MJ, Peterson JJ *et al*. Mass-like extramedullary haematopoiesis: imaging features. *Skeletal Radiol* 2012; 41 (8): 911-6.
2. Ini' C, Foti PV, Duminuco A *et al*. Renal extramedullary haematopoiesis as an epiphenomenon of bone marrow dysfunction in a patient with primary myelofibrosis: A rare case report. *Radiol Case Rep* 2024; 19 (6): 2286-91.
3. Choi H, David CL, Katz RL, Podoloff DA. Case 69: extramedullary haematopoiesis. *Radiology* 2004; 231 (1): 52-6.
4. Galperin-Aizenberg M, Volchek Y, Even Sapir E *et al*. Case report: Renal extramedullary haematopoiesis mimicking renal lymphoma on computed tomography. *Clin Radiol* 2006; 61 (10): 896-8.
5. Kurien A, Abraham A, Thanigachalam *et al*. Extramedullary haematopoiesis. An Unusual Cause for Acute Kidney Injury. *Ind J Nephrol* 2021; 1 (3): 322-3.
6. Stanchina M, Rosenbaum E, Pronko AJ *et al*. Extramedullary haematopoiesis of the Renal Pelvis in a patient with Myelofibrosis. *J Oncol Pract* 2019; 15 (8): 458-9.
7. Ricci D, Mandreoli M, Valentino M *et al*. Extramedullary haematopoiesis in the kidney. *Clin kidney J* 2012; 5 (2): 143-5.
8. Ahuja S, Grover G, Jha AK *et al*. Extramedullary haematopoiesis presented as solitary renal mass: a case report with review of literature. *Diagn Cytopathol* 2010; 39: 435-7.
9. Mubeen B, Charak Annu, Feroz I *et al*. Extramedullary haematopoiesis presenting as a solitary renal mass and mimicking a malignant tumour: A rare case report. *Archives of Medicine and Health Sciences* 2013; 1 (2): 159.
10. Imai K, Kitai H, Endo N *et al*. A case of perirenal extramedullary haematopoiesis in a patient with primary myelofibrosis. *CEN Case Reports Internet* 2017; 194-9.

CASE REPORT

JIACM 2024; 25 (4): 230-31

Pure Red Cell Aplasia

Heli Kapoor, Varun Shahi**, Rajesh Satyapal Taneja***, Praveen Malik*****

Abstract

Pure Red Cell Aplasia (PRCA) is an uncommon haematological disorder characterised by normocytic anaemia with reticulocytopaenia and preserved leucocyte and platelet counts. PRCA may be congenital or acquired. Acquired PRCA can be primary or secondarily associated with thymoma, lymphoproliferative diseases, infections, autoimmune disorders and certain drugs. Very rarely, it can be idiopathic. We report a case of chronic acquired idiopathic PRCA in a 60-year-old male. The patient had severe anaemia for which he had received multiple blood transfusions in last three months. The bone marrow aspiration and biopsy were done which revealed isolated depression of erythroblasts with final impression of acquired PRCA. A battery of tests and CT (chest and abdomen) were done to rule-out possible aetiologies but none were found. Hence, a diagnosis of idiopathic PRCA was established. Treatment was initiated with cyclosporin A and steroids in combination; remarkable improvement in haemogloblin and reticulocyte count was achieved.

Introduction

Pure Red Cell Aplasia (PRCA) is an exceedingly rare bone marrow disorder with an incidence of 1.06 patients per million per year[1]. It was first reported by Paul Kaznelson in 1922[2]. This condition manifests as isolated depression of erythroid series and is characterised by normocytic normochromic anaemia, reticulocytopaenia (reticulocyte count <1%) and diminished marrow erythroblasts (<0.5%)[2]. It can occur at any age with equal prevalence in both genders. PRCA may be congenital or acquired. Acquired PRCA may be primary or secondarily associated with Thymoma, Lympho-proliferative diseases (chronic lymphocytic leukaemia, lymphoma), solid organ malignancy, Infections (Parvovirus B19 infection, HIV, hepatitis, tuberculosis), Autoimmune disorders (SLE, Rheumatoid Arthritis) and Drug-induced (Erythropoietin)[3]. This case report delves into a unique instance of acquired PRCA, where the aetiology remained elusive, and was ultimately labelled as idiopathic.

