C O N T E N T S

Journal, Indian Academy of Clinical Medicine ● Vol. 23, Number 3-4, July-December, 2022

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Journal, Indian Academy of Clinical Medicine

Indian Association of Clinical Medicine

Headquarters:
Post-Graduate Department of Medicine, Sarojini Naidu Medical College,
Mahatma Gandhi Road, Agra - 282 002 (U.P.)

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GOVERNING BODY

Neutrophil to Lymphocyte Ratio: A Simple, Quick, and Independent Predictor of Severity and Outcome in COVID-19 Disease

Rajnish Kaushik*, Anubhav Gupta**, Devyani Thakur***, MPS Chawla****, Amit Suri*****, Parkash Chugh******

Abstract

Coronaviridae belongs to an enveloped RNA virus family and is kenned to cause the common cold and sometimes astringent illnesses. The most recently discovered coronavirus is COVID-19, referred to as severe acute respiratory syndrome caused by SARS-CoV-2. Current classification criteria for moderate and severe disease are respiratory rate, oxygen saturation, and PaO_2/FiO_2. These markers are significant but have no COVID-19 specificity. NLR is suggested as a simple marker of the systemic inflammatory response in critically ill patients and is an independent indicator of both short-term and long-term mortality in critical patients. The ease of using NLR as a systemic inflammatory marker and a potential predictor of clinical risk and outcome in critically ill patients reinforce its use in the COVID scenario. The aim of our study was to evaluate NLR as a COVID-19 disease severity marker and to evaluate the role of NLR in COVID-19 disease outcome. We included the demographics and clinical characteristics of 117 admitted patients who were RT-PCR positive for COVID-19. As per age and gender-wise distribution, 74 patients were male, and 43 were female, with a mean age of 49.11 ± 18.63 years. Mild patients had a mean NLR of 4.76 (2.03 to 7.77), the moderate disease had a mean NLR of 5.21 (2.00 to 9.88), and severe disease had a mean NLR of 6.19 (0.2 to 25) at admission. Our results show a strong relationship between higher NLR values with mortality (AUC = 97.4) with a sensitivity of 92.3% and specificity of 86.6% and is statistically significant. We recommend that NLR can be a quick, inexpensive, accessible, reproducible marker for gauging severity and outcome in COVID-19.

Introduction

Coronaviridae belongs to an enveloped RNA virus family and is kenned to cause the common cold and sometimes astringent illnesses. Sometimes zoonotic coronaviruses infect humans and propagate further, as shown in the MERS-CoV, SARS-CoV and recently COVID-19, via human-to-human transmission. The most recently discovered coronavirus is COVID-19, referred to as severe acute respiratory syndrome caused by coronavirus 2 (SARS-CoV-2)[1]. In mid-December 2019, Wuhan city in Hubei Province of China reported a COVID-19 outbreak, declared as the Public Health Emergency of International Concern (PHEIC) by the World Health Organisation (under International Health Regulations) on 30 January 2020, and later on 11 March 2020 as a pandemic[2]. COVID-19 halted the world, with over 20.5 million of lives being lost to COVID-19 globally[3]. While the majority of the cases are self-limiting, the disease has a 2 - 3% death rate[4]. Though COVID-19 has been chiefly characterised and noticed as a respiratory condition, up to 20% of patients with COVID-19 have a severe infection, including coagulopathy and septic shock, with severe extra-pulmonary symptoms[5]. which can have consequences, such as severe pneumonia, ARDS, and multi-organ failure that ultimately lead to death[6]. The gold standard for the diagnosis of COVID-19 is the RT-PCR test,

which detects viral RNA and usually produces a result within two to five hours. Nevertheless, the severity of the condition remains clinically based. The current classification criteria for moderate and severe disease are respiratory rate, oxygen saturation, and PaO_2/FiO_2. These markers are significant but have no COVID-19 specificity. In severe disease, patients rapidly deteriorate to respiratory distress/failure, metabolic acidosis, coagulation defects, and septic shock. Early identification of severe risk factors enables adequate assistance to provide support and quick access to a health facility or an intensive care unit (ICU) if necessary. The early identification would assist decrease mortality and avoid medical scarcity in the early triage. COVID-19 associated sepsis demonstrates decreased eosinophils, CD4+, CD8+, CD19+, and total lymphocytes; and significantly elevated liver enzymes, C-reactive protein, erythrocyte sedimentation rate (ESR), ferritin, and IL-6 levels in severe disease[7], with an exceptionally high incidence of lymphopenia in COVID- 19 patients[8]. Separately testing all these markers becomes challenging, particularly in resource-poor settings. The world is facing considerable challenges in the management of COVID patients and preventing further disease spread. Lockdowns and curfews have created a resource crunch. Resource-poor settings struggle to match the expanded need for appropriate diagnosis and management, which has also

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sparked fears of global economic crisis and recession. In such a scenario, the simplest objective tests gain utmost importance. One such test is the neutrophil to lymphocyte ratio or NLR. The NLR is suggested as a simple marker of the systemic inflammatory response in critically ill patients and is an independent indicator of both short-term and long-term mortality in critical patients[9-11]. The ease of using NLR as a systemic inflammatory marker and a potential predictor of clinical risk and outcome in critically ill patients reinforce its use in the COVID scenario.

To our knowledge, there is still no validation of the usefulness of NLR for predicting mortality in COVID-19 patients, especially in Indian patients. Hence, we discussed the role of NLR as a valuable, inexpensive, readily available, reproducible objective marker for the assessment of clinical severity and outcome in COVID-19.

Aims and objective

1. To evaluate NLR as a COVID-19 disease severity marker.
2. To evaluate the role of NLR in COVID-19 disease outcome.

Material and methods

Study design

We analysed the data retrospectively on clinical characteristics of RT-PCR confirmed COVID-19 patients admitted in corona wards between March 2020 to August 2020 in Dr Ram Manohar Lohia Hospital, New Delhi, India. Informed consent was taken from relatives of the patients to use their data for this research. Based on the COVID-19 management guidelines published by the Ministry of Health and Family Welfare (MoHFW), from time to time, patients were characterised on clinical severity as mild, moderate, and severe[12]. Mild COVID-19 was defined as patients presenting with uncomplicated upper respiratory tract infection; mild symptoms such as fever, cough, sore throat, nasal congestion, malaise and headache. Moderate COVID-19 was defined as clinical features of dyspnoea and or hypoxia, fever, cough, including $SpO_2 < 94\%$ (range 90 - 94%) on room air, the respiratory rate more than or equal to 24 per minute. Severe COVID-19 was defined as clinical signs of Pneumonia plus one of the following: respiratory rate > 30 breaths/min, severe respiratory distress, $SpO_2 < 90\%$ on room air. Two other patient groups were made based on the clinical outcomes, i.e., Discharge and Death. The following patients were excluded from the study:-

1. Patients on any prolonged immunosuppression, prolonged steroids (prolonged being defined as > = four weeks),

2. Pregnant women,
3. Patients with known malignancy and autoimmune diseases,
4. Patients with HIV/AIDS,
5. Patient's age less than 18 years.

Blood samples for NLR were collected during admission and again after seven days in EDTA vials. The samples were processed in Medonic M series analysers within 1 hour of collection. The neutrophil to lymphocyte ratio was calculated as the absolute neutrophil count and absolute lymphocyte count ratio (ALC). The normal value of NLR identified in healthy, non-geriatric adults ranges from 0.75 to 3.53[13].

Continuous and normally distributed variables were described by the mean and standard deviation (SD). Variables that did not show a normal distribution were defined using medians and interquartile ranges. Comparisons between all three groups were performed using the Kruskal-Wallis H-tests, followed by Bonferroni-corrected Mann–Whitney U-tests as a post-hoc analysis. With $P < 0.05$, the results were considered statistically significant, and the predictive value of NLR was calculated by using ROC.

Results

Table I shows the demographics and clinical characteristics of 117 admitted patients who were RT-PCR positive for COVID-19. As per age and gender-wise distribution, 74 patients were male, and 43 were female, with a mean age of 49.11 ± 18.63 years. Of these 117 patients, 59.8% (n = 70) had co-morbidities in the form of diabetes mellitus, hypertension, coronary artery disease, chronic kidney disease, bronchial asthma, etc. Severity categorisation into mild, moderate, and severe was 32%, 31%, and 37%, respectively. Of these 117 patients, 67% (n = 78) were discharged and 33% (n = 39) died.

Table I: Demographic profile of COVID patients.

Total number of patients (N)	117
Gender (M/F)	74 /43
Mean age	49.11 ± 18.63
Patients with co-morbidities	70
Clinical category	Mild - 38
	Moderate - 36
	Severe - 43
Clinical outcome	Discharged - 78
	Death - 39

Table II shows the trend of severity correlation of NLR in COVID-19 patients. Mild patients had a mean NLR of 4.76 (2.03 to 7.77), the moderate disease had a mean NLR of 5.21 (2.00 to 9.88,) and severe disease had a mean NLR of 6.19 (0.2 to 25) at admission. However, the NLR ratio further decreased at seven days to 3.59 (0.9 to 8.8) for mild patients, 3.64 (1.3 to 18.4) for moderate patients but increased to 6.70 (0.02 to 21.6) in severe disease. Likewise, the discharged patients showed a decreasing trend of NLR from a mean of 5.14 at admission to 3.32 at seven days. The NLR of patients who scummed the COVID-19 increased from a mean value of 5.99 at admission to 7.59 at seven days (Table III).

Table II: NLR as per disease severity (severity correlation).

Outcome	Early NLR	Late NLR	Significance
Discharge	4.76 ± 1.52	3.59 ± 1.95	0.042
Death	5.21 ± 2.06	3.64 ± 3.59	0.001
Severe COVID	6.19 ± 4.77	6.70 ± 4.96	0.04

Table III: Neutrophil lymphocyte ratio (NLR) as per disease outcome.

Outcome	Early NLR	Late NLR	Significance
Discharge	514 ± 2.07	3.32 ± 1.90	0.00
Death	5.99 ± 4.82	7.59 ± 5.50	0.004

ROC plot showed the cut-off value of the NLR as 7.69 at the time of admission. The result shows a strong relationship between higher NLR values with mortality (AUC = 97.4) with a sensitivity of 92.3% and specificity of 86.6% and is statistically significant P = 0.00 (Fig. 1).
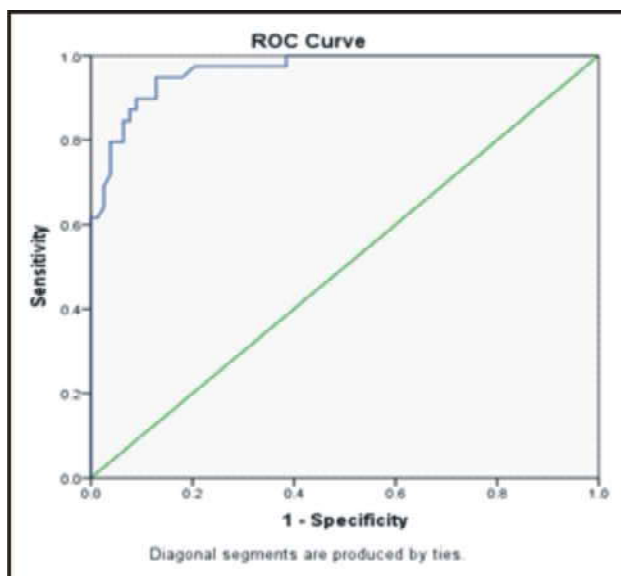
Fig. 1: ROC for prognostic value of early NLR.

Furthermore, we found that the cut-off value of the NLR after seven days was reduced to 4.65, providing strong evidence that patients with values above this are more likely to develop mortality (AUC = 96.5) with a sensitivity of 94.9% and specificity of 83.6%, at a significance of p = 0.00 (Fig. 2).
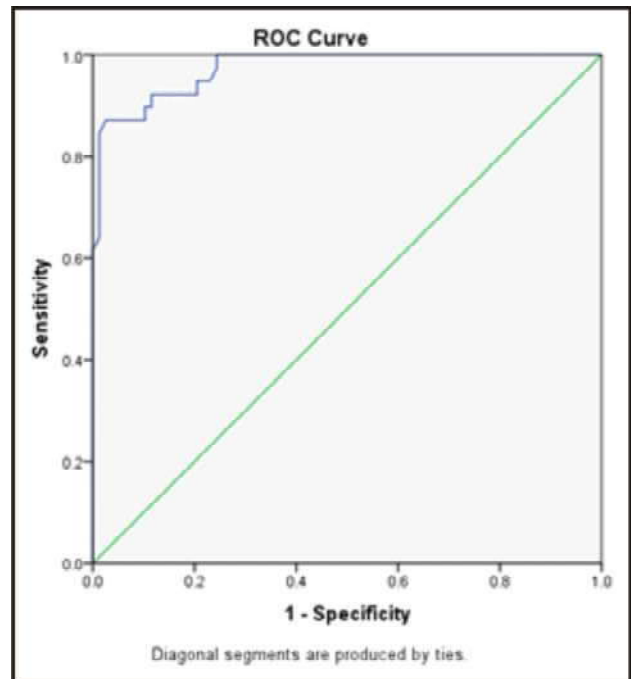
Fig. 2: ROC for prognostic value of late NLR.

Discussion

COVID-19, a highly infectious disease, has been spreading rapidly throughout the world and continues to pose a severe threat to global public health. The prognosis for patients with severe COVID is poor. As a result, we must identify potentially severe cases early and treat these patients as soon as possible. In our hospital's clinical practice of treating COVID-19 patients, we've found that the NLR is a quick and inexpensive predictor of severity and outcome.

There is strong evidence that immune system dysregulation plays a role in the development of viral hyper-inflammation. Serum Ferritin, IL-6 levels, lactate dehydrogenase (LDH), C-reactive protein (CRP), D Dimer levels, and serum Procalcitonin are some of the available laboratory parameters to monitor this hyper-inflammation[14]. The difficulty of obtaining most laboratory tests makes early, objective prognostication difficult and, as a result, morbidity and mortality rise. The lack of standardised management protocols emphasizes the value of early risk assessment even more. The absolute value of peripheral white blood cells in patients with COVID-19 is usually normal or low in laboratory examinations, and lymphopenia is common[4]. As

the lymphocyte count declines, the neutrophil count rises[15], owing to severe COVID-19's biphasic event of hyper-inflammation and immunosuppression, similar to severe sepsis[16]. Sepsis has two phases: an early phase (which lasts 5 days) and a late phase[17]. The distinction between early and late sepsis is increasingly being researched, and the two conditions may have different underlying mechanisms that require different treatment approaches[18-20]. Using the same reasoning as we did with COVID-19, we tested the NLR in the early phase and then in the late phase of the study. NLR has been studied in the context of sepsis as a diagnostic and prognostic marker, according to the reviewed literature[21]. Although there have been several studies, none have examined the NLR in early and late COVID-19 sepsis to determine whether it is a predictor of severity or outcome. Using NLR cut-off values of 4.3, Shang et al evaluated 443 patients and reported in their study that the AUC was 0.74, the sensitivity was 56.3 per cent, and the specificity was 83.7 per cent, indicating that the NLR cut-off value of 4.3 was indicative of severe disease. 22 Li et al discovered an NLR cut-off value of 11.3 in 93 patients to predict severe COVID-19, with a sensitivity of 78.1 per cent and specificity of 92.0 per cent in patients with severe COVID-19, respectively. 23 In another study, Basbus et al discovered that an NLR cut-off value of 3.0 among 131 patients could predict severe disease with 80.9 per cent sensitivity and 67.3 per cent specificity, indicating that the NLR cut-off value was accurate[24].

Wang et al proposed a still higher NLR cut-off value of 13.4 to indicate severe disease, with an AUC of 0.89 and sensitivity and specificity of 83.3 and 82.4 per cent, respectively, indicating severe disease[25]. Qin et al also found that a high NLR was associated with severe illness in 452 patients admitted to the hospital with COVID[26]. A study by Cheng et al looked at 456 patients and found that at an NLR of 3.2 and an AUC of 0.81, they could predict mortality with an accuracy of 78.3 percentage points and a specificity of 73.9 per cent[27]. As an example, Tatum et al studied 125 patients and found that an NLR cut-off value of 10 was associated with an AUC of 0.71, a sensitivity of 52.4 per cent, and a specificity of 96.7 per cent (with an AUC of 0.71)[28].

Despite the fact that cut-offs vary depending on the patient's demographic profile and have varying sensitivity and specificity, several researchers have come to the conclusion that a raised NLR is a predictor of COVID-19 severity and, in some cases, mortality.

Conclusions

NLR can be a quick, inexpensive, accessible, reproducible marker for gauging severity and outcome in COVID-19.

The higher the NLR, the greater the risk. High NLR at admission in early disease is a predictor of disease severity and can objectively help triage and guide management. An elevated NLR at the end of the first week is a predictor of mortality. Additional studies are needed to strengthen its role.

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Efficacy Of DNA-based Customised Diet and Exercise Plan for Weight Management

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Abstract

The change in the requirement of the nutrients is highlighted by gene-environment studies, depending upon changes in the genes of a person which influences metabolism and transport of different nutrients. Changes in weight of an individual throughout one's life are dependent on the interplay of various behavioral, genetic, and environmental factors. The present study will look into whether the inclusion of information of genes to personalise the diet of an individual and exercise plan would be fruitful or otherwise. The study analyses the nucleotide sequencing in various genes observed in preparing diet charts in group A and group B.

Aim: To determine the efficacy of DNA-based customised diet and exercise plan for weight management.

Material and methods: 30 subjects were included in the study and divided into 2 groups: Plan A – Number of subjects - 15 (n = 15) provided with DNA-based diet and exercise plan; Plan B – Number of subjects - 15 (n = 15) followed the conventional diet and exercise plan matched for age, gender, and BMI.

The study adopted moderate fat balanced nutrient, high protein and moderate carbohydrate diet as conventional therapy advocated by the hospital dietician. The study composed 1,500 kcal standard diet.

Results

1. *Weight loss was observed in individuals both in plan A and plan B. The difference in the weight loss after 3 months in plan A and plan B was not significant. However, at 6 months, the subjects in plan A showed significant reduction in weight when compared with plan B.*

2. *BMI of individuals of plan A at the end of 3 months was insignificant, but however, observations were statistically significant at the end of 6 months.*

3. *The reduction in mean waist line of subjects at the end of 6 months was observed to be statistically significant in subjects of plan A in comparison to that of subjects of plan B (P = 0.042S).*

Conclusion: DNA test based customised diet and exercise plan helped to loose weight more effectively as compared to the conventional diet plan among obese subjects. Adding a genetic personalised component to the weight loss programme, improved motivation and compliance among the subjects.

Key words: Genomic diet, SNPs, exercise, weight management.