Case report

A 60-year-old male, presented to the medicine emergency with complaints of generalised weakness, progressive exertional dyspnoea and swelling of feet over the past one year, which had worsened over last three months. There were no complaints of fever, jaundice, weight loss, loss of appetite, skin rash, joint pains, blood loss from any site or prolonged drug intake. The patient had a medical history of hypertension. Past history was notable for multiple blood transfusions in last 3 months.There was no significant family history. Physical examination revealed severe pallor, bilateral pedal oedema (pitting) and tachycardia without any lymphadenopathy and hepatosplenomegaly. On routine haemogram, his haemoglobin was 5.9 gm/dL, Reticulocyte count was 0.6%, RBC count - 1.82 million/cumm, haematocrit 18%, total leucocyte count - 9,010/cumm, DLC-Neutrophils - 38.50%, Lymphocytes - 40.1%, Eosinophils - 6%, platelets - 3.87 lac/cumm. On peripheral blood film examination, normocytic normochromic RBCs were seen. His liver function tests and renal function tests were within normal range. Stool for occult blood was negative. Bone marrow aspiration and biopsy was suggestive of significant erythroid suppression with an M: E ratio of 30:1. Erythroid series showed maturation arrest, few in early megaloblast phase with only 3% erythroid cells in marrow. Myeloid series showed normal differentiation and maturation with adequate and functional megakaryocytes. Differential count of the non-erythroid series showed myeloblasts - 00%, myelocyte - 06%, metamyelocyte - 3%, neutrophil - 80%, lymphocyte - 06%, eosinophil - 1%, plasma cells - 1%. Keeping in view the clinical presentation, examination, laboratory and bone marrow biopsy findings, a diagnosis of acquired PRCA was made. To find the underlying cause of acquired PRCA, blood samples were sent for infectious (Parvovirus B19, Hepatitis B, C, A, EBV, CMV virus) and autoimmune aetiologies (RF, ANA); all of which were negative. His CECT Chest and abdomen was normal which ruled-out the possibility of thymoma. Extensive work-up eliminated known causes, leading to the diagnosis of idiopathic PRCA. The patient was started on combination therapy of cyclosporin A and steroids. His haemoglobin increased to 9.8 gm/dL with significant improvement in reticulocyte count on follow-

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up after about four weeks of therapy.

Discussion

PRCA is an uncommon single lineage cytopaenia characterised by decreased RBC precursors with normal granulopoiesis and megakaryopoiesis in the bone marrow, presenting clinically as anaemia[4]. The aetiology of PRCA is as follows[5]:

- Congenital PRCA (Diamond-Blackfan anaemia)
- Acquired PRCA
- Primary

I. Primary autoimmune PRCA (includes transient erythroblastopenia of childhood)

II. Primary myelodysplastic PRCA

- Secondary (associated with)

I. Autoimmune/collagen vascular disorders (SLE, RA)

II. Lymphoproliferative disorders (CLL, Lymphoma, Angioimmunoblastic lymphadenopathy)

III. Solid Tumours (Thymoma)

IV. Infections (Parvovirus B19; Hepatitis A, B, C, E; HIV; EBV; CMV; TB)

V. Drugs and toxins (Erythropoietin)

The pathogenesis is heterogeneous and involves immune dysfunction with antibodies directed against erythroid precursor cells or erythropoietin[6], or due to T-cell-mediated suppression of erythropoiesis[7]. Clinical presentation of patients of acquired PRCA can be variable; severe anaemia, fever, anorexia, nausea, vomiting, headache and abdominal pain. Bone marrow examination reveals a complete absence of erythroblasts but normal granulocytic and megakaryocytic series.Thorough investigation is crucial to identify underlying causes, including a viral screen, serological studies for autoimmune diseases and CT thorax to rule-out thymoma.

Majority of acquired PRCA cases show clinical and haematological improvement on removal of the underlying offending agent. However, in Idiopathic PRCA most effective first-line treatment is Cyclosporin A (CsA) administered at a starting dose of 2 to 6 mg/kg per day (in divided doses) combined with steroid (prednisone at 30 mg/day) with a rapid taper, yielding an overall response rate (ORR) of about 65% to 87%[2]. The second-line therapy includes antithymocyte globulin (ATG) and cyclophosphamide. Following treatment, response to therapy is assessed by serial evaluation of reticulocyte count and haematocrit. The goal of treatment is to induce remission to attain an optimal haemoglobin concentration with the recovery of erythropoiesis, without any requirement for blood transfusion and associated problems.

In our case, even after extensive investigations none of the established causative factors for PRCA could be established; thus the case was labelled as idiopathic PRCA. The patient was then started on treatment with cyclosporin A and steroids, which induced remission and a significant increase in haemoglobin concentration after 4 weeks of therapy.

Conclusion

Any anaemia of prolonged duration, not responding to conventional therapy should be evaluated by bone marrow studies to rule-out ineffective erythropoiesis, dysplastic syndromes, a selective erythroid suppression/PRCA or infiltrative diseases of the bone marrow. PRCA is a rare disorder with varied aetiology. Whenever a diagnosis of PRCA is made, an underlying cause should be sought. A rapid response follows treatment of the underlying cause or withdrawal of the incriminating drug. In cases where no cause can be established, idiopathic PRCA may be treated with Cyclosporin A and steroids.