Introduction

Nutrition is considered as a basic human need and a pre-requisite for living a healthy life. Nutrigenomics is the science for studying the response of nutrition and diet on the physiological and genetic variations of one's body. Every individual has a unique set of genes that are attributable to variations in regards to different dietary components[1]. The variations in the genes of different individuals decide the metabolic traits such as response to diet and exercise plan which helps us to understand why some people can eat as much as they want but remain thin and why a certain type of exercise results in more weight reduction in one individual as compared to another. Testing one's genetic profile helps to determine which kind of food to be eaten and exercise to be done in ensuring a healthy lifestyle. The new field of nutrigenomics guides us to what specific foods you must consume, as per your genes. What you consume has a direct correlation to the genetic signals your body collects. These signals, one after the other, have the power to command all the molecules that add up to one's metabolism: the molecules that gives command to one's body either to burn calories or stockpile them. If the consumption of food is changed, it can even drastically change the manner in which the food interconnects with the body.

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The present study will look into whether the inclusion of information of genes to personalise the diet of an individual and exercise plan would be fruitful or otherwise.

Methods and material

In the present study we included 30 subjects (n = 30) and divided them into 2 groups as follows:-

- Plan A: Number of subjects – 15 (n = 15) who were provided with DNA-based diet and exercise plan.

- Plan B: Number of subjects – 15 (n = 15) following the conventional diet and exercise plan matched for age, gender, and BMI.

In the study we have adopted a moderate fat, balanced nutrient, high protein, and moderate carbohydrate diet as conventional therapy advocated by our hospital dietician for group B. A total of 1,500 kcal standard diet was prepared, out of which 20 - 30% energy was obtained from fats, 55 - 60% from carbohydrates and 15 - 20% from proteins. The diet restrictions were advocated along with suitable exercises.

Inclusion criteria

- Gender: both males and females.

- Ethnicity/food habits/economic background: no restrictions.

- Age (years): 18 - 60 years.

- BMI: between 25 to 38 kg/m^2.

- Ability to provide informed consent and complete health risk assessment prior to participation.

Exclusion criteria

- Individuals with diabetes mellitus, bariatric surgery, heart failure, cancer, liver, or renal disorders, HIV, and medical conditions that could affect body weight or ability to engage in structured physical activity of the study.

- Psychological or psychiatric medications within the previous twelve months.

- Reported pregnancy or cases of abortion/still birth in the last 6 months; or lactating mothers.

Study duration: 6 months follow-up.

Table I: List of genes under study with their physiological functions.

ADRB2[2]	Weakens the breaking-down of neutral fat (slower metabolism) causes needless energy accumulation in the body.
FABP2[3]	FABP plays a role in the transport of long-chain fatty acids and their acyl-CoA esters intracellularly. FABP2 plays a significant role in the synthesis of triglyceride-rich lipoprotein.
ADIPOQ[4]	The ADIPOQ gene comprises the instructions for production of the hormone adiponectin. This hormone is made solely by adipocytes (fat cells) and travels through the blood to reach the muscle and liver cells.
APOA2	Apolipoprotein A-II (APO-AII) is the second most frequently found protein in high density lipoproteins. APO-AII seems to damage the reverse cholesterol transport and antioxidant purpose of high-density lipoprotein.
PPARG[5]	PPARG plays an important physiological part as a regulator of central transcriptional adipogenic and lipogenic activity, insulin sensitivity and glucose homoeostasis.
TCF7L2[6]	TCF7L2 codes for a high mobility group (HMG). The protein plays an important role in blood glucose homoeostasis.
ACTN3[7]	Alpha-Actin binding protein is primarily expressed in skeletal muscle and functions as a structural component of sarcomere Z line. This protein helps in cross-linking actin for workout and athletic performance.
ADRB3	Beta-3 plays a part in the regulation of lipolysis and thermogenesis.

Table II: Events studied and their respective genes while prescribing DNA based genetic diet.

Metabolism of fat and glucose and risk of obesity	• ADRB2 • PPARG • FABP 2 • ADRB 3
Carbohydrate metabolism on fitness	• TCF7L2
Response to mono-unsaturated fats and poly-unsaturated fats (MUFA and PUFA)	• ADIPOQ • PPARG
Satiety behaviour: fat mass and obesity	• FTO
Type of sports, workouts, and exercises	• ACTN3
Response to exercise: metabolic efficiency, weight loss, and weight regain	• ADRB3

We consider for the genotype of the DNA sequence of a particular gene depending on the nucleotide sequences in the DNA and varied RNA formed; subsequent amino acids and the nature of the protein formed. Different proteins formed vary in their structure and function, catering to the different needs of the body (Table II).

Many studies have been done stressing upon the importance of the individual gene responsible in obesity management.

Ours is a unique study, incorporating all the genes as stated in Table I and II with their nucleotide sequences and single nucleotide polymorphisms affecting fats, carbohydrates, mono-saturated and poly-saturated fatty acids. Other parameters considered are satiety behaviour, types of sports, workouts, and response to exercises.

In the "DNA based genetic diet and exercise plan", we have studied SNP at ACTN3 gene, and depending upon the genotype workout, an exercise plan is prescribed.

Depending upon the genetic sequence of ACTN3 gene, following three types of variations in the SNPs have been noted.

Those with genotype CT were given endurance-based exercises and physical activity while those with CC and TT were given power-based exercises and aerobic exercises respectively.

Results

Table III: Gender of obese subjects in plan A and B in age group 20 - 50 years.

Gender	Plan A		Plan B		Total (Plan A + Plan B)
	No.	Percentage	No.	Percentage	
Male	11	73.33%	09	60.0%	20
Female	04	26.67%	06	40.0%	10
Total	15	100%	15	100%	30

Table IV: Comparison of mean weight of subjects in groups at baseline, after 3 months and 6 months of diet therapy.

Weight	Plan A Mean ± SD	Plan B Mean ± SD	t-value	P-value
Base line	91.67 ± 14.53	90.93 ± 14.80	0.137	P=0.892NS
After 3 Months	83.20 ± 12.72	86.73 ± 13.70	0.732	P=0.470NS
After 6 Months	75.07 ± 11.15	83.13 ± 14.18	1.94	P=0.04S

Table V: Comparison of mean waist line of subjects in groups at baseline, after 3 months and 6 months.

Waist Line	Plan A Mean ± SD	Plan B Mean ± SD	t-value	P-value
Base line	142.93 ± 19.96	147.07 ± 26.23	0.708	P=0.485NS
After 3 Months	131.33 ± 18.19	139.13 ± 19.82	1.12	P=0.271 NS
After 6 Months	120.06 ± 13.95	134.13 ± 21.42	2.13	P=0.042S

Table VI: Comparison of mean BMI of subjects in two studied groups at baseline, end of 3 months and 6 months of diet therapy.

BMI in Kg/m2	Plan A Mean ± SD	Plan B Mean ± SD	t-value	P-value
Base line	32.49 ± 2.37	32.93 ± 3.55	0.401	P=0.691NS
After 3 Months	29.35 ± 2.11	31.30 ± 3.39	1.89	P=0.069 NS
After 6 Months	27.05 ± 2.05	29.97 ±3.43	2.93	P=0.008S

Discussion

The present study has been undertaken using DNA amplification and nucleotide sequencing by Sanger's method. Reference genome has been utilised in the labs for sequencing purpose.

The basis of determination of amino acids are chiefly based on nucleotide studies. SNPs which are mapped with reference genomes, the resultant SNPs so observed could either be excitatory or inhibitory, or may have combined functioning. This perhaps is the reason why a particular kind of genetic diet so provided to an individual remains unsuitable for others despite having the same physique but the person is different genetically. This is possibly the precise reason that these SNPs decide the diet/edible oils in diet as PUFA and MUFA. These oils may be fruitful in one and unfruitful in others having same genomic nucleotide. Therefore, the suitability of the diet and the fruitful result could only be apparent after having genomic mapping of DNA-based chart for detecting SNPs. In the present study, we utilised the codon charts for mapping various DNA factors which have shown the presence of various combination of nucleotides in the gene/SNPs.

The study has analysed the nucleotides in various genes observed while preparing the diet charts of group A and B subjects. The combination of various nucleotide codon, contribute towards translation process, hydrophobic and hydrophilic region affinity, and the activation of base pairs, to normalise the pKa values towards normalcy. This normalised pKa value helps in proper functioning and cell metabolism of the obese person.

Different genes in the study as named are the sequence of nucleotides in DNA/RNA that encodes the different synthesis of gene products.

Our study has provided results that indicated statistically significant weight reduction in group A at the end of 6 months, as compared to group B.

The observations that the SNPs which are having appropriate functioning are based on the mapping results. The genetic diet provided on this basis, has given better results in the category of plan A (genetic diet subjects)

than that of conventional diet, wherein also DNA-based profiling studies were undertaken.

Further, values observed for the reduction in weight in group A and group B at 3 months were statistically not significant. However, the reduction in weight at the end of 6 months is found statistically significant (Table IV). While validating the results with SNPs, one may draw the conclusion for results of group A and B noticed at 3 months that satisfactory outcome is evident in either groups, but is statistically insignificant. The satisfactory outcome noticed in group B, the SNPs, were still active and partly dormant. Those which were dormant did not activate for functioning in group B. We find the results noticed were significant statistically at the end of 6 months for genetic diet (Group A). Herein, the observation (Group A) signified that those SNPs functioning as dormant or were inactive at the end of 3 months might have resumed the activity towards normality at the end of 6 months, with statistically significant results. Perhaps, the genetic diet had helped to activate the dormant and the inactive SNPs towards better functioning and outcome at 6 months. Previous workers have done studies with individual SNPs and did not discuss the probable reasoning of reduction in weight with genomic diet. The late response in the reduction of weight beyond 3 months could be based on upgradation and down-gradation phenomenon of individual SNPs, activation of dormant non-functioning SNPs.

Summary

A comparative study was conducted to assess the efficacy of DNA based customised diet and exercise plan for weight management in obese individuals. 30 obese persons were selected and divided into two groups: Plan A (15 individuals following genetic diet) and Plan B(15 individuals following standard diet).

Strict diet charting and exercise plan was followed by the individuals in the study. The subjects were evaluated at 0, 3, and 6 months on the parameters of mean weight of individuals, percentage of weight loss, mean difference of weight loss over 3 and 6 months, comparison of BMI and mean waist circumference and mean difference of waist circumference at 3 and 6 months.

- Weight loss was observed in individuals both in plan A and plan B. However, the difference in the weight loss after 3 months in plan A and plan B was not significant. However, at 6 months observation of plan A showed significant reduction in weight in comparison to

reductions observed in patients with plan B (Table IV).

- The reduction in mean waistline of subjects at the end of 6 months was observed to be statistically significant in subjects in plan A in comparison to that of subjects in plan B (Table V).

- BMI of individuals in plan A and B from baseline, decreased at the end of 3 months. The difference of BMI in plan A and B was not found to be significant at the end of three months. However, the results were statistically significant at the end of 6 months and more reduction was noticed with plan A genetic diet (Table VI).

Conclusion

DNA-based customised diet and exercise plan has helped in losing weight more effectively in comparison to the conventional diet and exercise plan amongst obese subjects. Adding a genetic personalised component to the weight loss programme, improved the motivation and compliance among the subjects.

Future prospects

Further studies can be undertaken via satellite RNA, protein structure analysis in obese persons for determining specific location on chromosome and genetic factor, besides identifying the SNPs.

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ORIGINAL ARTICLE

Study of Pulmonary Function Tests in Diabetic Nephropathy

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Abstract

Background: Type 2 diabetes mellitus is a universal public health problem with complications of diabetes on the eyes, kidneys, and nerves well documented, but studies on lung involvement in diabetes are sparse. This study was done to assess pulmonary function tests in patients with diabetic nephropathy and to know the correlation of pulmonary function with duration of diabetes and assess the prevalence of pulmonary hypertension in diabetic nephropathy.

Methodology: This was a cross-sectional study with 50 diabetic patients without nephropathy, 50 diabetic patients with nephropathy, and 50 healthy subjects without diabetes as the control group. Diabetics were age, sex and BMI matched to the control group. Diabetic nephropathy was diagnosed with the presence of diabetes and 24 hours urine protein excretion > 500 mg. Results of pulmonary function tests by spirometry were compared between groups. Pulmonary artery pressure was assessed by 2D ECHO. SPSS 21.0 version for Windows was used for all the statistical analysis. P value less than 0.05 was considered significant.

Results: Mean FEV 1% was 98.12 (+/-10.06), 75.88 (+/- 14.10) and 57.64 (+/- 13.49), mean FVC% was 86.78 (+/-8.77), 69.82 (+/- 13.88) and 53.02 (+/- 13.41) and mean PEF% was 88.62 (+/- 14.47), 59.40 (+/- 18.59) and 48.96 (+/- 20.94) among healthy subjects with no diabetes, diabetes mellitus group with no nephropathy and diabetic nephropathy group respectively. The difference observed between the groups was statistically significant (p value < 0.001). A restrictive pattern of lung function impairment was observed in diabetic patients which were more pronounced in diabetic nephropathy group.

Mean FEV1% was 71.03 (+/- 13.19), 66.74 (+/- 18.34) and 60.29 (+/- 15.25), mean FVC% was 65.66 (+/- 11.06), 60.79 (+/- 18.00) and 56.38 (+/- 16.55) among the participants having diabetes less than 10 years, 10 to 20 years and > 20 years respectively. The difference in mean FEV1%, FVC% with different duration of diabetes was not statistically significant. PAH was present in 3 patients (6%) in diabetes mellitus group without nephropathy and in 20 patients (40%) in diabetes nephropathy individuals, and the observed difference was statistically significant (p < 0.001).

Conclusion: Pulmonary function tests are impaired in diabetics, showing a restrictive pattern, and impairment is more pronounced in diabetics with nephropathy. There is no statistically significant relationship between the duration of diabetes and the derangement of pulmonary functions. Pulmonary hypertension is more common in the diabetic nephropathy group.

Introduction

Diabetes causes microvascular and macrovascular complications which involve organs such as the retina, kidney, nerve, and cardiovascular system, which makes it a major cause for renal failure, stroke, myocardial infarction, blindness, and amputation of limbs. The lungs, although not a classic organ involved in diabetes, may be affected by chronic hyperglycaemia due to its abundant connective tissue and pulmonary capillary network[1]. Pulmonary and other complications of diabetes share a common microangiopathic background, and lungs can be a target organ of diabetic complication[2].

Pulmonary diabetic microangiopathy usually remains clinically under-recognised as a substantial loss of the micro-vascular bed may not result in dyspnoea or any other clinical symptoms since the lungs have an extensive pulmonary reserve. Pulmonary functions assessment can also be useful in determining the progression of diabetic microangiopathy[3]. The use of spirometry remains a simple, non-invasive diagnostic tool that can provide a warning signal so that patients can take early preventive measures[4].

Derangement of pulmonary functions and pulmonary pressure in diabetics with nephropathy are least studied. Hence, this study was undertaken to study pulmonary functions by spirometry and pulmonary pressure in diabetic nephropathy.

Methodology

This was a cross-sectional observational study done at JSS hospital, Mysuru which included a total of 150 subjects, who were enrolled in our study using purposive sampling methods. Subjects were divided into 3 groups: 50 diabetic

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patients without nephropathy, 50 diabetic patients with overt nephropathy, and 50 healthy non-diabetic individuals. Both males and females with age more than 18 years were included in the study. Individuals with a history of connective tissue disorders or cardiopulmonary problems, history of smoking, end-stage renal disease (GFR < 15 ml/min), and urinary tract infection at the time of urine sample collection were excluded from the study.

Controls were matched according to their age, gender, and body mass index with subjects with diabetes. Diabetics without nephropathy were considered as individuals with a history of diabetes who were taking medications for diabetes and urine (routine test) showing no proteinuria.

Diabetic nephropathy was defined by including all of the following:

i.   History of diabetes on medications.

ii. Urine (routine test) showing proteinuria.

iii. 24-hours urine protein estimation > 500 mg per day.

iv. Fundoscopy showing evidence of diabetic retinopathy.

Pulmonary functions were assessed using CONTEC 10 spirometer at room temperature in a sitting position. Subjects were made to undergo pulmonary function tests 3 times at an interval of 5 mins and the best of the three readings was taken. ERS/ATS guidelines for performing spirometry were followed. FVC, FEV1, and PEF were measured. Values were expressed as a percentage of the predicted values according to Knudson's standard predicted values[5,6]. Pulmonary pressure was measured with 2D ECHO by TR jet and calculating RVSP. Pulmonary hypertension was diagnosed if RVSP > 40 mmhg[7].

Statistical analysis

For categorical/binary variables, we used proportions, and for continuous variables, we used interquartile range (IQR), standard deviation, mean, and median. Inferential statistics were done by using fisher exact test/Chi-square test, one-way ANOVA, independent t-test, and person correlation. SPSS 21.0 version for Windows was used for all the statistical analysis. A p-value less than 0.05 was considered significant. Two or more independent proportions were compared using the Chi-square test/Fisher exact test. Comparing means among independent groups versus mutually exclusive groups was done using an independent t test. One-way ANOVA test compared the difference in means between multiple independent groups.

Results

The mean age of the study participants with no diabetes

group, diabetes group without nephropathy and diabetic nephropathy group were 58.60 (+/- 10.26), 59.80 (+/- 9.53) and 59.96 (+/- 9.76) respectively. In all three groups, the proportion of males and females were the same and males were more compared to females in each group.

Mean BMI was 25.10 (+/- 3.42), 24.82 (+/- 3.61), and 24.92 (+/- 3.58) among the nil diabetes group, diabetics without nephropathy, and diabetics with nephropathy group respectively.
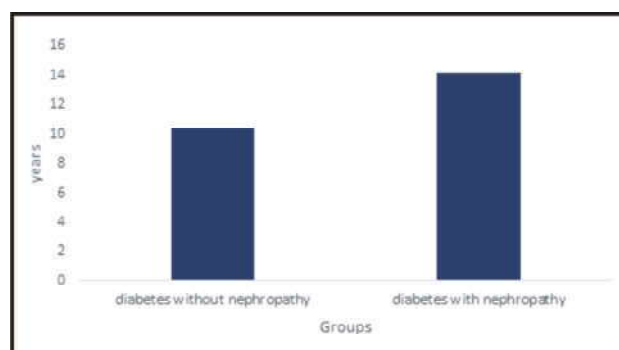
Fig. 1: Mean duration of diabetes among study groups.

The mean duration of diabetes mellitus among the diabetics without nephropathy and diabetic nephropathy group were 10.42 (+/- 5.57) and 14.18 (+/- 6.83) respectively. The mean duration of DM among the diabetic nephropathy group was higher than the diabetic group without nephropathy and the difference observed was statistically significant.