Reference

1. Nakazawa H, Sakai K, Ohta A *et al*. Incidence of acquired pure red cell aplasia: a nationwide epidemiologic analysis with 2 registry databases in Japan. *Blood Adv* 2022; 6282-90.
2. Sawada K, Fujishima N, Hirokawa M. Acquired pure red cell aplasia: updated review of treatment. *Br J Haematol* 2008; 505-14.
3. Fisch P, Handgretinger R, Schaefer HE. Pure red cell aplasia. *Br J Haematol* 2000; 1010-22.
4. Kaznelson P. Zur Entstehung der Blutpla Èttchen. Verhand lungen der Deutschen Gesellschaft. *Fur Innere Medizin* 1922; 34: 557-8.
5. Lipton J, Glader B, Means B. Red cells aplasia: acquired and congenital disorders. In: Greer JP, Arber DA, Glader B *et al.* (eds). *Wintrobe's Clinical Hematology*. 13th ed. Lippincott Williams & Wilkins 2014; 975-6.
6. Rahbar M, Chitsazian Z, Abdoli F *et al*. Pure red cell aplasia due to antibody against erythropoietin in haemodialysis patients. *J Nephropathol* 2017; 6 (1): 25-9.
7. Wilson HA, McLaren GD, Dworken HJ. Transient pure red-cell aplasia: cell-mediated suppression of erythropoiesis associated with hepatitis. *Ann Intern Med*.

CASE REPORT

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Sheehan's Syndrome

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Abstract

Sheehan's syndrome, after severe post-partum haemorrhage, is now less commonly encountered with the advent of modern care, but occurs frequently in developing countries. Initial presentations include hyponatraemia, asthenia and weight loss. It can have a gradual onset of partial to complete pituitary insufficiency over months to years. The diagnosis of this rare disease is often delayed because the symptoms are vague and the pituitary dysfunction is insidious in nature. This case report deals with a patient who had repeated admissions for hyponatraemia, hypotension and eventually was diagnosed as Sheehan's syndrome.

Introduction:

Sheehan's syndrome classically described after severe post-partum haemorrhage is now less commonly encountered with the advent of modern obstetric care, but it occurs much more frequently in developing countries[1]. Patients present with a range of symptoms occurring due to reduced secretion of pituitary hormones like GH, LH, FSH, TSH, ACTH, and PRL. Such symptoms include breast atrophy, weight loss, amenorrhoea, failure of lactation, weakness, dry skin, loss of axillary and pubic hair and psychiatric disturbances[2]. The presentation varies from acute development of hypovolaemic shock resulting in adenohypophyseal vessel vasospasm and pituitary necrosis to the gradual onset of partial to complete pituitary insufficiency over months to years. Due to its delayed diagnosis, clinical presentation (which usually impairs quality-of-life) and potentially life-threatening complications, (e.g., coma or death), Sheehan's syndrome still remains an important entity among pregnant women, clinicians and public health services around the world.

Case report

A 46-year-old lady came to the emergency department with complaints of giddiness, vomiting, easy fatiguability and loss of weight and appetite. Patient was a known case of hypothyroidism for the past 15 years for which she was on Thyroxine for 9 years. Her initial thyroid function test reports were not available. She stopped taking drugs for the past 6 years. Patient was a known case of Hepatitis C related chronic liver disease for the past 6 years for which she was treated with sofusbuvir and velpatasvir for 6 months in view of high viral load. Her latest HCV RNA PCR had no detectable levels of viral RNA. For the past 4 years she had recurrent admissions for complaints of giddiness and easy fatiguability during which she was found to have hypotension and hyponatraemia. She was treated symptomatically and discharged during those admissions. Her obstetric history was significant because of post-partum haemorrhage following the third delivery for which she received multiple blood transfusions and hysterectomy was also done. She also had a history of lactation failure following the third delivery.

On examination, her GCS was 15/15 with general examination showing pallor, breast atrophy and loss of axillary and pubic hair. Her blood pressure was 80/50 mmHg which improved to 90/60 mmHg following a 20 mL/kg fluid bolus of normal saline. Further investigations showed normocytic normochromic anaemia, hyponatraemia, a low free T4 with low normal TSH (Table I) and ultrasound abdomen showed altered liver echoes. Her Echocardiogram was normal. In view of recurrent admissions for hyponatraemia and hypotension alongwith evidence of central hypothyroidism, possibility of adrenal insufficiency was considered. Her 8 A.M. serum cortisol and serum ACTH were low (Table I) thereby confirming the diagnosis of secondary adrenal insufficiency. With a history of post-partum haemorrhage in the third delivery followed by lactation failure, and secondary hypoadrenaltim along with regression of secondary sexual characteristics, the possibility of pan-hypopituitarism was considered. Pituitary profile showed low levels of serum FSH, LH and prolactin (Table I). MRI brain showed hypoplastic pituitary (Fig. 1, 2, 3 and 4). She was treated with physiological doses of thyroxine and steroids and is currently on follow-up.