Mean FEV 1% was 98.12 (+/- 10.06), 75.88 (+/- 14.10) and 57.64 (+/- 13.49) among no diabetes, diabetes without nephropathy and diabetic nephropathy groups respectively, and the lowest was seen with diabetic nephropathy and the second lowest among diabetics without nephropathy and the difference observed was statistically significant. Mean FVC% was 86.78 (+/- 8.77), 69.82 (+/- 13.88) and 53.02 (+/- 13.41) with no diabetes, diabetes mellitus with no nephropathy and diabetic nephropathy groups respectively, the difference observed was statistically significant. FEV1/FVC was 91.74 (+/- 2.65), 88.88 (+/- 7.06) and 89.48 (+/- 6.08), the difference observed between nil diabetes group and diabetics with nephropathy, was not significant. The mean PEF% was 88.62 (+/- 14.47), 59.40 (+/- 18.59) and 48.96 (+/- 20.94) among nil diabetes group, diabetes without nephropathy and diabetic nephropathy group respectively. The difference observed among three groups was statistically significant.

As FEV1/FVC was more than 70% and FVC reduced, a restrictive pattern of lung functions was observed in diabetics which was more pronounced in diabetic nephropathy group.

Table I: Lung function parameters among the study groups.

Spirometry variables	Group							P value		
	NDM		DM without nephropathy		DN			NDM	DM without nephropathy	NDM
	Mean	SD	Mean	SD	Mean	SD	PValue	DM without nephropathy	DN	DN
FEV1%	98.12	10.06	75.88	14.10	57.64	13.49	<0.0001	<0.0001	<0.0001	<0.0001
FVC %	86.78	8.77	69.82	13.88	53.02	13.41	<0.0001	<0.0001	<0.0001	<0.0001
FEV1/FVC	91.74	2.65	88.88	7.06	89.48	6.08	0.03	0.035	1	0.135
PEF%	88.62	14.47	59.40	18.59	48.96	20.94	<0.0001	<0.0001	0.014	<0.0001
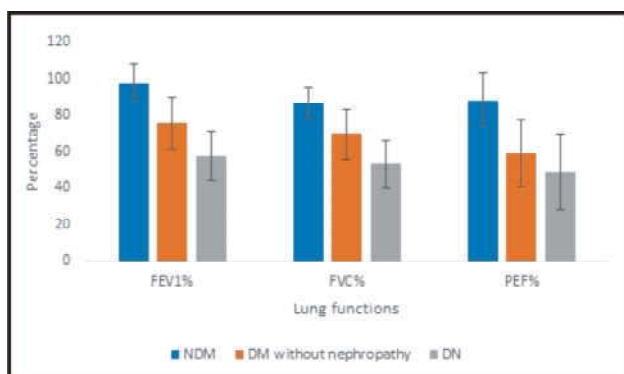
Fig. 1: Lung functions among study groups.

Table II: Correlation between lung function tests and duration of DM

		Duration of DM years
FEV1%	Pearson correlation	- .276
	P value	.060
	N	100
FVC%	Pearson correlation	- .245
	P value	.014
	N	100
FEV1/FVC	Pearson correlation	- .005
	P value	.958
	N	100
PEF%	Pearson correlation	- .216
	P value	.051
	N	100

FEV1% showed negative linear relationship with duration of DM in years: i.e, FEV1% decreases with increase in duration of DM.

FVC% showed a negative linear relationship with duration of DM, i.e., FVC% decreases with increase in duration of DM.

FEV1/FVC showed a negative correlation with duration of DM, i.e., FEV1/FVC decreases with increase in duration of DM.

PEF% showed a negative correlation with duration of DM, i.e., PEF% decreases with increase in duration of DM.

FEV1%, FVC%, FEV1/FVC and PEF% with duration of diabetes was not statistically significant (p value > o.o5).

Table III: Prevalence of PAH among study participants.

		Group					
		No DM		Diabetes Mellitus with no nephropathy		Diabetes Nephropathy	
		Count	Column N%	Count	Column N%	Count	Column N%
PAH	No PAH	48	96.0%	47	94.0%	30	60.0%
	PAH	2	4.0%	3	6.0%	20	40.0%

P< 0.0001

PAH was present in 2 subjects with no diabetes (4%), in 3 patients with diabetes with no nephropathy (6%) and in 20 patients with diabetes nephropathy (40%). The prevalence of PAH was highest among diabetic nephropathy and the difference observed between the groups was significant p < 0.0001 using chi square.

Discussion

Globally, diabetes has reached epidemic proportions. More than eight per cent of the world's population suffers from diabetes, which is estimated to be over 350 million people. By 2,035, the number of diabetics is expected to rise to over 550 million. Diabetics are likely to develop CKD in more than 40% of cases, and many will develop ESRD requiring renal replacement therapy[8].

Loss of recoil of lungs due to elastin and collagen changes, autonomic neuropathy of respiratory muscles, chronic inflammation as well as small vessel changes of lung capillaries can cause pulmonary dysfunction. Lung complication and other complications of diabetes share a common microangiopathic background showing the lung can be a target organ of diabetic complications[2].

Measurement of pulmonary functions by spirometry can be a basic screening test for assessing pulmonary dysfunction in diabetics. Hence, in this study, we have measured pulmonary function tests by spirometry to assess the alteration of PFTs in diabetics with and without nephropathy and looked into its correlation with the duration of diabetes.

Studies done by Swati *et al*, Sonali *et al*, Shafiee *et al* and the Framingham heart study by Robert *et al* showed that FEV1, FVC, and PEF by spirometry were reduced significantly in diabetics when compared to normal individuals[2,3,9,10]. In this study we have observed that, the mean FVC% predicted, mean FEV1% predicted, and mean PEF% predicted were significantly less in individuals with diabetes with and without nephropathy in comparison with controls (P < 0.001). Shafiee *et al* and He *et al* showed that FVC% predicted, FEV1% predicted, PEF% predicted values, when compared to diabetics without nephropathy and healthy controls, diabetic nephropathy patients showed a significantly lower value (P < 0.05)[1,2].

In this study, we observed that pulmonary functions (FVC% predicted, FEV1% predicted, PEF% predicted) were significantly reduced in the diabetics with nephropathy in comparison to diabetics without nephropathy and non-diabetic subjects (p value < 0.001). Shafiee *et al*, He *et al*, and Gilmour 1 *et al* in their respective studies showed a restrictive pattern of lung functions in diabetics with and without nephropathy, changes were more pronounced in the diabetic nephropathy group[1,2,11]. An analysis by Selvaraj *et al* showed a predominant restrictive pattern of lung disease in type 2 diabetics[12]. In this study, we have observed a restrictive pattern of lung functions in diabetics with and without nephropathy patients, significant changes were seen in diabetic nephropathy patients when compared to diabetics without nephropathy and normal healthy individuals (P value < 0.001).

The results of this study showed that type 2 diabetics have a restrictive pattern of lung functions even though they do not seem to have any respiratory symptoms. Spirometry remains a simple and cost-effective and non-invasive diagnostic tool that can be used as an indicator that serves as a warning to patients so they can take early preventive measures.

A study conducted on 106 patients of type 2 diabetes by Mittal *et al* showed that the mean duration of patients with diabetes was 10.18 ± 5.17 years. Diabetes duration and lung functions exhibited a significant negative correlation[3]. A study done by Shah *et al*, who enrolled sixty males with T2 DM and 60 healthy subjects as controls, showed that PFTs were reduced significantly in diabetic patients when compared to healthy subjects. There was no correlation between pulmonary functions and the duration of diabetes[13].

Pande *et al* enrolled 100 type 2 diabetics who came to hospital and 100 healthy individuals from general population. Testing of PFTs revealed a significant decline in PFT parameters in comparison to non-diabetic controls, but a decline in FVC, FEV1, and FEV1% was not significant with diabetes duration[9].

In the present study, it was found that the mean duration of diabetes was 14.18 years among the diabetic nephropathy group and 10.42 years among the diabetics without nephropathy. In this study, it was seen that in diabetics with and without nephropathy, FEV1% predicted, FVC% predicted and PEF% predicted showed a negative correlation with duration of diabetes. As diabetes duration increased, pulmonary functions were reduced. (r-value was negative). But, the correlation of pulmonary functions FEV1% predicted, FVC% predicted, FEV1/FVC, and PEF% predicted with different duration of diabetes was not statistically significant (p value > 0.05).

Mehta *et al* in their study showed that the prevalence of pulmonary HTN in diabetic nephropathy was around 60% and PH increases as the CKD stage increases[14]. This study revealed that pulmonary hypertension was commonly seen in diabetic nephropathy individuals (40%) when compared to diabetics without nephropathy (6%).

Conclusions

Pulmonary functions (FVC, FEV1 and PEF by % predicted values) were significantly lower in diabetic patients with and without nephropathy when compared to the healthy nondiabetic controls which was statistically significant. Diabetes subjects with nephropathy had much lower FEV1 and FVC values compared to diabetics without nephropathy. Restrictive type of pulmonary functions was seen in diabetics and the changes were more pronounced in the diabetic nephropathy group. There was a negative correlation between pulmonary functions and diabetes duration, but the changes observed was not statistically significant. Pulmonary hypertension was more commonly seen in diabetic nephropathy. Alteration in pulmonary functions was evident proving that lung is an organ involved as a part of micro and macrovascular complications in diabetics.

As lung function impairment was more pronounced in diabetic nephropathy patients compared to diabetic patients without nephropathy, strict glycaemic control may help in preventing the progression to diabetic pulmopathy.

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CSF Lactate: An Equal Diagnostic but a Superior Prognostic Marker Than CSF Cortisol in Acute Bacterial Meningitis

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Abstract

Objective: Meningitis remains a serious clinical problem in developing countries. Delayed diagnosis and treatment result in significant morbidity and mortality. Case fatality can be as high as 25% in bacterial meningitis. Early antibiotic therapy is crucial for improving the outcome of bacterial meningitis. There is a need for a reliable and cost effective method to differentiate among various types of meningitis. There is a need for a test which can distinguish bacterial meningitis from other meningitis during the acute phase of the disease, so as to avoid complications due to delayed treatment. Apart from this, a test which predicts the ultimate prognosis in such type of patients is also needed. Thus, determination of unstimulated endogenous CSF-cortisol and CSF lactate activity may be an early diagnostic and prognostic marker in bacterial meningitis patients and to determine the cut-off level of cortisol and lactate for the diagnosis of bacterial meningitis.

Methods: Adults with clinical findings compatible with meningitis and no prior treatment were studied. Seventy patients with conventional CSF parameters consistent with acute bacterial meningitis were evaluated. CSF lactate and CSF cortisol were recorded.

Results: The peak incidence of bacterial meningitis was seen in the 36 - 40 years age group and the mean Glasgow Coma Scale score on admission was 4.35. The mean cortisol concentration in cerebrospinal fluid (CSF) was 159 (59 to 277) nmol/l and the mean CSF lactate concentration was 8.8 (5.6 to 12.1) mmol/l. Furthermore, CSF lactate levels correlated with Glasgow Coma Scale score (f = 11.635, P = < 0.0001) but not the CSF cortisol levels (f = 2.008, P = 0.104). The CSF cortisol concentration of 50 nmol/l and CSF lactate concentration of 3 mmol/l was found to be the optimal cut-off values for diagnosis of bacterial meningitis.

Conclusion: CSF cortisol levels and CSF lactate levels in patients with bacterial meningitis are highly elevated and may serve as valuable marker in diagnosing bacterial meningitis. Furthermore, CSF Lactate but not CSF cortisol correlate with disease severity.

Key words: Bacterial meningitis, CSF lactate, CSF cortisol, Glasgow coma scale.

Introduction

Meningitis is an inflammation of the leptomeninges and underlying subarachnoid cerebrospinal fluid (CSF). So, bacterial meningitis is a common infectious disease of the CNS in developing countries like India, and also a major global health problem even in the developed world. Its causative organisms mainly Pneumococcus, haemophilius influenza and meningococcus have a worldwide distribution. It represents a serious disease associated with significant morbidity and mortality[1]. Furthermore, long-term sequelae such as hearing loss, palsies, and personality changes affect approximately 40% of survivors[2].

Signs and symptoms, results of routine CSF analysis and radiological finding are often inadequate in making a definitive diagnosis. Gram's stain and AFB stain of CSF are rapid methods of detection of the pathogenicorganism, but lack sensitivity. Similarly, culture of CSF is another method of diagnosis, but it is time consuming. PCR test is a highly sensitive and specific test, but is very costly and not widely available. There is a need for a test which can distinguish bacterial meningitis from other meningitis during the acute phase of the disease, so as to avoid complications due to delayed treatment. Apart from this, a test which predicts the ultimate prognosis in such types of patients is also needed.

In view of all these limitations, determination of CSF-cortisol and CSF-lactate activity may be a valuable marker in diagnosing bacterial meningitis and also determining its prognostic value in these patients. The main purpose of this study is to evaluate utility of CSF cortisol and CSF lactate in bacterial meningitis.

Material and Method

This was a prospective study including adults more than 16 years of age, who presented to the emergency department of the Sarojini Naidu Medical College, Agra during the

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period between Sept 2018 to March 2020 with clinical findings consistent with meningitis, (e.g., fever, headache, vomiting, nuchal rigidity, and impaired consciousness). Blood samples were drawn and a lumbar puncture was performed after initial clinical assessment. Biochemical and cytological examination of CSF samples were performed, including the measurement of leukocyte counts, neutrophil counts, glucose level, protein. Only those patients who were diagnosed as having acute bacterial meningitis on the basis of conventional CSF parameters were included (Fig. 1) in the study. CSF lactate, CSF cortisol and culture for bacterial meningitis was done. In blood samples collected at the same time, serum leukocyte count, serum glucose, and blood culture were done. The exclusion criteria comprised partially treated cases (which included treatment with antibiotics and steroids), traumatic lumbar punctures for CSF collection, meningitis in HIV patients, or those who were on ART.

Once the diagnosis of bacterial meningitis was made, the following data were recorded: demographics (age, gender, weight), total number of patients admitted, medical history, clinical findings, and the results of the tests performed.

Reference values for CSF-cortisol: 8 - 12 nmol/l or,

Normal	–	< 2 µg/dl
Nonbacterial	–	2 - 10 µg/dl
Bacterial[3]	–	> 10 µg/dl

Reference values for CSF-lactate: 0.9 - 3.0 mmol/l.

The clinical outcome and neurological disability of the patients was assessed using GLASGOW OUTCOME SCORE (Table I). The study was approved by the hospital's ethical committee. A written informed consent was obtained from all parents/guardians. SPSS (SPSS Statistics for Windows, Version 20.0, NY, USA) and XLSTAT 2016 (Microsoft® Excel/

Fig. 1: *Flow chart for diagnosing bacterial meningitis in adults. CSF, cerebrospinal fluid; TLC, total leukocyte count.*

XLSTAT© 2016, Addinsoft, Inc., Brooklyn, NY, USA) were used for statistical analysis. The Chi-square test was applied and $p < 0.05$ was taken as statistically significant. The results were expressed as means and standard deviation. Prognostic efficiency of CSF lactate and CSF cortisol was analysed using Chi-square test.

Table I: Glasgow Outcome Score (GOS).

Score	Description
1.	Death
2.	Persistent Vegetative State: Patient exhibits no obvious cortical function.
3.	Severe disability: Conscious but disabled. Patient depends on others for daily support.
4.	Moderate disability: Disabled but independent. Patient is independent as far as daily life. Disabilities include varying degrees of dysphasia, hemiparesis or ataxia as well as intellectual and memory deficits and personality changes.
5.	Good recovery: Resumption of normal activities even though there may be minor neurologic or psychologic deficits.

Results

During the study period, among the clinically suspected meningitis patients, 70 patients were diagnosed as acute bacterial meningitis. The demographics, clinical and CSF parameters are summarised in Table II. The mean CSF lactate in bacterial meningitis study population was 8.81 with a Standard Deviation of 2.3 mmol/l, and the minimum and maximum values were 5.6 and 12.1 mmol/l respectively.

The mean CSF Cortisol level in bacterial meningitis study population was 159.43 nmol/l with a Standard Deviation of 62.12, and the minimum and maximum values were 59.0 and 277.0 nmol/l respectively.

CSF culture was positive in 26 (37.14%) patients. The following bacteria were identified in CSF cultures: *Streptococcus pneumoniae*[11] and *Neisseria Meningitidis*[15]. The mean CSF lactate (Table III) in patients with positive and negative CSF culture were 10.31 ± 1.75 (6.6 - 12.10) and 7.92 ± 2.12 (5.6 - 12.10) mmol/l, respectively ($p < 0.0001$). There was statistically significant difference observed in CSF lactate concentrations between patients with CSF culture positive and negative meningitis.

There was a significant statistical difference in CSF Cortisol levels (Table IV) in culture-positive bacterial meningitis and culture-negative bacterial meningitis. The mean CSF Cortisol was lower in culture-positive bacterial meningitis (140.96 ± 48.38) as compared to culture-negative bacterial meningitis (170.35 ± 62.32) with a p value of 0.0429.

Table II: Demographic, clinical and CSF characteristics of the patients.

Variable	n = 70
Age, years	33.44 (21 - 41)
M/F	49/21
Fever	70 (100%)
Vomiting	37 (52.85%)
Signs of meningeal irritation	70 (100%)
Mental status changes	23 (32.85%)
Convulsions	10 (14.28%)
Focal deficit (Hemiparesis)	3 (4.28%)
6th CN palsy	5 (7.14%)
CSF TLC/mm^3	1713 (705 - 3814)
CSF protein (mg/dl)	440 (230 - 630)
CSF glucose (mg/dl)	30.06 (10.8 - 53.6)
CSF lactate (mmol/l)	8.8 (5.6 - 12.1)
CSF cortisol (nmol/l)	159 (59 - 277)
CSF culture positivity	26 (37.14%)
Glasgow outcome score (mean)	4.35
Outcome (death at day 28; n)	4
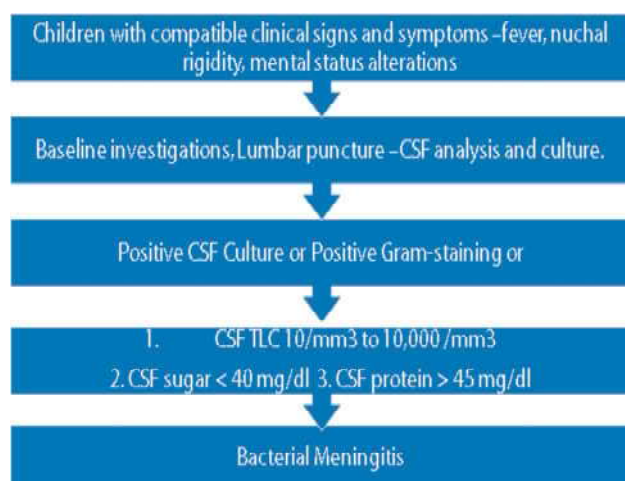
CSF: Cerebrospinal fluid; CN: Cranial nerve; TLC: Total leukocyte count.

Table III: Microbiological corelation of CSF lactate (mmol/l) levels in bacterial meningitis study population.

	CSF lactate (mmol/l)	
	Culture and/PCR positive	Culture and/PCR negative
N	26	44
Mean ± SD	10.31 ± 1.75	7.92 ± 2.12
Mini.	6.6	5.6
Max.	12.10	12.10

t-value = -4.850; p-value = < 0.0001.

Table IV: Microbiological corelation of CSF cortisol levels in bacterial meningitis study population.

	CSF cortisol (nmol/l)	
	Culture and/PCR positive	Culture and/PCR negative
N	26	44
Mean ± SD	140.96 ± 48.38	170.35 ± 62.32
Mini.	59	69
Max.	248	277

t-value = 2.063; p-value = 0.0429.

Table V: Mean levels of CSF lactate (mmol/l) in respective GOS were as follows.

CSF lactate	Glasgow Outcome Score (GOS)				
	1	2	3	4	5
n	4	3	5	10	48
Mean ± SD	11.28 ± 1.40	11.90 ± 0.17	11.50 ± 0.94	10.17 ± 1.36	7.85 ± 1.99
Mini.	9.2	11.7	9.9	7.1	5.6
Max.	12.10	12	12.10	11.7	12.0

f-value = 11.635; p-value = < 0.0001; CD = 0.81.

There was a significant statistical difference in mean CSF lactate levels in different Glasgow outcome score patients. The mean CSF lactate was lower in patients with GOS-5 as compared to patients with GOS-1 with a p value of < 0.0001.

It shows that CSF lactate levels had significant relationship with the prognosis of the patients, levels being less raised in patients with improved outcome and more raised in patients with worse outcome.

Table VI: Mean levels of CSF cortisol (nmol/l) in respective GOS were as follows.

CSF cortisol	Glasgow Outcome Score (GOS)				
	1	2	3	4	5
n	4	3	5	10	48
Mean ± SD	146.25 ± 65.96	223.67 ± 30.02	132.40 ± 71.30	127.10 ± 53.54	166.06 ± 61.20
Mini.	93	189	59	59	73
Max.	238	241	238	202	277

f-value = 2.008; p-value = 0.104.

There was no significant statistical difference in mean CSF cortisol levels in different Glasgow outcome score patients (p value 0.104).

It shows that CSF cortisol levels had no significant relationship with the prognosis of the patients.

Discussion

The neurological outcomes of Bacterial Meningitis (BM) are often poor, making the early diagnosis and treatment important[5]. Nuchal rigidity, fever, and altered mental state are among the most commonly reported signs and symptoms in adults with BM[6], although one or more of these signs and symptoms is commonly absent[7,8]. We reported fever (100%) as the most commonly presenting symptom, followed by headache (74.3%) and vomitting (37%). In the present study, the classical triad (fever, nuchal rigidity, and altered mental status) was observed only in 23% of patients with BM. Van de Beek *et al*, reported that all

the three features were present in only 44% of 696 adults with proven BM[9], but the absence of all three excluded the diagnosis, with a sensitivity of 99%. Berkley *et al*, observed that 50% to 90% of patients with BM reported neck stiffness[10]. Thomas *et al* further concluded that the poor diagnostic value of neck stiffness is not improved by the presence of Kernig's or Brudzinski's signs, because neither has a sensitivity of more than 10%[7]. The present results showed that, overall, clinical history and examination have a low diagnostic accuracy when used alone. This observation is in agreement with the findings of earlier studies in children and adults[11,12]. Therefore, the onus of final diagnosis lies on CSF examination and bacterial isolation through cultures, in a clinically compatible case. Nigrovic *et al*, reported that the combined assessment of history, CSF microscopy, and CSF biochemistry had a sensitivity of 100% and a specificity of 66% in differentiating between BM and VM in children[13]. However, the atypical manifestation of CSF examination, including culture negative and negative Gram-staining, can result in a missed diagnosis of BM. Studies in adults have indicated that adding CSF lactate to routine CSF examination is better in estimating the chance of BM in a very short time[12,14]. The mechanism of the increase in concentration of lactate in the CSF of patients with meningitis is not clear, but it has been linked with anaerobic glycolysis of brain tissue due to a decrease cerebral blood flow and oxygen uptake[15]. In the present study, a statistically significant increase in CSF lactate was observed in patients with BM. A cut-off value of 3 mmol/l for CSF lactate was found to be optimal for diagnosing bacterial meningitis. The cut-off values studied for CSF lactate concentration ranged from 2.1 to 4.44 mmol/l, in different studies in adults and children[14,16]. Although the epidemiology of BM differs by age[17], the diagnostic value of CSF lactate is similar between children and adults[12]. Huy *et al*, in a systemic review on assessment of CSF lactate concentration to distinguish BM from aseptic meningitis, reported a sensitivity ranging from 0.86 to 1.00 (mean: 0.96; 95% CI: 0.95 - 0.98), and a specificity that varied widely from 0.43 to 1.00 (mean: 0.94; 95% CI: 0.93 - 0.96). The mean positive likelihood ratio (LR+) was calculated at 14.53 (95% CI: 8.07 - 26.19), and the mean negative likelihood ratio (LR"), at 0.07 (95% CI: 0.05 - 0.09). Sakushima *et al*, in their systematic review, found that CSF lactate had LR+ of 22.9 (95% CI: 12.6 - 41.9), LR" of 0.07 (95% CI: 0.05 - 0.12), and diagnostic odds ratio of 313 (95% CI: 141 - 698). They concluded that the very low LR" indicated that lack of CSF lactate is particularly good for discarding BM[12]. Moreover, in the present study, there was a significant statistical difference in mean CSF lactate levels in different Glasgow outcome score patients. The mean CSF lactate was lower in patients with GOS-5 as compared to patients with GOS-1 with a p value of < 0.0001. It shows that CSF lactate levels had significant relationship with the prognosis of the patients, levels being less raised in patients with improved outcome and more raised in patients with worse outcome. Since the CSF lactate concentration is neither specific for BM nor for any specific bacteria in patients with BM, the results should always be interpreted in line with clinical findings and the results of conventional assays, including CSF concentrations of protein, cells, and glucose, as well as a microbiological CSF[18]. Furthermore, CSF lactate cannot be used for antibiotic selection, which must be based on the results of microscopic smear examination and/or culture for bacteria.

Studies in adults have indicated that adding CSF cortisol to routine CSF examination is better in estimating the chance of BM early[19]. In the present study, the mean CSF cortisol level in bacterial meningitis study population was 159.43 nmol/l with a Standard Deviation of 62.12, and the minimum and maximum values were 59.0 and 277.0 nmol/l respectively. Manjunath, BV (2015)[3] found out that mean cerebrospinal fluid cortisol activity was 13.06:g/dl, 4.44:g/dl, 2.29:g/dl and 1.05:g/dl in neutrophilic meningitis, lymphocytic meningitis, aseptic meningitis and controls respectively. Mean CSF cortisol level in neutrophilic meningitis was significantly higher as compared to other groups.

In the Holub *et al* study, the mean CSF cortisol was significantly elevated in neutrophilic meningitis (133 nmol/l) compared to aseptic (17 nmmo/l) and controls (10 nmol/l)[19], p value being signigicant (< 0.001).

There was a significant statistical difference in CSF cortisol levels in culture-positive bacterial meningitis and culture-negative bacterial meningitis. The mean CSF cortisol was lower in culture-positive bacterial meningitis (140.96 ± 48.38) as compared to culture-negative bacterial meningitis (170.35 ± 62.32) with a p value of 0.0429. This is in controversy to the study by Holub *et al*, in which it was observed that there was reverse significant difference in CSF cortisol levels in culture positive (162 nmol/l) and culture negative percent (103 nmol/l)[19]. In present study, there was no significant statistical difference in mean CSF cortisol levels in different Glasgow outcome score patients (p value 0.104). It shows that CSF cortisol levels had no significant relationship with the prognosis of the patients.

It can be concluded that a cut-off value of 3 mmol/l for CSF lactate and 50 nmol/l for CSF cortisol, had high diagnostic accuracy for BM but CSF lactate was found to be a better prognostic marker than CSF cortisol as patients with comparatively higher CSF lactate levels had severe neurological disability or died as compared to lower CSF lactate levels. This relationship was not seen in case of CSF cortisol. The present study had a number of limitations. Only a single measurement of lactate and cortisol was

made, upon hospital admission; repeat assessments to monitor treatment and response were not performed. Further, the results were not compared with conventional serum markers and CSF biomarkers (such as CRP).

Conclusion

The present study indicated that for diagnosing BM, the CSF lactate concentration and CSF cortisol concentration are good independent indicators and better markers compared to other conventional markers including CSF glucose, CSF protein, and CSF total number of leukocytes. CSF lactate was found to be better prognostic marker than CSF cortisol in that it correlated with patients having adverse clinical outcome better than CSF cortisol. Cost effectiveness studies should be performed to investigate the economic impact of using this technique as a routine assay in hospital to diagnose BM.

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Profile of Urinary Tract Infections in Catheterised Patients in the Critically Ill Population in a Tertiary Care Hospital

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Abstract

Urinary tract infection (UTI) is commonly seen in women, diabetics, immune-compromised individuals, anatomic abnormalities, and history of instrumentation for surgical or medical conditions. Catheter-associated bacteriuria is commonly encountered in the medical health care system especially with prolonged catheterisation.

Objective: The main objective of the study was to do risk stratification of patients requiring urinary catheterisation, correlate it with the clinical profile and study the microbiological profile of catheterised adult patients with Catheter-associated Urinary tract infections (CAUTI) in a tertiary care hospital.

Material and Method: This was a cross-sectional, hospital based, observational study done at a tertiary care hospital in New Delhi on critically ill patients requiring indwelling urinary catheters for medical or surgical illness.

Results: A total of 200 patients formed the study population, and amongst them 139 were males and 61 were females. CAUTI was diagnosed in patients with indwelling catheters for at least 48 hours who developed symptoms or signs of infection. It was observed that E. Coli was the most common pathogen involved in infection followed by Enterococcus spp, Pseudomonas spp. and Klebsiella pneumoniae. There was very high level of resistance to various broad spectrum antibiotics.

Conclusion: A universal definition of CAUTI needs to be accepted as the present criteria emphasize too much on the presence of symptoms. High-risk patients need to be identified and a guarded approach towards catheterization in critical patients needs to be followed. Hospital policies need to be more stringent towards the catheter insertion and its maintenance.

Introduction

Urinary tract infection (UTI) is an infection that affects part of the urinary tract. When it affects the lower urinary tract (urinary Bladder and urethra), it is known as cystitis and urethritis respectively; and when it affects the upper urinary tract (kidneys and ureters) it is known as pyelonephritis.

Patients at an increased risk for UTI include women, diabetics, immune-compromised patients, those with anatomic abnormalities, impaired mobility, incontinence, advanced age, and a past history of instrumentation[1].

Catheter-associated bacteriuria accounts for almost 40% of all nosocomial infections. It appears to be a result of the widespread use of urinary catheterisation, most of which is inappropriate (in hospitals and long-term care facilities). Early studies , along with recently published reports estimate the incidence of healthcare associated urinary tract infection at around 2 - 3 patients per 100 admissions (anywhere between 15% to 25% of patients admitted to a general hospital have a urethral catheter inserted at one time or another during their stay). Urinary catheter use is more common in acute care and referral hospitals, with approximately 1 in 5 patients admitted receiving an indwelling catheter once during their hospital stay. Bacteriuria and funguria are acquired in up to 25% of patients with indwelling urinary catheters left in place for more than 7 days. Urinary catheterisation for more than 6 days is by far the most important risk factor for acquisition of a urinary tract infection UTI; each day of catheter use is associated with approximately 5% increase in bacteriuria[2]. By one month, nearly all patients with an indwelling catheter will be bacteriuric and/or funguric. Catheter- associated urinary tract infections (CAUTI) remains a leading cause of nosocomial infections with significant morbidity, mortality, and additional healthcare related costs. 80% of UTIs in a hospital setting in the US are estimated to be due to a catheter, leading to a longer length of hospital stay. About 13,000 deaths (mortality rate 2.3%) are attributed to urinary tract infections (UTI) in the US[3].

Other risk factors associated with catheter-associated bacteriuria include a variety of states including female sex, catheter insertion outside the operating room, catheter care violations, rapidly fatal underlying illness, older age, diabetes mellitus, elevated serum creatinine at the time of

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catheterisation and poor general physical conditions as assessed by different scales like SAPS II score (Simplified Acute Physiological Score)[4].

Approximately 15% cases of nosocomial bacteraemia are attributed to the urinary tract, and bacteriuria is the most common source of Gram-negative bacteraemia among hospitalised patients.

Complications of long-term catheterisation (> 30 days), in addition to almost universal bacteriuria, include lower and upper urinary tract Infection, bacteraemia, frequent febrile episodes, catheter obstruction, renal and bladder stone formation associated with urease producing uropathogens, local genitourinary infections, fistula formation, incontinence, and even bladder cancer[5].

Bacteriuria in patients with short-term catheters is usually caused by a single organism[6]. *Escherichia coli* is the most frequent species isolated, although it comprises fewer than one-third of isolates[7]. Other Enterobacteriaceae, such as, Klebsiella species, Serratia species, Citrobacters species, non fermenters such as *Pseudomonas aeruginosa*, and Gram-positive cocci, including coagulase-negative Staphylococci and Enterococcus species, were also isolated.

In contrast to patients with short-term catheterisation, urinary tract infections in patients with long-term catheterisation are usually polymicrobial[8].

Funguria, mostly candiduria, is reported in 3% - 32% of patients catheterised for short periods of time. The recovery of Candida species from urine samples presents the clinician with a dilemma, because the presence of Candida can signify either simple colonisation of lower urinary tract and the catheter which may not need treatment or an upper UTI including both ascending pyelonephritis and renal candidiasis, which requires aggressive treatment[9].

Catheter-associated bacteriuria comprises a large reservoir of antimicrobial-resistant organisms, particularly in critical care units, and can be the source of cross-infection. It is reported that 15% of episodes of hospital-acquired bacteriuria occur in clusters, and these often involve highly antimicrobial-resistant organisms. Widespread use of third-generation Cephalosporins and Fluoroquinolones has created selective advantage for the spread of multiple drug resistant extended-spectrum beta-lactamase producing *E. coli*, *Klebsiella*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Methicillin-resistant *Staphylococcus aureus* among the Intensive care units across the globe[10,11]. There have been reports of Imipenem resistant isolates of *Pseudomonas aeruginosa* isolated from urine samples of catheterised patients of a tertiary care referral and teaching hospital of New Delhi, India[12]. The genotypic presence of an extended-spectrum beta-lactamase (ESBLs) producing and/or metallo-beta-lactamase (MBL) producing organism in severe infections can result in treatment failure even in cases in which the minimal inhibitory concentration (MIC) of the cephalosporin or carbapenem chosen for treatment is in the susceptible range[13,14]. This gives an alert to the Clinical Microbiology Laboratory as there are considerable number of multi-drug resistant Gram-negative isolates, which are not usually screened for ESBL or MBL as a routine.

Various studies done worldwide have shown changing patterns in the aetiology of UTIs[15]. However, studies on CAUTI in India are few[16]. The objective of this prospective cohort study was to record the clinical profile of adults who develop CAUTI in the Intensive Care Unit of a tertiary care hospital, along with the determination of the causative bacteria, and their respective antimicrobial sensitivity and, resistance patterns, which would help in better understanding of the current trends at tertiary care hospitals and assisting in formulation of guidelines for empirical treatment of CAUTI while awaiting the culture sensitivity.

Material and Method

This was a cross-sectional, hospital based, observational study done at tertiary care hospital in New Delhi. The study was approved by the ethical committee of the hospital and was performed after taking written informed bilingual consent from the subjects for the study.

The primary objective was to study the clinico-microbiological profile of catheterised adult patients with catheter-associated Urinary tract infections (CAUTI) and to determine the significant predictors or risk factors associated with catheter-associated urinary tract infections (CAUTI) in the above group of patients.

Previous studies performed for assessing catheter-associated urinary tract infections showed an incidence between 2.45% to 24.7%. Thus an assumption of 14% as the incidence of CAUTI was made and, with an error margin of 5%, a minimum required sample size at 5% level of significance is 180 patients. However, we collected a sample data of 200 patients over a period of 18 months from critical care area of the hospital that includes various intensive care units (ICUs), operation theater (OT) complex, and post-anaesthesia care unit (pre-op). All patients were catheterised by trained staff (Senior resident doctors, Consultants, or Nursing staff trained for the procedure) under aseptic precautions as per the standard operating procedures of Infectious disease control department of the hospital. Infection control guidelines are in strict adherence to IDSA guidelines. All patients having an indication for catheterisation with Foley's catheter over 18 yrs were included. Patients already diagnosed as case of UTI before

catheterisation, baseline urine routine and microscopy examination revealing significant pyuria, condom catheters, suprapubic catheters and percutaneous nephrostomy tubes, Foley's catheter having been removed within 48 hours of catheterisation after procedure, catheterised outside our hospital prior to admission, history of recurrent UTI, anatomical abnormality causing urethral obstruction/ urethral stricture, etc., and not giving consent were excluded from the study.

Data was collected by direct observation, interviewing the treating physicians, and from the patient's medical records. It was recorded for each patient using a structured proforma, which included the following parameters: age, sex, diagnosis at admission, functional status, mental status, indication for catheterisation, place of catheterisation (emergency/ICU), duration of catheterisation, analysis of urine R/M and urine C/S reports. Functional status was classified as either ambulatory or non-ambulatory (subjective assessment of the observer) and mental status classified as alert or impaired (confused, drowsy, stuporous, and comatose). The indications for catheterisation were documented.

Urine specimens were collected from the indwelling catheter by observing all aseptic precautions and transferred and subsequently transported in a sterile, leak-proof container, secured with a lid and sent immediately to the microbiology lab or if in the rare case that immediate transport to the laboratory was not possible, the sample was refrigerated at 2 - 8°C and sent within the next 24 hours.

Specimen after catheter removal from patient (after 48 hours of removal of catheter or after 7 days of catheterisation – whichever is earlier) was a midstream clean catch urine sample.

The following samples were collected under all aseptic conditions using universal precautions and were sent for urine routine and microscopy (R/M):-

1. A baseline sample at the time of catheterisation in order to exclude those with a pre-existing urinary tract infection,

2. Second sample at 48 hrs, and

3. A third sample after 7 days of catheterisation or 48 hrs of catheter removal (whichever was earlier).

Sample for urine culture and sensitivity (C/S) was sent immediately following a positive 2nd or 3rd urine routine/ microscopy report showing significant pyuria ($\geq$ 10 leucocytes/HPF).

Urine routine and microscopy (R/M) examinations were performed using auto-analyser-COBAS A urine culture was performed after inoculating each sample on a CLED agar plate and density of isolates (colony count) was established by counting the number of colonies and expressing the same in terms of Colony Forming Units per ml (CFU/ml) after multiplying with 100. All isolates were identified by their colony characteristics and Gram-stained appearance, and then processed in the VITEK 2 COMPACT automated system (BIOMERIEUX) for identification and antibiotic susceptibility.