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Table I: Hormonal profile of the patient.

Hormone	Patient Value
Free T4	0.8 ng/mL↓
TSH	0.9 mIU/mL
Serum cortisol (8 A.M.)	1.28 µg/dL↓
Serum ACTH	6.05 pg/mL↓
LH	1.69 mIU/mL↓
FSH	8.22 mIU/mL↓
Prolactin	1.76 ng/mL↓

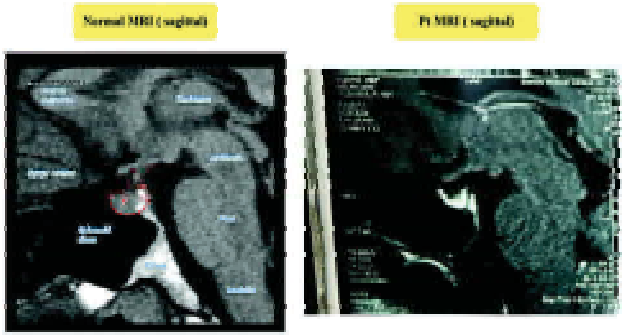


Fig. 1: *Pituitary gland measures 4.7 x 8.2 x 2.8 mm (APXTRXCC). Volume: 62 CC. No Evidence of obvious mass lesion/haemorrhage/abnormal enhancement noted.*

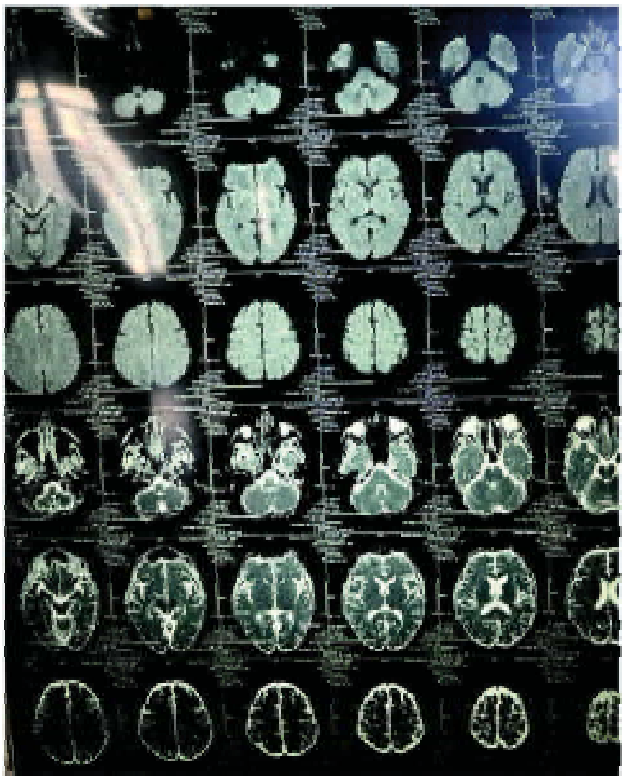


Fig. 2: *MRI brain: Axial view.*

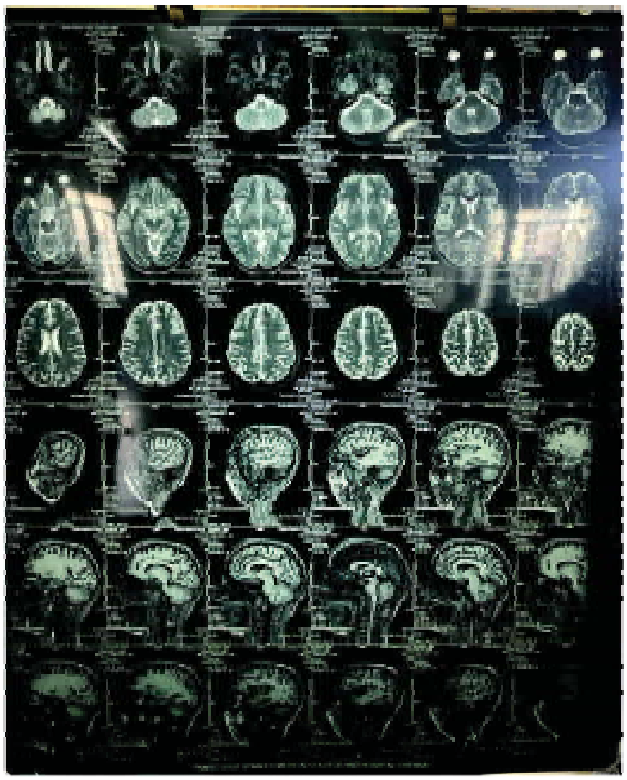


Fig. 3: *MRI brain: Axial and sagittal view.*

Fig. 3: *MRI brain: Sagittal view.*

Discussion

Even with the advent of modern obstetric care, Sheehan's syndrome is a significant cause of morbidity and mortality in developing nations[1]. An epidemiological study from India (Kashmir) has documented a prevalence of around 3.1% in adult women[3]. Normally during pregnancy, under the influence of hormones secreted by the placenta, the pituitary gland enlarges in size. At the end of pregnancy, its volume

increase by 136%. The gland enlargement is predominantly due to hyperplasia of lactotrophs within the gland. The portal vessels supplying the gland are extremely sensitive to volume and pressure changes in the systemic circulation. The anterior pituitary has a low pressure portal system in contrast to the posterior pituitary which functions at a higher pressure. Consequently, the cells of the anterior pituitary are more prone to necrosis in pregnancies complicated by significant postpartum haemorrhage[4]. Though being a post-partum event, the broad spectrum of presentation of Sheehan's syndrome serves as a major road block on the path to diagnosis. In a retrospective analysis, the mean diagnostic delay was 20.37 ± 8.34 years[5]. Presentation of the disease can be acute or chronic. In its acute form, the disease can often be fatal where the patient presents with headache, visual disturbances, loss of consciousness, failure of lactation and features of acute adrenal insufficiency such as hypotension, hypoglycaemia, extreme fatigue, nausea, vomiting and hyponatraemia[6]. Patients presenting with the chronic form on the other hand, often have non specific clinical findings. In a study by Diri *et al* involving 114 participants, 52% had non specific symptoms[7]. Secondary hypothyroidism and secondary adrenal insufficiency are among other presentations of Sheehan's syndrome, as was the case in our patient. Findings like failure to lactate, loss of axillary and pubic hair, secondary hypothyroidism and hyponatraemia have been shown to be quite common in a number of studies. In one such study by Guo-li *et al*, 85.6% had loss of axillary and pubic hair, 74.2% were unable to lactate, 70% had secondary hypothyroidism and 33.7% had hyponatraemia[8]. Hyponatraemia was a consistent and important finding in our patient, given the fact that it was the reason for her recurrent hospital admissions. The aetiology of hyponatraemia in Sheehan's syndrome is not known, but might involve increased antidiuretic hormone (ADH) release as a consequence of reduced blood pressure and cardiac output. However, a more important mechanism may be that cortisol deficiency results in increased hypothalamic secretion of corticotropin-releasing hormone (CRH), an ADH secretagogue. Hyponatraemia might even be the initial presentation of the disease[9]. With respect to radiological investigations, MRI plays a pivotal role in the diagnosis and the findings vary with the duration of disease. Early in the disease, we see an enlarged pituitary, with low T1 and high T2 homogenous signal. A ring enhancement is also a possibility during early stages of the disease. In the later stages, patients with Sheehan's syndrome can have partially (25 - 30%) or completely (70 - 75%) empty sella turcica on imaging studies[10]. In view of the varied presentations of the disease, diagnostic criteria have been proposed by Diri *et al*[7] (Box 1).

Treatment of Sheehan's syndrome is aimed at appropriate replacement of deficient hormones, though such replacement does not improve the pituitary function nor does it delay the progression of pituitary necrosis. Recombinant human growth hormone (GH) is not recommended as routine treatment for all patients with adult-onset GH deficiency, because the small clinical benefits do not seem to warrant daily injections and high cost[11]. Treatment of adrenal crisis involves IV hydrocortisone at a bolus dose of 100 mg followed by a dose of 200 mg over the next 24 hours along with treatment of the precipitating factor. For chronic secondary adrenal insufficiency, oral hydrocortisone should be given at a standard replacement dose of 10 to 20 mg on awakening and 5 to 10 mg at 3 to 6 pm[12]. For hypothyroidism, in young patients who have no evidence of ischaemic heart disease, a starting dose of 100 micrograms should be used. In older patients or patients known to have cardiovascular disease, caution should be used with an initial dose of 25 to 50 micrograms and the aim of replacement is to place the fT4 in the normal range. When hypothyroidism and hypocortisolism coexist, steroid replacement should precede the replacement of thyroxine in view of preventing an adrenal crisis[13]. Once hormone replacement is initiated and optimised, patient assessments should be on an annual basis to determine their cardiovascular risk. Constant supervision is needed to optimise the long-term outcome of patients.

Box 1: Diagnostic Criteria for Sheehans's syndrome as proposed by Diri *et al*.