Case definition

CAUTI was defined in patients with indwelling urethral catheterisation for a period of at least 48 hours along with presence of symptoms or signs (new onset or worsening of fever, rigors, altered mental status, malaise, or lethargy with no other identified cause; flank pain; costovertebral angle tenderness; acute haematuria; pelvic discomfort; or in those whose catheters have been removed, dysuria, urgent or frequent urination, or suprapubic pain or tenderness) compatible with UTI, with no other identified source of infection along with e"10^3 colony forming units (CFU)/ml of e"1 bacterial species in a single catheter urine specimen or in a midstream voided urine specimen from a patient whose urethral catheter has been removed within the previous 48 hours[17].

Catheter-associated asymptomatic bacteriuria (CAASB) was defined in patients with indwelling urethral catheterisation by the presence of e"10^5 CFU/ml of e"1 bacterial species in a single catheter urine specimen in a patient without symptoms compatible with UTI.

Statistical analysis

The data was analysed, for various categorical variables and was expressed as frequencies and percentages. The various risk factors for catheter-associated UTI (variables) have been compared using Chi-square/Fisher's exact test. Also, Odds ratio has been computed for every such comparison. A p-value < 0.05 is considered statistically significant. Statistical Package for Social Sciences (SPSS) version 15.0 software has been used for data analysis.

Results

A total of 200 patients formed the study population, and amongst them 139 were males and 61 were females. The average age was 57.3 ± SD 17.92 years with range of 19 - 93 years. Most of the patients included in the study were elderly with age more than 60 years (45.5%). The highest numbers of male patients as well as female patients were > 60 years of age (64 patients and 27 patients respectively).

A total of 25 (12.5%) patients in our study developed an episode of catheter-associated urinary tract infection within

7 days of catheterisation. Most of the patients with CAUTI were from the Medical ICU (18 out of 25, i.e., 72.00%). After categorising all the patients, irrespective of their gender, it was seen that the age group of > 60 years contributed the largest share to the burden of CAUTI cases (64.00% of all CAUTI cases) followed by patients in the sixth decade of life (20.00%). Male patients over the age of 60 years contributed to maximum number of CAUTI cases (13 patients, 52%) of the total CAUTI cases in our study. This age group also had the highest rate of CAUTI among all the male catheterised patients of different age groups.

In the females second decade patients had highest rate of CAUTI (50.00%, 1 of the total 2 female patients), followed by females in the sixth decade (16.67%). On an aggregate, males contributed to the maximum burden of CAUTI cases (18 out of 25, i.e., 72.00%), while females contributed to a lesser extent (7 out of 25, i.e., 28.00%). The most common indication for catheterisation in our study was for critically ill patients, (i.e., 127 out of 200). This group also contributed to the maximum burden of CAUTI cases (19 out of 25). While as a preoperative preparation only 4 out of 55 (rate of 07.27%) patients developed CAUTI.

Table I: Indication for catheterisation and occurrence of CAUTI.

Indication for catheterisation	CAUTI Absent		CAUTI Present	
	No. of patients (n)	%	No. of patients (n)	%
Critically ill patients requiring accurate measure of urinary output	108	61.71	19	76.00
Need to measure urine output accurately in an uncooperative patient	4	2.29	0	0.00
Preoperative catheter insertion	51	29.14	4	16.00
Trauma	11	6.29	2	8.00
Patients with neurogenic bladder or retention	1	0.57	0	0.00
TOTAL	175	100	25	100

Association of risk factors in CAUTI

Various risk factors were hypothesised from the review of literature, and were tested for significance in our study. The statistical analyses to test for significance, of the most important risk factors are stated below.

Age: Association of age of the patient as a risk factor for the development of CAUTI within 7 days of catheterisation was evaluated and it was found that 16.67% of catheterised patients e" 60 years of age developed CAUTI within 7 days of catheterisation, compared to 8.65% of patients aged less than 60 tears. Age e" 60 years significantly affects the development of CAUTI (p-value = 0.043) and it was observed that patients 60 years of age or older were 2.1 times more likely to develop CAUTI than those aged less than 60 years.

Gender: It was seen that 18 out of 139 male patients (12.95%) developed CAUTI within 7 days of catheterisation, compared to 11.48% female patients, although the rate of occurrence of CAUTI among both genders was similar, our study did not show female gender as a statistically significant risk factor for the development of CAUTI (p-value = 0.386). It was possibly due to lesser number of female patients in the study.

Duration: Duration of catheterisation was an important risk factor for the development of CAUTI. It was seen that 18 out of 110 patients (16.36%) with duration of catheterisation of 4-7 days developed CAUTI, compared to only 7.78% of patients who were catheterised for only 2 - 3 days. The duration of catheterisation significantly affects the development of CAUTI (p-value = 0.034). Patients with duration of catheterisation of more than 3 days, were 2.3 times more likely to develop CAUTI, than were the patients who were catheterised for less than 3 days.

Diabetes: It was observed that 19.23% of patients with diabetes mellitus as a primary or secondary diagnosis, developed CAUTI within 7 days of catheterisation, compared to 10.14% of patients without diabetes mellitus. "Diabetes mellitus" as a primary or secondary diagnosis significantly affects the development of CAUTI (p- value = 0.044). Patients with documented diabetes mellitus are 2.1 times more likely to develop CAUTI than non-diabetic patients with indwelling urethral catheters.

Creatinine: High serum creatinine level was an independent risk factor for the development of CAUTI within 7 days of the catheterisation. 18.31% of patients with high levels of serum creatinine (e" 1.2 mg/dl) developed CAUTI within 7 days of catheterisation, compared to 09.30% of patients with lower or normal values. The "serum creatinine level" significantly affects the development of CAUTI (p-value = 0.033). Patients with documented high creatinine levels are 2.1 times more likely to develop CAUTI than patients with indwelling urethral catheters having normal creatinine levels.

Mental status: Level of consciousness of patients who were catheterised revealed that 16.84% patients with impaired level of consciousness developed CAUTI within 7 days of catheterisation as compared to 08.57% of alert patients. The "level of consciousness" significantly affects the development of CAUTI (p-value = 0.039). Patients with impaired consciousness are 2.2 times more likely to develop CAUTI than alert patients with indwelling urethral catheter.

Ambulation: As functional status showed that 15.97% of

non-ambulatory patients developed CAUTI within 7 days of catheterisation, compared to 07.41% of ambulatory patients. The "functional status" significantly affects the development of CAUTI (p-value = 0.036) and it showed that non-ambulatory patients were 2.4 times more likely to develop CAUTI than ambulatory patients with indwelling urethral catheters.

Bacterial isolates obtained in CAUTI

A maximum of three urine routine and microscopy samples were collected from each patient, the first one at time of catheterisation, the second one at 48 hours of catheterisation and the third one after a week of catheterisation. Urine culture was sent following report of significant pyuria seen in second or third samples. Many patients were unavailable for the third sample due to catheter withdrawal, death or discharge from the hospital.

After computation of all the bacterial isolates of different CAUTI patients, it was observed that *E. coli* was the most commonly grown isolate (13 out of 25; 52.00%). *Enterococcus spp.* were the second highest contributing organisms (20.00%) followed by *Pseudomonas spp.* (04 out of 25; 16.00%). *Klebsiella pneumoniae* was isolated from 03 cases (12.00%). High levels of resistance to various broad spectrum antimicrobials were seen in case of the Gram-negative and Gram-positive isolates. Overall, between 23 to 39% of the *Escherichia coli* isolates, 67 to 100% of the *Klebsiella pneumoniae* isolates, and 75% of the *Pseudomonas* isolates were resistant to one or more carbapenems tested for susceptibility. The consequence of high rate of fluoroquinolone prescription in the wards and ICUs was reflected in all islolates of *E. coli, Klebsiella,* and *Pseudomonas* (100% each) showing resistance to Ciprofloxacin. The most effective drug against the Gram-negative isolates was Colistin. All the isolates of *E. coli, Klebsiella,* and 03 out of 04 isolates of *Pseudomonas* were susceptible to those two drugs. Two out of five strains (40.00%) of *Enterococcus spp.* were resistant to Vancomycin and Teicoplanin. All the strains were susceptible to Linezolid.

Discussion

Catheter-associated urinary tract infection (CAUTI) remains a leading cause of nosocomial infections with significant morbidity, mortality, and additional healthcare related costs. The increasing drug resistance among nosocomial pathogens, particularly the Gram-negative bacilli, has raised a serious cause of concern in dealing with CAUTI. Holistic approach of good catheter care, hand hygiene of care givers, proper maintenance of perineal hygiene of the patients, adherence to strict antimicrobial prescription policies and proper antimicrobial stewardship by infection control units have become extremely necessary for controlling the emergence of nosocomial outbreaks of CAUTI with multi-drug resistant isolates.

In the Indian context, few studies appear to have been conducted on detailed clinical and microbiological profile of CAUTI. Various studies from India have given information on the resistance patterns of the responsible bacterial isolates[18].

A total of 200 patients with indwelling urethral catheter were included in our study and catheters were placed in intensive care units (ICUs) or the post-anaesthesia care unit (also called the pre-op) under supervised expert care. Paraplegia, cerebrovascular disease and female sex were found to statistically increase the chances of a CAUTI[19]. In our study lesser number of female patients were catherised thus explains lower number of CAUTI in females.

The age of the patients included in the study varied between 18 to 93 years. Most of the patients included in the study were elderly aged ≥ 60 years of age (45.5%). Wide range of variation in observations was evident on reviewing the literature. It was that the incidence was as low as 8.5% to 73.3%, which has been published worldwide for CAUTI, among patients with an indwelling catheter's. Some of the studies have expressed their rates of infection against a denominator of 1000 catheter days. Thus, due to the discrepancy of denominators, it was not possible to compare the rate of CAUTI from all the studies reviewed with our own study.

The wide variation in the observations from various studies could be due to:-

1. Results being collected from various studies reported from developed countries, which exclusively included the patients from the ICUs, the private hospitals and nursing homes, had lower rates than the studies reported from public hospitals of developing countries, including patients from general medical and surgical wards.

2. The different definitions of CAUTI and HAUTI (hospital acquired urinary tract infection) that the various studies have adapted in labeling their cases, also varied to a great extent.

3. Various terms and acronyms like "asymptomatic bacteriuria", "catheter associated urinary tract infection (CAUTI)", "symptomatic catheter associated urinary tract infection (SUTI)", "acute bacteremic catheter-associated urinary tract infection (ABUTI)", "complicated urinary tract infection" etc are used extensively in the literature. Thus studies often failed to clarify them objectively.

Table II: Risk factors: Various risk factors were found in different studies.

Study	Age > 60 yrs	Female sex	Non ambulatory	Impaired mental status	Diabetes mellitus	Ser. creatinine > 1.5 mg/dl
Kamat et al[13]	Significant p < 0.05	insignificant	–	insignificant	–	–
Bhatia et al[20]	Significant p < 0.05	insignificant	Significant p < 0.05	insignificant	–	–
Puri et al[21]	Significant p < 0.05	Significant p < 0.05	–	–	–	–
Danchaivijitir et al[22]	–	Significant p < 0.05	–	–	–	–
Zacharias et al[23]	insignificant	insignificant	Significant p < 0.05	Significant p < 0.05	insignificant	–
Present study	Significant p < 0.05	insignificant	Significant p < 0.05	Significant p < 0.05	Significant p < 0.05	Significant p < 0.05

Almost all the studies, excluding ours experienced a significantly higher rate of CAUTI among female catheterised patients. Kamat *et al* have stated that the incidence of CAUTI was more among the catheterised females patients when compared with their male counterparts[13]. In our study it was not statistically significant (P = 0.652) and we documented higher number of male patients than the female patients (139 versus 61).

All the risk factors hypothesized to affect infection rate in urinary catheterised patients were not uniformly studied in all the reports. Neither could we take into account all the possible risk factors, but there were some statistically significant findings in our study. Patients 60 years of age or older were 2.1 times more likely (p-value = 0.043) to develop CAUTI than those aged less than 60 years.

Duration of catheterisation was an important risk factor and patients with catheterisation of more than 3 days were found to have 2.3 times more incidence of CAUTI (p-value = 0.034) when compared with patients who were catheterised for less than 3 days. Diabetic were 2.1 times more likely (p-value = 0.044) to develop CAUTI than non-diabetic patients with indwelling urethral catheters. Similarly individuals with impaired consciousness had 2.2 times more likelihood (p-value = 0.039) of developing CAUTI. A similar observation was made with non-ambulatory patients who had 2.4 times incidence of CAUTI (p-value = 0.036) in comparison with ambulatory patients. We also observed in our study that patients who had documented high creatinine levels, had higher incidence (up to 2.1 times) of CAUTI (p-value = 0.033). Though, we could not ascertain in our study the exact cause of this it is most likely that these patients already had an element of pyelonephritis or renal compromise secondary to occult lower Urinary tract infection or minimal outflow obstruction. In order to reduce the risk of CAUTI, duration of catheterisation, antibacterial coated catheter and strict infection control measures during catheterisation have been recommended[24]. CAUTI are seen more frequently in patients where insertions have been done under poor aseptic techniques, improper maintenance and socio adaptive factors. Cultural and behavioural changes in hospitals policies go along way in preventing catheter-associated UTI. It was documented that such policies reduced both unadjusted and adjusted analysis of catheter-associated UTI rates per 1000 catheter-days[25]. Provider based studies have shown poor knowledge about ideal aseptic technique based catheterisation. A study showed that the knowledge of doctors was statistically significantly better (P < 0.05) than nurses in identifying the indications for catheterisation in critically ill patients. Similarly in same study only 57% out of all the respondents could identify preventive measures for the development of CAUTI[26].

Conclusion

A universally accepted definition of CAUTI needs to be adhered to while defining a case of CAUTI. The existing criteria emphasizes too much on the presence of symptoms, but with a decree of "no other recognised cause". This approach creates practical confusion in the field level, particularly for two groups of patients especially the ones who are critically ill and unconscious, hence proper elicitation of pain and tenderness becomes impractical and those that may have other non-UTI causes of fever, supra-pubic tenderness or flank pain. Hospitals need to have more stringent catheter insertion and catheter care policies. Stringent policies need to be formulated for insertion of indwelling catheters in health care facilities and they need to be adhered to when treating these patients.

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ORIGINAL ARTICLE

Predicting the Disease Severity and Mortality in COVID-19 Patients based on Disease Characteristics: An Observational Study in Indian Setting

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Abstract

Introduction: The emergence of newer mutated variants of COVID-19 virus has posed a significant challenge. The present study is aimed at investigating the clinical characteristics of COVID-19 and the parameters that may serve as predictors of severity and mortality related to COVID-19 in an Indian setting.

Methods: The observation study was carried-out by using the data of COVID-19 patients admitted between July 2020 to June 2021 at JLN Medical College, Ajmer, Rajasthan, India. The demographic and clinical data of clinically significant parameters were collected. The statistical difference between recovery and death and between patients who required long-term oxygen and those who did not was evaluated for various demographic and clinical variables. Chi-square and Fisher exact test were performed for categorical variables and t-test for continuous variables. Regression analyses were also carried-out for different variables with respect to survival and death, and for oxygen dependency.

Results: Variables namely age, duration of hospital stay, overweight, breathlessness, O_2 mask therapy, BiPAP support, and ventilator usage were found to be significantly different between recovered and expired subjects (P 0.00). The study has noted hypertension (25.06%) and diabetes (23.73%) as the common comorbidities noted in COVID patients, followed by coronary artery disease (2.98%) and asthma. The study has validated the role of oxygen saturation and requirement of oxygen in predicting mortality among COVID-19 patients. The study identified age as a significant predictor of mortality, obesity as a risk factor in COVID-19 patients, gender as a factor influencing the requirement of oxygen, and fever as an independent factor related to oxygen therapy. Bilevel positive airway pressure was given to majority of expired patients (83%) compared to 10% in recovered patients.

Conclusion: Variables namely age, BMI, duration of hospital stay, breathlessness, O_2 mask therapy, BiPAP support, and ventilator usage could be predictive in COVID-19 severity and mortality. The variables to be considered for predicting oxygen dependency are age, urban/rural, gender, duration of hospital stay, weight, height, BMI, fever, cough, breathlessness, diabetes, hypertension, and CAD.

Key words: COVID-19, BMI, diabetes.

Introduction

Corona virus disease (COVID-19) has emerged as a global pandemic causing significant catastrophic effects on world demographics. According to WHO data for 28 October 2021, nearly 244,897,472 confirmed cases including 4,970,435 deaths were reported. The corresponding number of confirmed cases and deaths reported for India were 3,42,31,809 including 4,56,386 deaths[1,2]. Early detection of disease and timely treatment are paramount to control severity and reduce mortality among COVID-19 patients[3]. The illness can be classified as severe in patients with the following clinical signs: SpO_2 < 94% on room air at sea level, ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) < 300 mmHg, respiratory frequency > 30 breaths/min, or lung infiltrates > 50%, and breathlessness. Respiratory failure, septic shock, and/or multiple organ dysfunctions are seen in critically ill patients[4].

Age, duration of fever, interval from illness onset to viral clearance, dyspnoea, lung capacity of patients, pre-existing co-morbidities such as obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, respiratory disease, kidney disease, and malignancy are important factors associated with severity and mortality. Hence, they can be considered as high-risk factors among COVID-19 patients[5,6]. Oxygen saturation and requirement of oxygen support play a vivid role in mortality among COVID-19 patients. A study by Lee *et al*, reported that CRP, neutrophil, lymphocyte count along with age and hypertension status were able to predict risk of supplement oxygen requirement among COVID-19 patients[7]. Several studies have suggested

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various laboratory markers and factors that are found to be beneficial for the early recognition of severe illness. Laboratory markers such as neutrophil-to-lymphocyte ratio, lymphocyte and neutrophil count, platelet volume, albumin, c-reactive protein, ferritin, lactate dehydrogenase level, and red blood cell distribution width have been found to be associated with severity and mortality in COVID-19 patients[8,-12]. However, no significant and authentic data are available to correlate the clinical marker with the disease severity and mortality.

The rural healthcare setting in India is challenging with underdeveloped healthcare services, poor infrastructure, and lack of proper diagnostic facilities[13]. With the emergence of newer mutated variants of COVID virus, identifying the symptoms and factors that are associated with severity in COVID-19 patients is beneficial in predicting the disease severity and referring the patients who are at risk to a higher healthcare centre. The present study is intended to investigate the clinical characteristics of COVID and the parameters that may serve as predictors of severity and mortality related to COVID-19 in an Indian setting.