Essential criteria for the diagnosis:	Criteria that are not essential, but, if present are strongly suggestive of the diagnosis:
• Typical history of severe post-partum uterine bleeding, particularly at last delivery	• Severe hypotension or shock at index delivery
• At least one pituitary hormone deficiency	• Post-partum amenorrhoea
• A partially or completely empty sella turcica on a CT or MRI scan in the chronic phase	• Failure of postpartum lactation

Conclusion

Hypopituitarism is a rare, chronic disease associated with considerable morbidity and reduction in life span. Though the incidence of Sheehan's syndrome has declined in the past few decades as a result of modernised obstetric care in the developed world. The syndrome has been increasingly recognised as one of the leading causes of hypopituitarism in developing countries. The clinical impact of hypopituitarism can be variable and is determined by the age at which the condition occurs, its rapidity of onset, the gender of the patient, the underlying cause, and the pattern of hormone deficiencies. Non-specific symptoms being a common presentation, affect the quality-of-life, especially because of long diagnostic delay. In such instances, patients often remain undiagnosed or misdiagnosed for a long time and receive inappropriate treatments. This was the case in our patient where non-specific symptoms and recurrent hyponatraemia led to her diagnosis almost 15 years after her initial insult of post-partum haemorrhage. Increased awareness of this condition can facilitate earlier diagnosis and prompt treatment, hence improving the quality-of-life and lowering morbidity and mortality.

References

1. González G, Gerardo J, Almaguer B *et al*. Sheehan's Syndrome Revisited: Underlying Autoimmunity or Hypoperfusion? *Inter J Endocrinol* 2018; 8415860: 8.
2. Feinberg EC, Molitch ME, Endres KK *et al*. The incidence of Sheehan's syndrome after obstetric haemorrhage. *Fertility and Sterility* 2005; 84 (4): 975-9.
3. Zargar AH, Singh B, Laway BA *et al*. Epidemiologic aspects of post-partum pituitary hypofunction (Sheehan's syndrome). *Fertil Steril* 2005; 84 (2): 523-8.
4. Schury MP, Adigun R. Sheehan's Syndrome. [Updated 2023 Sep 4]. In: Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459166/
5. Gokalp D, Alpagat G, Tuzcu A *et al*. Four decades without diagnosis: Sheehan's syndrome, a retrospective analysis. *Gynecol Endocrinol* 2016; 32 (11): 904-7.
6. Roberts DM. Sheehan's syndrome. *Am Fam Physician* 1988; 37: 223-7.
7. Diri H *et al*. Extensive investigation of 114 patients with Sheehan's syndrome: a continuing disorder. *Eur J Endocrinol* 2014; 171: 311-8.
8. Du GL, Liu ZH, Chen M *et al*. Sheehan's syndrome in Xinjiang: Clinical characteristics and laboratory evaluation of 97 patients. *Hormones (Athens)* 2015; 14 (4): 660-7.
9. Schrier RW. Body water homeostasis: clinical disorders of urinary dilution and concentration. *J Am Soc Nephrol* 2006; 17: 1820-32.
10. Gokalp D *et al*. Four decades without diagnosis: Sheehan's syndrome, a retrospective analysis. *Gynecol Endocrinol* 2 June 2016 (epub ahead of print).
11. Frohman LA. Controversy about treatment of growth hormone-deficient adults: a commentary. *Ann Intern Med* 2002; 137 (3): 202-4.
12. Bornstein SR, Allolio B, Arlt W *et al*. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2016; 101 (2): 364-89.
13. Toogood AA, Stewart PM. Hypopituitarism: Clinical Features, Diagnosis, and Management. *Endocrinology and Metabolism Clinics of North America* 2008; 37 (1): 235-61.