Material and Methods

This observational study was carried by extracting the data of COVID-19 patients admitted between July 2020 to June 2021 (including 1st and 2nd wave) at JLN Medical College, Ajmer Rajasthan, India. The study included data of adult patients of both the gender, diagnosed with COVID-19 infection by RT-PCR technique and excluded the subjects with missing details or insufficient information. The demographic and clinical data collected were age, gender, clinical symptoms, presence of comorbidities like hypertension, diabetes, COPD/asthma, coronary artery disease and obesity, details of oxygen requirement, duration of hospital stay, and survived or succumbed to death. The statistical difference between recovery and death and between patients who required long-term oxygen and those who did not was evaluated for various demographic and clinical variables. Chi-square and Fisher's exact test were performed for categorical variables and t-test for continuous variables. Regression analyses were also carried-out for different variables with respect to survival and death, and for oxygen dependency by running python code in Jupyter Notebook (6.2.0).

Results

The study recruited a total of 1513 patients and 607 patients were excluded due to missing information. The study considered the data of 906 patients with a mean age of 51.59 years and male-to-female ratio of 1:0.58. Requirement of long-term oxygen was noted for 510

(56.29%) patients. The corresponding proportion of subjects noted with comorbidities hypertension, diabetes, COPD/ asthma and coronary artery disease were 25.06%, 23.73%, 3.09%, and 2.98% respectively. Categorisation according to BMI showed that 25.06% of the patients were overweight and 5.52% were obese. The details of clinical and demographic characteristics and the distribution of comorbidities are briefed in Table I. The distribution of co-morbidities demonstrated that around 61% of patients did not have any associated co-morbidities. Hypertension (25.06%) and diabetes (23.73%) were the most common co-morbidities noted among the COVID patients followed by obesity (5.52%), coronary artery disease (2.98%) and COPD/asthma (3.09%) (Table I).

Table I: Demographic/clinical characteristics and distribution of co-morbidities.

Clinical factors		
Variables		**Mean ± SD***
Age		51.59 ± 18.12
Gender (M/F)		572/334
Urban		626 (69.09%)
Rural		280 (30.91%)
Weight (kg)		65.32 ± 10.33
Height (meter)		1.65 ± 0.09
BMI	Underweight	31 (3.42%)
	Ideal	598 (66.0%)
	Overweight	227 (25.06%)
	Obese	50 (5.52%)
Duration of hospital stay		8.25 ± 4.87
Distribution of co-morbidities		
(n = 906)		
Co-morbidities		n (%)
Diabetic mellitus		215 (23.73%)
Hypertension		227 (25.06%)
COPD/asthma		28 (3.09%)
Coronary artery disease		27 (2.98%)

Mean and standrad deviation for all continues variables. Number and percentage for categorical variables.

Comparison between recovered and expired subjects demonstrated that the incidence of hypertension was more in recovered than in expired patients (P < 0.05), and this may be due to more number of subjects belonging to the recovered category than expired. Whereas, the duration of hospital stay was more in expired patients than recovered (P < 0.05, Table III). Most of the subjects received at least one form of oxygen treatment, and the requirement of

mechanical ventilation was more among expired patients (P < 0.05). Statistically significant difference was noted for the variables namely advanced age, overweight, longer duration of hospital stay, longer O₂ therapy, bilevel positive airway pressure (BiPAP) support, and ventilator usage upon comparison between the 2 groups. Among the co-morbidities, hypertension was found to be statistically significant between the groups (P 0.00, Table II).

Table II: Comparison of the variables for the significance level between recovered and expired subjects.

Predictors (n = 906)		Recovery (n = 801)	Expired (n = 105)	P-value
Age		49.97 ± 17.97	63.94 ± 14.1	< .0001
Urban/rural		569 (232)	57 (48)	0.00
Gender (M/F)		503 (298)	69 (36)	0.56
BMI	Underweight	29 (3.62%)	2 (1.90%)	0.53
	Ideal	545 (68.04%)	53 (50.48%)	0.00
	Overweight	185 (23.10%)	42 (40.0%)	0.00
	Obese	42 (5.24%)	8 (7.62%)	0.44
Duration of hospital stay		8.06 ± 4.8	9.66 ± 5.05	0.00
Fever		606 (75.66%)	95 (90.48%)	0.00
Cough		538 (75.66%)	78 (74.29%)	0.14
Breathlessness		483 (67.17%)	96 (91.43%)	0.00
O₂ mask therapy		401 (50.06%)	96 (91.43%)	0.00
BiPap support		84 (10.49%)	88 (83.81%)	0.00
Ventilator usage		5 (0.62%)	49 (46.67%)	0.00
Diabetic mellitus		185 (23.10%)	30 (3.75%)	0.21
Hypertension		183 (22.85%)	44 (5.49%)	0.00
Coronary artery disease		15 (1.87%)	5 (0.62%)	0.07

Mean and standard deviation for all continuous variables. Number and percentage for categorical variables. ‡Chi-square and Fisher-exact test was used for categorical variables and t-test for continuous variables.
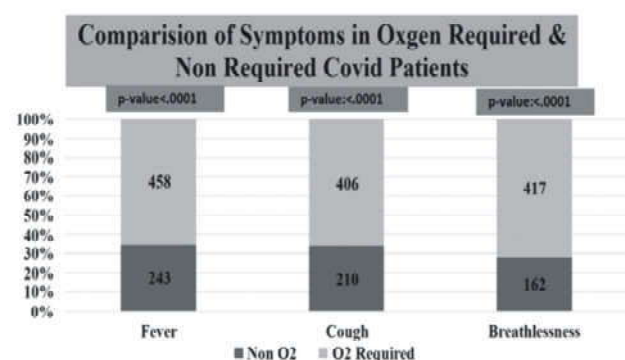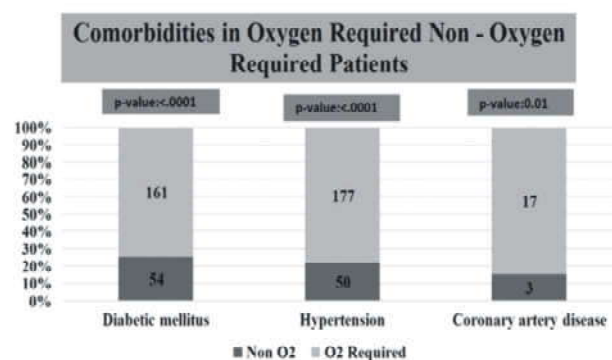

Fig. 1: The above is the graph for comparison of symptoms oxygen required and not required patient.

Fig. 2: The above is the graph for comparison in oxygen required and non-oxygen required patients.

Comparison of variables for oxygen dependency demonstrated statistically significant difference for variables namely, fever, cough, breathlessness, diabetes, hypertension and coronary artery disease (Fig. 1 and 2).

Around 54% of the variance was explained by the model and had higher effect size according to criteria. The model was statistically significant (P 0.00) and the variables that showed the significance difference between survival and death (P < 0.05) were rural/urban, presence or absence of fever, cough, BiPap support, ventilator usage, and duration of hospital stay (Table III).

Table III: Results of regression analysis with respect to survival and death.

	COEF	Std ERR	t	P > |t|	(0.025)	(0.975)
Intercept	0.9277	0.04	23.095	0	0.849	1.006
Gender	-0.0041	0.016	-0.266	0.791	-0.035	0.026
Rural/urban	0.045	0.016	2.823	0.005	0.014	0.076
BMI ideal	0.02	0.026	0.763	0.446	-0.031	0.071
Overweight	0.0059	0.029	0.205	0.837	-0.051	0.062
Fever	0.0069	0.02	0.34	0.734	-0.033	0.047
Breathlessness	-0.0278	0.018	-1.57	0.117	-0.062	0.007
O₂ mask therapy	-0.0233	0.018	-1.294	0.196	-0.059	0.012
Cough	0.0494	0.017	2.849	0.004	0.015	0.083
BiPap support	-0.3159	0.022	-14.113	0	-0.36	-0.272
Ventilator usage	-0.6079	0.034	-17.732	0	-0.675	-0.541
Hypertension	-0.0305	0.018	-1.664	0.096	-0.066	0.005
CAD	-0.0858	0.049	-1.761	0.079	-0.181	0.01
Age	-0.0003	0	-0.669	0.503	-0.001	0.001
Duration of hospital stay	0.0032	0.002	2.049	0.041	0	0.006

Regression analysis based on oxygen dependency showed variance of 32% and higher effect size. The variables that showed statistical difference with reference to oxygen

requirement status were presence or absence of fever, cough, breathlessness and age (Table 4).

Table IV: Results of regression analysis with respect to oxygen dependency.

	COEF	std ERR	t	P > (t)	(0.025)	(0.975)
Intercept	-0.0188	0.02	-0.941	0.347	-0.058	0.02
Gender	-0.0095	0.008	-1.17	0.242	-0.025	0.006
BMI ideal	-0.0308	0.014	-2.236	0.026	-0.058	-0.004
BMI overweight	-0.0147	0.015	-0.978	0.328	-0.044	0.015
Fever	-0.0041	0.011	-0.388	0.698	-0.025	0.017
Breathlessness	0.0106	0.009	1.146	0.252	-0.008	0.029
O_2 mask therapy	0.9336	0.009	101.03	0	0.915	0.952
Cough	0.0157	0.009	1.723	0.085	-0.002	0.034
Hypertension	0.0085	0.01	0.885	0.376	-0.01	0.027
CAD	0.0292	0.026	1.141	0.254	-0.021	0.079
Age	0.0013	0	5.03	0	0.001	0.002
Duration of hospital stay	0.0016	0.001	1.953	0.051	-0.0000077	0.003

Discussion

In the current study, variables – namely age, duration of hospital stay, overweight, breathlessness, O_2 mask therapy, BiPAP support, and ventilator usage – were found to be significantly different between recovered and expired subjects. In concurrence with the current findings, an Uttar Pradesh-based retrospective observational study has reported that advancing age, gender, and duration of stay may influence the COVID-19 outcome. The association between the hospital duration and clinical outcome was found to be statistically significant (P < 0.001)[14].

The study noted hypertension (25.06%) and diabetes (23.73%) as the common co-morbidities noted in COVID-19 patients, followed by coronary artery disease (2.98%) and asthma. Patients with comorbidities are at higher risk for critical illness due to COVID-19. Co-morbidities such as coronary artery disease, diabetes and hypertension are associated with worst outcome in COVID-19[7,15-17]. A meta-analysis by Pititto *et al* has concluded that diabetes, hypertension and cardiovascular disease are important risk factors influencing severity and mortality in COVID and their intensive treatment is paramount while managing the infection[18]. Similarly, another meta-analysis of 40 studies concluded that coronary artery disease (CAD) in COVID-19 patients is linked to poor prognosis[19]. CAD was found to be related to ICU admission (P = 0.002), disease progression (P = 0.003), severe/critical COVID-19 (P < 0.001), and mortality (P < 0.001)[19]. The current study has noted a significant association between the presence of co-

morbidities and oxygen requirement. CAD was present in 3.33% patients with oxygen requirements as opposed to 0.76% patients not requiring oxygen. Similarly, diabetes was noted in 31.57% patients requiring oxygen compared to 13.64% patients without oxygen requirement.

Several studies have validated the role of oxygen saturation and requirement of oxygen in predicting mortality among COVID-19 patients. The present study has also corroborated the same, as most of the patients received at least one form of oxygen treatment, and the need for mechanical ventilation was more among seriously ill expired subjects. A retrospective cohort study among 369 adult patients with COVID-19 admitted to a tertiary care hospital in Lima, Peru has also noted that oxygen saturation values < 90% on admission and age > 60 years were significantly correlated with mortality. The study has highlighted the need for early identification of hypoxaemia and timely hospital care to reduce COVID-related mortality[20].

Studies have reported age as significant predictor of mortality and it is also considered as a major factor while estimating the need for oxygen supplement among COVID-19 hospitalised patients[21,20,7]. In the present study, mean age of fatalities was 64 compared to 50 years for the recovered patients, and 59 vs 42 with regard to the requirement of oxygen. A significant difference in incidence and mortality rate of COVID-19 has been observed between urban and rural population[22,23]. In line with this finding, the current study has noted significant difference between expired and recovered patients with regard to urban/rural status.

Several studies have reported obesity as a risk factor in COVID-19 patients and the association of high BMI with mortality and oxygen requirement during hospitalization in COVID-19 patients[24-27]. The present result has noted that among the expired subjects, 40% were overweight and 7.62% were obese. A meta-analysis involving by Cai *et al*, has concluded that obese patients have higher risk of infection, hospitalisation, disease severity, ICU admission, mechanical ventilation, and COVID-19-associated mortality[28].

Present study has identified gender as a factor influencing the requirement of oxygen. Biole *et al*, reported that requirement of oxygen supplementation was more among men than women. In contrast, Raimondi *et al*, has noted that women required more oxygen nasal cannula than men[29,30]. The incidence of hypertension was found to be more in recovered than in expired patients (P < 0.05). However, this finding is not generalisable, as it could be attributed to more number of recovered patients (801) than expired (105). Mostly tterminally ill patients experience shock and reduced blood pressure, so this finding may not

be clinically useful.

Fever has been identified as an independent factor related to oxygen therapy and high-grade fever is more common among critically ill COVID-19/deceased patients[31,32]. In the current study, fever was common in majority of deceased and patients with oxygen requirement than in recovered and patient without oxygen requirement. Breathlessness was found to be significantly associated with the risk of mortality and considered as independent factor in relation to oxygen therapy[33,31]. In the present study, breathlessness was prevalent among expired and oxygen-required patients compared to deceased patients and patient with oxygen requirements. Cough is considered as most prevalent symptoms in COVID-19 patients[34-36]. Cough is significantly different between patients with or without oxygen requirement. The mean duration of hospital stay is significantly difference between expired and recovered patients and patients with or without oxygen requirement.

Bilevel positive airway pressure is considered in COVID-19 patients with type 2 respiratory failure such as chronic obstructive pulmonary disease[37]. In the present study, bilevel positive airway pressure was provided to majority of expired patients (83%) compared to 10% in recovered patients. Oxygen therapy is significantly associated with mortality in COVID-19 patients[31]. In the present study, the requirement of oxygen mask therapy was more in expired patients (91%) compared to recovered subjects (50.06%). Richardson et al in a study from New York reported that oxygen therapy was required by 27.8% of the patients who were admitted to the hospital. Among the hospitalised subjects, 12% of the patients required mechanical ventilation and 88% of them succumbed to death[38]. In our study, requirement of mechanical ventilation was more among expired patients as compared to recovered patients.

Although several studies have tried to develop statistical prediction models for COVID-19, very few studies have evaluated the data from real-world settings to understand the parameters that may serve as predictors of COVID severity. The present study holds significant relevance, as literature review shows that very few studies from India have evaluated the factors predictive of COVID-19. The major limitations of the current study were observational design and not evaluating the role of biochemical parameters in predicting COVID-19 severity and outcome. Although the study has reported urban/ rural and gender status as factors the requirement of oxygen, it has not studied the effect of each parameter separately such as male and female, and urban and rural.

Conclusion

Variables namely age, BMI, duration of hospital stay, breathlessness, O_2 mask therapy, BiPAP support, and ventilator usage are beneficial in predicting COVID-19 severity and mortality. The variables to be considered for predicting oxygen dependency are age, urban/rural, gender, duration of hospital stay, weight, height, BMI, fever, cough, breathlessness, diabetes, hypertension and CAD. Developing prediction models based on these variables may assist in triaging the patients effectively during hospital admission. However, further studies are needed for the development and verification of such prediction models.

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Urinary Hepcidin Levels in Iron Deficiency Anaemia and Correlation with Severity in Children up to 12 Years of Age: Report from a Tertiary Care Centre

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Introduction

Iron Deficiency Anaemia (IDA) remains the commonest nutritional deficiency in developing countries with the highest prevalence in preschool children[1]. IDA in infants and toddlers may significantly contribute to diminished mental, motor, and behavioural effects[2]. Iron is an essential element for every living organism, as it has a vital role in oxygen transport in the form of haemoglobin, myoglobin and catalysis of oxidation-reduction reactions, involved in energy kinetics[3].

IDA is diagnosed by reduction in levels of haemoglobin, ferritin, transferrin saturation, and mean corpuscular volume (MCV) along with other iron parameters[4]. The three stages in IDA as per increasing severity are described by Al-Mazahi *et al* as stage 1 (deficiency depicted by iron store depletion; decreased serum ferritin levels), stage 2 (impaired erythropoiesis, low transferrin saturation) and stage 3 (impaired red blood cell synthesis and Hb concentration. microcytic anaemia)[5]. Serum ferritin is commonly deployed as an indicator of iron reserves besides acute phase reactant in response to inflammation. The standard clinical and laboratory methods for diagnosis of IDA include clinical symptoms such as pallor, breathlessness, fatigability and laboratory evidences from complete blood count (CBC), iron profile, and serum ferritin test[6].

Hepcidin, a 25 amino acid peptide hormone, synthesized in hepatocytes and excreted through kidney. It facilitates control of iron uptake and movement in the form of ferritin, inhibition of absorption of dietary iron in the duodenum and blocks the release of iron from macrophages and controls the movement of iron stored in enterocytes, hepatocytes and macrophages. Studies suggest the role of hepcidin levels in serum and urine in iron pathophysiology[1]. More advanced understanding of the hepcidin kinetics and iron regulation, its role in conditions of disturbed iron metabolism, refractory and chronic anaemias, haemoglobinopathies and hemolytic anaemia[10].

Hepcidin levels can be measured in plasma, serum and urine. Urinary hepcidin seems more useful than serum hepcidin as three variants (hepcidin 20, 22 and 25) are found in urine in comparison to only 2 in serum (while only Hepcidin 25 and 20) and free of diurnal variation[11,8]. Urinary hepcidin assay provides an indirect measure of the circulating hepcidin level and potential non-invasive means for diagnosing ID, which can be particularly useful in children[11,16]. In absence of sufficient data in Indian children, we planned to evaluate urinary hepcidin and its correlation with serum ferritin and serum iron parameters in diagnosis of IDA in children upto 12 years of age.

Methods

This study was a case-control study conducted in the Departments of Paediatrics and Biochemistry, in a tertiary care centre in northern India over a period of 18 months (Nov. 2018 - March 2020). Informed consent from parents/ guardians and institutional ethical clearance was obtained.

Inclusion criterion: All patients of IDA from age 6 months to 12 years.

Exclusion criterion: Age < 6 month or > 12 years, anaemia due to chronic disorders (tuberculosis, rheumatological disorders, kidney disease), hemolytic anaemias, malignancies, infections (Bacterial, viral, parasitic, fungal), megaloblastic anaemia, iron therapy in previous 3 months, or deranged liver or kidney function tests.

Sample size: Sample size in a previous study on urinary hepcidin was taken as reference. Results of this study concluded AUC of urinary hepcidin - 25 level for predicting stage 1, stage 2, and stage 3 were 0.84, 0.95 and 0.99. Taking these values as reference, ä as 0.06 and 5% level of significance, sample size was calculated as 87. We enrolled total 100 children with 1:1:1:1 ratio in 4 groups as described

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below we took 25 samples per group[23].

Informed consent/assent was obtained from parent or guardian of all children enrolled in the study and assent were taken wherever necessary. Approval from Institutional Ethics Committee was obtained. All enrolled children were divided in 4 groups of 25 children each including three groups of IDA with increasing severity:-

Group 1 (IDA stage 1): Low serum ferritin level (≥ 20 ng/ml) and normal serum transferrin and normal haemoglobin, Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin concentration (MCHC values).

Group 2 (IDA stage 2): Low serum ferritin (<12 ng/ml) and low serum transferrin saturation <16% and normal haemoglobin, MCV and MCHC values.

Group 3 (IDA stage 3): Low serum ferritin (<12 ng/ml) and low transferrin saturation (<16%) and microcytic hypochromic anaemia.

Group 4 (Control group): Healthy children, normal serum ferritin level >20 ng/ml and transferrin saturation >35% and normal haemoglobin, MCV and MCHC values.

After enrollment, clinical and demographic were noted. Samples were collected for estimation of:-

1. Haematological parameters: Around 7 - 10 ml of venous blood sample was collected using aseptic techniques for estimation of haemoglobin with RBC indices and peripheral smear, c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), kidney and lung function tests, serum ferritin, serum iron, transferrin levels, iron saturation, and total iron binding capacity (TIBC) and unsaturated iron-binding capacity (UIBC), serum Vitamin B$_{12}$ and folate levels.

2. Urine analysis: A total of 10 ml of urine sample was collected with standard methods for routine microscopy, urinary creatinine, urinary hepcidin. For urine hepcidin measurement, spot urine samples were taken and centrifuged at 3,000 rpm for 10 min. Supernatant was stored at -20° C till the batch was analysed by ELISA.

Statistical analysis was done using SPSS version 21.0. Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Quantitative variables were compared using ANOVA/ Kruskal Wallis Test (when the data sets were not normally distributed) between the four groups (control, stage I, stage II, and stage III). Qualitative variables were compared using Chi-Square test. Receiver operating characteristic curve was used to find out cut-off point and sensitivity, specificity, NPV and PPV of urinary hepcidin-2 for predicting stage 1, stage 2, and stage 3. Pearson correlation co-efficient/Spearman rank correlation co-efficient was used to correlate urinary hepcidin with various other quantitative parameters. A p value of < 0.05 is considered statistically significant.

Results

A total of one hundred children (6 months to 12 years) were enrolled to include 75 children with IDA and 25 healthy children as a control group. All children suspected clinically of iron deficiency anaemia were investigated. A total 89 sequential blood samples were sent to include consecutive 25 children in 3 groups that is total of 75 children. For the fourth group of 25 controls, a total of 30 samples were sent. The 100 children who were included in 4 groups were further investigated with urine and blood samples.

Baseline clinical and demographic parameters were comparable in all 4 groups. In the study most of the subjects in group 1 and group 4 were 5 - 10 years of age; while group 2 and group 3 were 1 - 5 years of age. The age-wise distribution of children showed 10 infants [group 1 (1), group 2 (3), group 3 (6), group 4 (0)] and most toddlers (n = 37) [(group 1 (5), group 2 (12), group 3 (15), group 4 (5)]. Thirty three children 5 years - 10 years [group 1 (11), group 2 (5), group 3 (4), group 4 (13)] and twenty children > 10 years [group 1 (8), group 2 (5), group 4 (7)]. Mean age of controls was 8.40 ± 2.97 years while in stage 1, stage 2 and stage 3 ID subjects mean age was 8.04 ± 3.65 years, 5.31 ± 4.00 years and 2.84 ± 2.82 years respectively. No statistically significant difference was observed in gender distribution in different groups (males 52% in group 1, 60% in group 2, 84% in group 3 and 48% in group 4; p > 0.001). Baseline laboratory parameters and for anaemia including mean haemoglobin, ferritin, transferrin, etc., are shown in different groups in Table I. Serum ferritin level in normal healthy control group was 115.80 ± 63.88 ng/ml and in stage 1, stage 2, and stage 3 ID subjects was 17.86 ± 1.68 ng/ml, 10.72 ± 1.34 ng/ml and 10.02 ± 1.51 ng/ml respectively. Serum ferritin, total iron and transferrin saturation levels were significantly lower in all stages of ID as compare to control group. More significant reduction in their level was observed with progression of severity of ID (P < 0.01). No correlation was seen with CRP, serum folate, and serum vitaminB12. While TIBC and UIBC were significantly higher in all stage of ID compare to controls and also with progress of severity of ID, TIBC and UIBC increased significantly.

Table I: Laboratory parameters and urinary hepcidin in iron deficient patients enrolled in study.

Parameter	Group 4 (n = 25)	Group 1 (n = 25)	Group 2 (n = 25)	Group 3 (n = 25)	P value
Mean Age (±SD)	8.40 ± 2.97	8.04 ± 3.65	5.31 ± 4.00	2.84 ± 2.82	< 0.001
Haemoglobin	12.44 ± 0.47	12.01 ± 0.55	11.32 ± 0.84	8.34 ± 0.92	< 0.001
Total leucocyte count (mm^3)	8446.40 ± 2254.48	8825.20 ± 2407.57	8407.6 ± 2650.63	9935.6 ± 2201.37	0.12
Polymorphs (10 ^{3}/uL)	6.15 ± 8.44	5.97 ± 6.56	6.22 ± 9.92	6.12 ± 7.29	0.57
Lymphocytes (10 3/uL)	3.36 ± 10.33	3.62 ± 6.77	3.26 ± 8.66	3.29 ± 8.24	0.26
Eosinophils (10 3/uL)	1.84 ± 1.43	2.12 ± 1.2	3.24 ± 3.56	2.16 ± 0.94	0.22
ESR	9.56 ± 5.40	8.48 ± 5.90	8.48 ± 6.03	10.04 ± 5.60	0.61
MCV (fl)	85.59 ± 6.21	79.74 ± 3.88	75.32 ± 4.20	64.44 ± 9.90	< 0.001
MCH (pg)	29.50 ± 2.35	27.57 ± 2.14	25.52 ± 2.06	20.12 ± 4.87	< 0.001
MCHC	33.78 ± 1.49	32.92 ± 1.41	31.56 ± 1.57	29.31 ± 2.50	< 0.001
PCV (%)	36.94 ± 2.56	36.42 ± 2.00	34.29 ± 2.73	27.27 ± 3.31	< 0.001
RDW (%)	12.28 ± 0.95	12.86 ± 1.02	14.38 ± 1.46	16.77 ± 1.82	< 0.001
Platelet (10^6/mm3)	3.02 ± 0.66	2.88 ± 0.67	3.89 ± 1.82	5.59 ± 1.69	< 0.001
Reticulocyte count (10^9/l)	1.99 ± 0.55	1.71 ± 0.64	2.06 ± 0.90	1.77 ± 0.65	0.32
LDH	219.96 ± 67.91	216.04 ± 59.28	227.12 ± 85.24	235.68 ± 50.88	0.39
S. Ferritin (ng/ml)	115.80 ± 63.88	17.86 ± 1.68	10.72 ± 1.34	10.02 ± 1.51	< 0.001
Total iron (ug/dl)	109.40 ± 43.16	72.40 ± 16.40	43.60 ± 13.33	27.76 ± 8.53	< 0.001
TIBC (μmol/l)	263.68 ± 40.75	287.76 ± 40.42	376.96 ± 90.75	455.12 ± 115.92	< 0.001
UIBC (ug/dl)	163.64 ± 67.39	205.96 ± 66.64	280.32 ± 89.12	359.80 ± 97.81	< 0.001
Transferrin Saturation (%)	50.08 ± 16.05	35.28 ± 12.34	11.98 ± 3.05	8.91 ± 3.77	< 0.001
CRP (mg/dl)	0.44 ± 0.27	0.42 ± 0.28	0.40 ± 0.23	0.54 ± 0.30	0.30
FA (ug/l)	14.36 ± 4.77	16.02 ± 9.39	13.46 ± 6.20	11.58 ± 9.54	0.05
Vitamin B12 (ng/l)	458.28 ±118.48	430.72 ± 158.48	399.84 ± 139.66	353.68 ± 115.88	0.02
Mean Urinary Hepcidin (ng/ml)	114.28 ± 88.94	99.86 ± 145.47	18.31 ± 27.66	16.03 ± 48.78	< 0.001
Median (range) urinary hepcidin (ng/ml)	111.77 (1.26 - 326.18)	34.47 (0 - 449.3)	32.03 (0 - 97.44)	0.06 (0 - 241.48)	< 0.001

Urinary hepcidin level in control group was 114.28 ± 88.94 ng/ml and in stage 1, stage 2 and stage 3 ID subjects was 99.86 ± 145.47 ng/ml, 18.31 ± 27.66 ml and 16.03 ± 48.78 ng/ml respectively. Urinary hepcidin levels were significantly reduced with increasing severity of IDA (P < 0.01). The median (range) urinary hepcidin values (ng/ml) also showed similar declining trends among control group: 111.77 (1.26 - 326.18); and stage 1 IDA: 34.47 (0 - 449.3), stage 2 IDA: 32.03 (0 - 97.44) and stage 3 IDA: 0.06 (0 - 241.48) (P < 0.001). From above, it is observed that urinary hepcidin levels were significantly lower in all stages of ID and from stage one to stage 3 (P < 0.05). Table II shows urinary hepcidin levels showed significant positive correlation with age, weight, haematological parameters (Hb, MCV, MCH, MCHC, PCV, total iron, ferritin level and transferrin saturation (P < 0.01). On the contrary, urinary

levels of hepcidin showed significant negative correlation with platelets (r value = -0.32), TIBC (r value = -0.63) and UIBC (r value = -0.57).

Receiver operating characteristics (ROC) curve was used to detect three cut-off points for urinary hepcidin level to differentiate IDA and its different stages, from healthy children (≥ 69.41 ng/ml, ≥ 16.63 ng/ml and ≥ 13.04 ng/ml). The area under curve (AUC) was 0.66 (p < 0.01), 0.89 (p < 0.001) and 0.91 (p < 0.001) at 3 cut-off points respectively with 95% CI as (0.49 - 0.81), (0.81 - 0.98) and (0.83 - 0.99) respectively. Sensitivity (72%, 72% and 84%), specificity (68%, 96% and 96%), positive predictive value (69.3%, 94.8% and 95.5%) and negative predictive value (70.0%, 84% and 90%) were highest for urine hepcidin value ≥ 13.04 ng/ml.

Table II: Correlation between urinary hepcidin and clinical laboratory parameters (n = 100).

|	Urinary Hepcidin (ng/ml)	
	R value	P value
Age (years)	0.28	0.005
Weight (kg)	0.31	0.002
Haemoglobin (g/dl)	0.55	0.000
MCV (fl)	0.62	0.000
MCH (pg)	0.64	0.000
MCHC (g/dl)	0.49	0.000
PCV (%)	0.46	0.000
Platelet (×10³/mm³)	- 0.33	0.001
Reticulocye count (%)	- 0.04	0.678
Lactate dehydrogenase (U/L)	-0.043	0.668
S. Ferritin (ng/ml)	0.63	0.000
Total iron (µ/dl)	0.66	0.000
TIBC (µ/dl)	-0.64	0.000
UIBC (µ/dl)	-0.57	0.000
Transferrin saturation (%)	0.71	0.000
C-reactive protein (mg/dl)	0.01	0.960
Urinary creatinine (mg/dl)	-0.07	0.451

Discussion

Due to rapid growth and development in young infants and children, the demand of iron exceeds the availability of iron rendering them more vulnerable to the effect of iron deficiency anaemia. For diagnosis of IDA, an ideal screening test should be non-invasive, low cost and with high sensitivity to identify IDA before the onset of clinical symptoms for early intervention and adverse motor, behavioural, and mental complications. Currently, conventional blood haemoglobin and serum ferritin levels are the available invasive tests used worldwide popularly[12]. Serum hepcidin has shown high sensitivity[14] in previous studies in adults and children with iron deficiency anaemia with a positive correlation between serum iron, serum ferritin, MCV and transferrin saturation suggesting it as a useful indicator of iron stores. The potential limitation of the use of serum hepcidin levels for diagnostic tests is diurnal variation[19]. Serum iron levels are significantly high at noon and 8 pm in comparison to that at 8 am. Serum hepcidin levels also show similar diurnal variation[18]. In absence of sufficient data, we planned to investigate urinary hepcidin level as a marker for iron deficiency anaemia in children which will have the advantage of being non-invasive and simple screening method.

The results in our study on urinary hepcidin showed low values in all 3 groups of IDA compared to control group (114.28 ± 88.94 ng/ml). The mean urinary hepcidin level further declines with increasing stages of iron deficiency (stage 1:99.86 ± 145.47 ng/ml, stage 2: 18.31 ± 27.66 ng/ml, stage 3: 16.03 ± 48.78 ng/ml) significantly (P < 0.01). Similar results were observed in previous studies by Sonia et al[13] (urinary hepcidin level: control group (443.92 ± 91.84 ng/ml, stage 1: 362.6 ± 31.36 ng/ml, stage 2: 273.16 ± 33.48 ng/ml and stage 3: 189.12 ± 21.14 ng/ml) and Sanad et al[11], (urinary hepcidin (nmol/mmol Cr): healthy: 2.8 ± 1.3 stage 1: 0.7 ± 0.22, stage 2: 0.3 ± 0.009, stage 3: 0.079 ± 0.009) respectively. Mouhamed et al[15] also showed similar significant decline in urinary hepcidin in IDA (stage I: 0.69 ± 0.16, stage II: 0.29 ± 0.05 and stage III: 0.08 ± 0.00) in comparison to control group (2.88 ± 0.82 nmol/mmol creatinine) (P < 0.001).

With a cut-off point of urinary hepcidin in different stages of IDA (Stage 1: ≥ 69.41 ng/ml, stage 2: ≥ 16.63 ng/ml, stage 3: ≥ 13.04 ng/ml). Area under curve (AUC) was significantly highest in stage 3 (0.91 vs 0.89 vs 0.66; p < 0.001). Sensitivity of these three cut-off points was 72%, 72% and 84% respectively while specificity was 68%, 96% and 96% respectively. Positive predictive value (PPV) of these three cut-off points was 69.3%, 94.8% and 95.5% respectively while negative predictive value (NPV) was 70.0%, 84%, and 90% respectively. Our results were similar to Sonia et al[13] who showed cut-off points for ID stage 1, stage 2, and stage 3 were ≥ 369 ng/ml, ≥ 315 ng/ml, and ≥ 293 ng/ml, with PPV of 80, 96 and 92%, respectively, and NPV of 85, 95, and 95% respectively. The sensitivity (84, 96, and 96%) and specificity (80, 96, and 92%) of these cut-off values were also reported to be high, but was showing trend of reducing levels of hepcidin. Similar results by Sanad et al[11] who reported the cut-off level for stage 1, 2 and 3 ID as ≥ 0.94, ≥ 0.42 and ≥ 0.08 nmol/mmol Cr respectively while the AUC was 0.838, 0.944 and 0.999 respectively (p < 0.001). At these cut-off sensitivity of urinary hepcidin to diagnose ID stage 1, 2, and 3 was 88, 96 and 96% respectively while specificity was 88, 92 and 100% respectively. Mouhamed et al[15] also used receiver operator curve (ROC) to know the best cut-off level of urinary hepcidin to detect stage 1, stage 2 and stage 3 iron deficiency in healthy children and got the cut-off level as ≥ 0.95, ≥ 0.38, ≥ 0.089 respectively. At these cut-off points, the sensitivity, specificity, PPV and NPV was 100% to detect stage 1, stage 2 and stage 3 ID respectively.

Correlation of urinary hepcidin with iron parameters
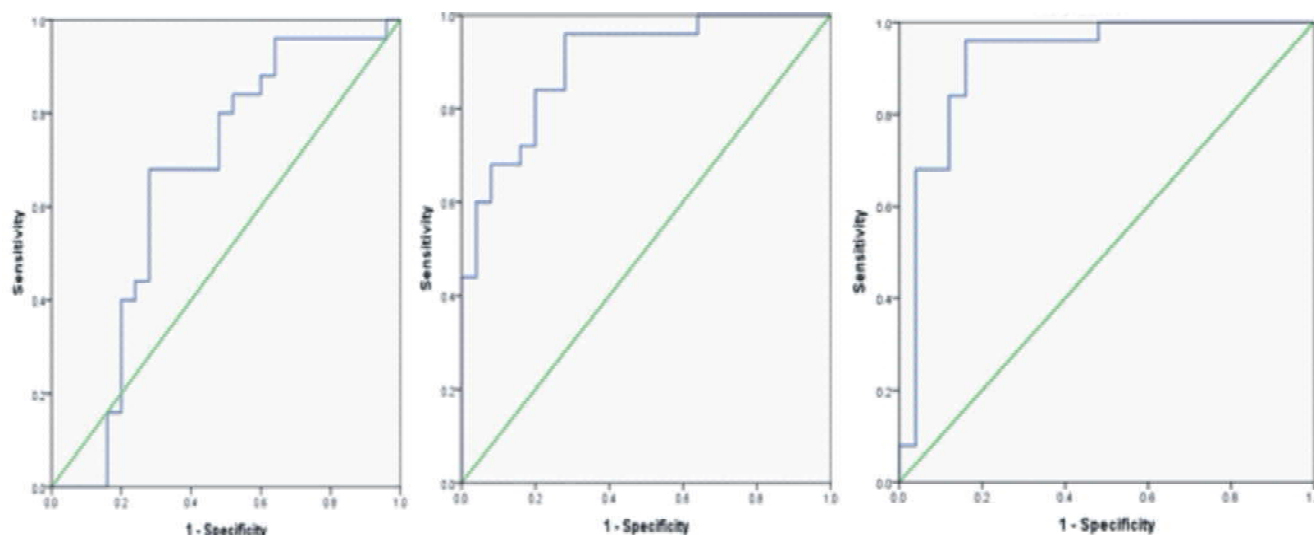
	Stage 1	Stage 2	Stage 3
Area under curve	0.66	0.89	0.91
Standard Error	0.08	0.04	0.04
95% CI	0.49-0.81	0.81-0.98	0.83-0.99
Cut off value of hepcidin (ng/ml)	69.41	16.63	13.04
Sensitivity	72.0%	72.0%	84.0%
Specificity	68.0%	96.0%	96.0%
PPV	69.3%	94.8%	95.5%
NPV	70.8%	77.4%	85.7%
Accuracy	70.0%	84.0%	90.0%

Fig. 1: Predictive values of urinary hepcidin level in detection of iron deficiency in three different stages.