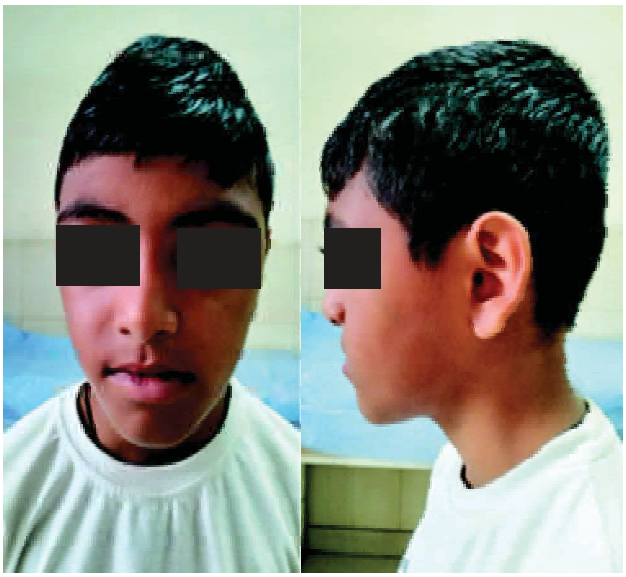
JIACM 2024; 25(4): 236

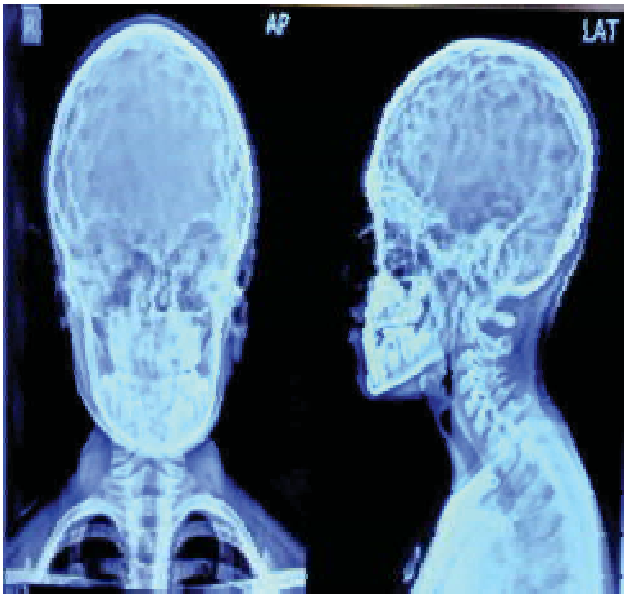
A Young Boy Presenting with Headache: Crouzon Syndrome

Ragini Ram, S Anuradha***

A 12-year-old boy presented with recurrent headaches for last three years. The headache was frontal in location and responded to analgesics. For the last 6 months he complained of persistent headache with blurring of vision and poor response to pain relievers. There was history of increasing proptosis for last few months. The antenatal and neuro-developmental history was normal with the child attending regular school. Examination revealed a conscious boy with normal intelligence. The child had an abnormal skull shape with proptosis as shown in the figure. Fundus examination revealed bilateral papilloedema. There were no focal neurological deficits and other systems were normal on examination. A Plain X-ray skull revealed a characteristic silver beaten appearance – Figure. A diagnosis of Crouzon syndrome was made.

Crouzon syndrome, also known as craniofacial dysostosis or acrocephalosyndactyly, is a complex genetic syndrome that occurs in about 1 in 61,000 newborns and ranks among the most prevalent types of craniofacial dysostosis[1]. It is caused by a mutation (inherited or sporadic) in the Fibroblast Growth Factor Receptor 2 (FGFR2) or the FGFR3 gene located on chromosome 10. Crouzon syndrome is characterised by a classic trio of an atypical skull structure, distinctive facial features, and protruding eyes. Crouzon syndrome causes the skull's sutures to fuse together too early (craniosynostosis) resulting in an abnormal head and face





shape. It can be syndromic or non-syndromic, the latter being more common. Non-syndromic craniosynostoses are not associated with other body dysmorphisms and usually affect only one suture of the skull, while syndromic craniosynostoses are known to affect multiple skull sutures and are associated with craniofacial dysmorphisms, abnormalities of extremities, and other bony anomalies[2].

Commonly, fusion of bilateral coronal sutures causes brachycephaly (short, wide skull) as seen in this case. The main mechanism behind papilloedema is postulated to be high intracranial pressure (ICP), secondary to anomalous skull development. Radiology reveals a copper beaten appearance of skull, lacunae in the skull and shallow orbits. A multidisciplinary team (typically including a pediatrician with dysmorphology training, a craniofacial surgeon, a neurosurgeon, an oculo-facial plastic surgeon, and an oral maxillofacial surgeon) is needed for optimum management such that patients can achieve maximum functional status and relief of chronic headache.

References

1. Tonpe S, Warbhe H, Banode P *et al*. Radiological Diagnosis of Crouzon Syndrome: A Case Study. *Cureus* 2024; 16 (6): e62564.
2. Rafique Ali AA, Ismail F, May May C *et al*. Ophthalmic features of craniosynostosis: A Malaysian experience. *Eur J Ophthalmol* 2022; 32 (3): 1417-23.

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VIDEO IN CLINICAL MEDICINE

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Opsoclonus in a Patient with Scrub Typhus Infection: A Rare Neurological Manifestation

Rashmi Meena, Gargee Ratan**, S Anuradha***, Sumeet Singla*****

A 36-year-old man, presented with a fever with chills for 1 week and abdominal pain and vomiting for 1 day. There was no history of cough, shortness of breath, abnormal body movements, altered sensorium, headache, decreased urine output, jaundice, or bleeding manifestations. On examination, the patient was conscious and oriented to time, place, and person. His vitals were stable. A typical *'cigarette burn'* eschar was noted over his chest (Fig. 1). The rest of the general physical and systemic examinations were unremarkable. On the 3rd day of hospitalisation, he developed chaotic, multidirectional eye movements consistent with opsoclonus (Video). Repeated neurological examination was normal with normal cerebellar function. Laboratory investigations showed thrombocytopenia (40,000), elevated ESR (91), hyperbilirubinaemia (Total bilirubin - 3.5 mg/dL), and abnormal liver (AST 133 U/L, ALP 388 U/L) and kidney function tests (urea - 106 mg/dL/1.3 mg/dL). Scrub typhus IgM ELISA was positive, while other infectious causes were ruled out. MRI of the brain revealed gliosis and encephalomalacia as sequelae of prior traumatic brain injury with a normal cerebellum.

The patient was treated with tablet doxycycline for 10 days, along with supportive management. The patient showed clinical improvement during hospitalisation and was discharged in stable condition.

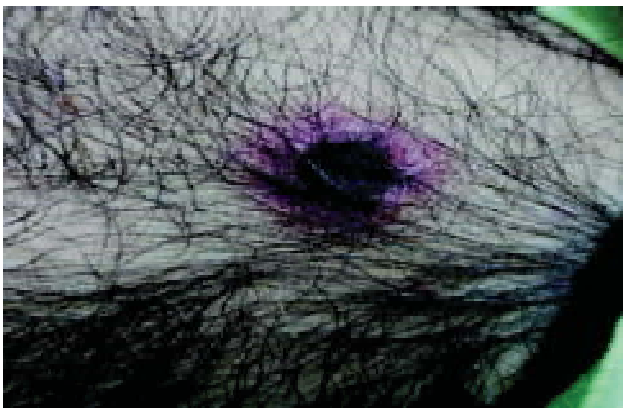

Fig. 1: *Eschar over his chest.*

Scrub typhus, a significant public health issue, is caused by the rickettsial bacterium *Orientia tsutsugamushi*, which is predominantly found within the Tsutsugamushi Triangle. Its incidence has been reported as 4.6 per 1,00,000 over a decade, with a case fatality rate of 13.6%[1]. Systemic manifestations typically emerge in the second week of illness and may include central nervous system involvement, such as meningitis, encephalomyelitis, encephalopathy, and occasionally cranial nerve palsies or ocular symptoms[2,3].

Opsoclonus, a rare neurological manifestation, is an uncommon presentation of scrub typhus. This condition, often accompanied by myoclonus, cerebellar dysfunction, or extrapyramidal signs, typically resolves during the febrile phase of the disease. Opsoclonus arises from dysfunction of Purkinje cells in the dorsal vermis, leading to disrupted inhibitory control of saccadic burst neurons in the pontine reticular formation. This disruption results in disinhibition of the cerebellar fastigial nucleus, causing the characteristic abnormal eye movements.

Ravikar Ralph *et al* reported a case series on opsoclonus, a rare neurological manifestation of scrub typhus, in 18 patients out of 1,650 cases over five years at a teaching hospital from India. Opsoclonus occurred after a median of 11 days from fever onset, with 94% showing myoclonus, 67% cerebellar dysfunction, 33% extrapyramidal syndrome, and 17% aseptic meningitis. CSF analysis revealed mildly elevated WBC counts, high protein levels, and normal glucose, while brain MRI was unremarkable in 75% of cases. The case-fatality rate was 5.5%, and complete neurological recovery occurred by 12 weeks post-discharge. Opsoclonus typically arises during the resolving febrile phase and resolves with appropriate treatment[4].

Opsoclonus is generally associated with parainfectious, paraneoplastic, or other causes. It is more commonly observed in children, where neuroblastoma is the most frequent malignancy, and paraneoplastic causes are more

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prevalent than parainfectious ones.

This case report and video underscores the growing variability and complexity of neurological presentations associated with scrub typhus. Opsoclonus-myoclonus syndrome (OMS) is typically recognized as a well-defined paraneoplastic syndrome, likely driven by an antibody-mediated mechanism. In contrast, parainfectious OMS appears to have a favourable prognosis, provided the underlying infection is effectively treated.

References

1. Bonell A, Lubell Y, Newton PN *et al*. Estimating the burden of scrub typhus: A systematic review. *PLoS Negl Trop Dis* 2017; 11 (9): e0005838.
2. Neela A, Gohil R, Tagore R, Ta V. Opsoclonus: A Rare Neurological Manifestation in a Patient With Scrub Typhus Infection. *Cureus* 2024; 16 (9): e70058.
3. Rana A, Mahajan SK, Sharma A *et al*. Neurological manifestations of scrub typhus in adults. *Trop Doct* 2017; 47 (1): 22-5.
4. Ralph R, Prabhakar AT, Sathyendra S *et al*. Scrub Typhus-Associated Opsoclonus: Clinical Course and Longitudinal Outcomes in an Indian Cohort. *Ann Indian Acad Neurol* 2019; 22 (2): 153-8.

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