showed significant positive correlation with Hb (r value = 0.55), MCV (r value = 0.610, MCH (r value = 0.64), MCHC (r value = 0.49), PCV (r value = 0.45), serum iron level (r value = 0.65), ferritin level (r value = 0.62) and Tsat (r value = 0.71) (P < 0.01) and significant negatively correlated with platelet count (r value = -0.32), UIBC (r value = -0.57) and total iron binding capacity (r value = -0.63). Similar observations were reported by Sanad *et al*[11] who reported significant positive correlation of urinary levels of hepcidin with Hb, MCV, MCHC, hematocrit value, serum iron level, ferritin level and Tsat (P < 0.01) and significant negative correlation with serum transferrin and TIBC (P < 0.01). Cherian *et al*[16] they also reported positive association of hepcidin with haemoglobin, MCV, serum iron, serum ferritin and transferrin saturation levels and negative correlation between hepcidin and transferrin. Sonia *et al*[13] also observed that the levels of urinary hepcidin were positively associated with MCV, serum iron, haemoglobin, MCV, serum ferritin level and transferrin saturation while urinary hepcidin was negatively correlated with total iron binding capacity.
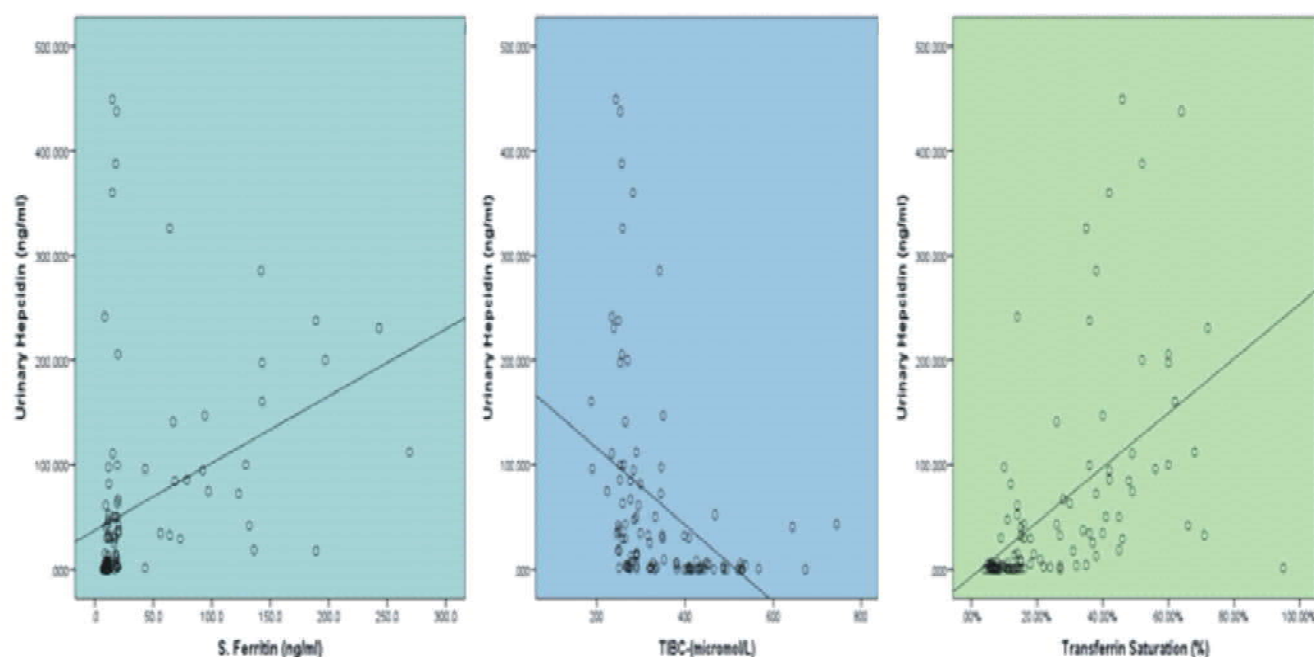
Fig. 2: *Scatterplot showing correlation of urinary hepcidin with serum ferritin, TIBC, and Transferrin saturation.*

Findings of our study was in concordance with Al-Mazahi *et al*[6] and Sonia *et al*[13]. Apart from that, study by Bregman *et al*[17] found positive correlation of hepcidin with ferritin levels. Mouhamed *et al*[15] also found statistically significant correlation between urinary hepcidin level with ferritin level and T. sat (P < 0.01). In contrast, urinary levels of hepcidin showed significant negative correlation with TIBC (P < 0.01).

The strength of our study was a strong study design. Urine specimen is associated with higher pre-analytical variability compared to serum which is a potential limitation of urinary hepcidin assay[19]. Therefore, further evaluation of urine hepcidin as non-invasive monitoring tool of iron status in children is necessary. The small sample size of this study prevents generalisability of results and warrants future studies with larger sample sizes are required to know the cut-off values of urinary hepcidin for diagnosing and differentiating different stages of ID for confirmation.

We conclude that with increasing severity of IDA, along with routine parameters (Hb, MCV, MCH, MCHC, transferrin saturation, total iron) levels of urinary hepcidin also decreased. Urinary hepcidin may be a useful non-invasive, easy, quick, and a low cost, test for mass screening of IDA.

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Monkeypox, a Re-emerging Infection: A Narrative Review

Ashutosh Garg*, Khyati Thapliyal**, Vivek Pal Singh***

Abstract

Monkeypox is a re-emerging infection caused by a zoonotic virus belonging to the Orthopoxvirus genus, same as smallpox and chickenpox. Since its discovery in humans in 1970, there have been outbreaks in African nations, the United States of America and Europe. The latest ongoing global outbreak has been declared a public health emergency. Traditionally, monkeypox has been a benign illness with spontaneous resolution in two to three weeks; however, atypical manifestations have raised concern for new challenges and fatality. No specific antiviral drug or vaccine is in current existence anywhere in the world. Vaccines for smallpox, antiviral drugs against viruses belonging to Orthopoxvirus genus and supportive treatment are the mainstay of therapy in the developed world. In the developing world, only supportive therapy is being employed. Vaccine development is in progress.

Key words: Monkeypox, re-emerging infections, monkeypox vaccine, smallpox vaccine.

Introduction

Humans have encountered infectious diseases since antiquity. But the agricultural age, which brought with it community dwelling and population growth, gave rise to conditions of new and continual microbial development[1]. Human intervention in the erstwhile wildlife has only accelerated these changes. Monkeypox is one amongst the many re-emerging infections. It has now been declared by the World Health Organisation as a global public health emergency on 23rd July, 2022[2]. Although so far a disease with low mortality, it is nevertheless a concern since it jumped out of its endemic zone in central and west Africa. Given the fact that the number of cases is on the rise everyday throughout the world, it is imperative for healthcare workers to apprise themselves of its clinical characteristics, transmission routes, preventive strategies, and methods of management. A brief discussion of other re-emerging infections is presented at the end.

Virology

Monkeypox is a deoxyribonucleic acid (DNA) zoonotic virus endemic to Central and West Africa. It belongs to the genus *Orthopoxvirus* of the *Poxviridae* family. It gets its name from the fact that it was isolated from laboratory monkeys in the 1950s in Denmark. The first human case was discovered in the Democratic Republic of Congo in the 1970s[3]. So far, two strains – Congo clade and West African clade – have been discovered. The current 2022 global outbreak is said to be the latter clade; however, it has been speculated that given its atypical presentation, it may be a new strain.

Transmission routes

Animal to human: Exposure via bites, scratches, body fluids and meat preparation can transmit the virus from animals to humans. Monkeys and humans are incidental hosts while rodents are suspected reservoirs.

Human to human: Amongst humans, it can spread through direct contact via sores, scabs, and body fluids. Soiled linens (fomites), prolonged respiratory exposure and vertical transmission has also been noted. It is not clear if semen and vaginal fluids are spreading agents, however in the 2022 global outbreak, viral DNA was discovered therein.

Clinical characterstics

Incubation period: It is usually 5 to 13 days with the range being 4 to 21 days.

Period of infectiousness: It is from the onset of clinical manifestations to the appearance of scabs and re-epithelialisation (appearance of new skin).

Signs and symptoms: It usually begins with a prodrome of fever, myalgia, severe headache, and lymphadenopathy. The prodrome can last for 5 days. About 1 to 4 days later, the characteristic rash appears which can continue for 2 to 3 weeks, followed by crusting in 1 to 2 weeks (Fig. 1). Rash without a prodrome has also been reported in the 2022 global outbreak. The lesions can range from a few to several thousands. It usually begins on the face and spreads centrifugally. Traditionally the lesions are synchronous but in the 2022 global outbreak, asynchronicity has been observed. Palms and soles, the oral mucosa, conjunctivae,

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and genitalia may also be involved. In severe cases, the lesions may coalesce and skin gets sloughed off. The lesion progresses from a macule, papule, vesicle to a pustule, followed by scab and culminating in the appearance of new skin. The rash is painful until it crusts (scabs), when it starts itching. It is usually a self-limiting infection.

Hospitalisation and case fatality: Hospitalisation rate is low with mortality for the Congo clade being 10% while it is 3 - 6% for the West African clade[4]. Mortality was not observed during the 2003 United States outbreak and in the current 2022 global outbreak, no death has been reported as of July 2022.

Complications: Secondary infections, bronchopneumonia, encephalitis, sepsis and *keratitis* which can lead to loss of vision.

Individuals at risk of severe disease:-

● Children younger than 8 years of age.

● Immunocompromised individuals (HIV-1 infection with

Fig. 1: *Stages of monkeypox rash. From: Antinori A, Mazzotta V, Vita S et al. Epidemiological, clinical and virological characteristics of four cases of monkeypox support transmission through sexual contact, Italy, May 2022. Euro Surveill 2022; 27 (22). (Accessed on July 30, 2022). Reproduced under the terms of the Creative Commons Attribution 4.0 International License.*

clusters of differentiation-4 cell count < 200 per microliters), leukaemia, lymphoma, organ transplantation, ongoing cancer chemotherapy or radiotherapy, high-dose corticosteroids, haematopoietic stem cell transplant < 24 months).

- Exposure to high viral load.
- Presence of complications.

Caveats about the current 2022 global outbreak: In the current outbreak, certain atypical manifestations were observed such as the rash beginning in the genital area, rectal bleeding, rectal pain and tenesmus, asynchronous rash and a possible lack of prodrome.

Definitions

Contact history: A case is considered as indexed when s/he, within 21 days of onset of illness, came in contact with a probable or a confirmed case; or has traveled to an endemic country; or has come in contact with animal sourced from an endemic country.

Suspected case: Characteristic rash plus contact history; or genital ulcer or proctitis not responding to standard treatment for sexually transmitted diseases (STDs).

Probable case: Orthopoxvirus DNA detected by polymerase chain reaction (PCR), immunohistochemistry or electron microscopy; or presence of immunoglobulin M (IgM) antibody against orthopoxvirus within 4 to 56 days after rash onset.

Confirmed case: Monkeypox virus DNA detected by PCR or isolated in a culture.

Clinical exclusion criterion: Alternative diagnosis is satisfactory or there is lack of development of rash 5 days after symptom onset.

Sample collection, precautions and transport

Samples that can be collected: Rash roof, rash fluid, rash base or crust in plain tube, nasopharyngeal swab in viral transport medium, blood in yellow top tube (serology), blood in purple top tube (viral DNA).

Sample collection precautions: Samples must be collected in full personal protective equipment (PPE) comprising full body gown, N95 mask, face-shield, and gloves.

Sample transport instructions: Please refer to Ministry of Health and Family Welfare guidelines for detailed instructions[5].

Testing laboratory: As of the writing of this article, all samples are being sent to the National Institute of Virology, Pune.

Diagnosis

History: Travel to endemic region(s), past history of chickenpox, vaccination history, contact history including sexual history, pattern of rash development.

Diagnostic algorithm: As explained in the case definitions above, a two-tier system is in place for confirmation of monkeypox virus. Suspected cases are tested for the genus orthopoxvirus DNA via PCR. A test negative for orthopoxvirus DNA rules-out monkeypox virus. A sample that is positive for orthopoxvirus DNA (now labeled as a probable case) is tested for monkeypox virus DNA. If monkeypox virus DNA is found to be positive, it is now labeled as a confirmed case. IgM antibodies may also be tested in the blood specimen from 4 to 56 days after rash onset.

Differential diagnosis

It is important to be aware of common differentials that can be confused with monkeypox:-

1. Varicella (Smallpox):
   - It does not manifest with lymphadenopathy and has a characteristically asynchronous rash.

2. Herpes simplex:
   - Ideally only PCR can distinguish between the two viruses.

3. Herpes zoster:
   - Dermatomal distribution (widespread, if disseminated infection).

4. Smallpox (vaccine-associated).

5. Secondary syphilis, lymphogranuloma venereum, chancroid:
   - Since a portion of the individuals in the current 2022 global outbreak of monkeypox are those with sexual contact history, STDs should be kept in mind.

6. Hand-foot-and-mouth disease.

7. Measles.

8. Molluscum contagiosum.

9. Infectious mononucleosis.

10. Non-infectious aetiologies.

Treatment

Asymptomatic individuals with contact history: It can

take up to 21 days for symptoms to develop. Healthcare workers are advised to continue working, while keeping a lookout for the development of symptoms. Government guidelines for the general public are awaited.

Treatment at home: Patients who are haemodynamically stable, those who are not at risk of severe disease, non-pregnant females, and those without complications can be treated at home with supportive treatment as described below.

Criteria for hospitalisation:

- Severe disease.

- Individuals at risk of severe disease (see under Clinical Characteristics).

- Younger than 8 years.

- Pregnant or breastfeeding.

- Presence of complications.

- Immunocompromised individuals.

Patient isolation: Patient must be isolated, must wear a mask, observe cough etiquette, and cover all skin lesions.

Virus containment measures:

- Aerosol generating procedures in hospital must be done in PPE.

- Linen of patients must be handled with minimal shaking and ruffling.

- Standard detergent and water can be used for laundry.

- Use hand-sanitiser or soap and water for handwashing.

Supportive treatment

Skin rash: Patient must not scratch; local antiseptic and emollients can be used.

Genital lesions and proctitis: Sitz bath may be used.

Oral ulcers: Warm saline gargles may be used.

Fever, itching, dehydration, nausea, vomiting: Antipyretics, anti-histamines, antiemetics and fluids.

Antiviral drugs: None of the antiviral drugs are approved by the Centres for Disease Control and Prevention (CDC), United States of America (USA) specifically for the treatment of monkeypox (as of July 2022)[6]. However, certain drugs have been shown to have activity against monkeypox. Tecovirimat and cidofovir can be specially procured from the CDC for use against monkeypox (none of these are currently available in India).

1. Tecovirimat: it is an antiviral against viruses belonging to Orthopoxvirus genus. It targets F13 protein (for viral envelope), thus inhibiting the virus from developing an envelope and from exiting the host cell[7]. It is currently only available through special procurement from CDC, USA. Both intravenous and oral preparations exist. It needs to be given for a duration of 14 days. No major adverse effects have been reported. Oral drugs are associated with headache, nausea, and abdominal pain.

2. Cidofovir/brincidofovir: Cidofovir has shown *in-vitro* activity against smallpox, monkeypox, and cowpox[8]. Human studies are lacking. Brincidofovir is an oral prodrug of cidofovir. In a United Kingdom study on seven human monkeypox patients, tecovirimat/brincidofovir was used[9]. Three patients were treated with brincidofovir, all of whom manifested transaminitis. One patient who was given tecovirimat did not develop any adverse reaction and had a shorter hospital stay.

Monitoring for complications: Patients must be closely monitored for development of complications by paying attention to the following symptoms and signs:-

- Recurrence of fever after it has subsided previously.

- Foul smelling pus from lesions.

- Cough, shortness of breath, chest pain.

- Altered sensorium, seizure.

- Blurred vision, eye pain.

- Bleeding.

Preventive measures

Personal protection protocols:

- Facemask.

- Cough etiquette.

- Avoid crowded places.

- Avoid unprotected contact with wild animals, dead or alive.

- A suspected animal with contact history must be isolated for 30 days[10].

- Avoid sexual contact with multiple partners or with partner(s) with relevant contact history.

- Exchange contact details with sexual partner for retrospective contact tracing.

- Use condom during sexual intercourse.

- Do not touch lesions of patients with bare hands.

- Isolate self in case of doubtful symptoms.

Vaccination: No vaccine specific for monkeypox is available as of now. Vaccines used for smallpox are being

procured and stockpiled. NIV, Pune successfully isolated the virus in end-July 2022 and with the help of the Indian Council of Medical Research (ICMR), New Delhi has sought assistance of pharmaceutical companies to develop vaccines.

Post-exposure prophylaxis (PEP): As of now, there are two vaccines which may potentially reduce the risk of developing monkeypox post-exposure[11].

1. **Modified vaccinia Ankara (MVA):** It is a non-replicating, attenuated vaccinia (smallpox) virus vaccine which can provide cross-protection against monkeypox (both vaccinia and monkeypox viruses belong to the Orthopoxvirus genus). It is being manufactured as JYNNEOS in the United States and IMVANEX in the European Union. It can be administered to immunocompromised individuals. Two doses are supposed to be given subcutaneously four weeks apart.

2. **ACAM2000:** It is replication-capable vaccinia virus vaccine. It cannot be administered to immunocompromised individuals.

3. **Vaccinia immune globulin:** Immune globulin against vaccinia may be given to immunosuppressed individuals as PEP.

Emerging and re-emerging infections

Emerging infections (EIs) are those that have either never happened in humans or have occurred only in an isolated population. While re-emerging infections (REIs) are those that at one point of time were a major concern in a geographical area or globally; they declined and then reappeared as outbreaks[12]. Most EIs and REIs can be traced back to have originated from animals. Population expansion, urbanisation, globalisation, wildlife interference, among others are the reasons why viruses and bacteria which were previously confined to their ecological niches spilled over to exotic locations. Hosts – both animal and humans – who were never intended to be recipients of these ended up acquiring and transmitting these organisms, which now became pathogens.

In a 2007 article in *Nature*, Wolfe, Dunavan and Diamond enlisted five stages when a pathogen which exclusively infects animals (stage 1) transforms into an exclusive human disease (stage 5). A brief discussion of these stages with examples of pathogens sheds light on how these transformations take place. Not all pathogens reach stage 5.

Stage 1: Microbes exclusively present in animals and not found in humans under natural conditions.

Examples: Plasmodium malaria (most).

Stage 2: Microbes transmitted from animals to humans under natural conditions but not between humans.

Examples: Rabies, anthrax, tularemia, Nipah virus, West Nile virus.

Stage 3: Microbes that can undergo transmission between humans for a few cycles and that eventually die out.

Examples: Ebola virus, Marburg virus, monkeypox virus (historical trend).

Stage 4: Microbes that can undergo prolonged cycles of transmissions between humans.

Examples: Yellow fever, dengue fever, cholera, influenza A, typhus.

Stage 5: Microbes exclusive to humans.

Examples: Falciparum malaria, measles, mumps, rubella, syphilis, smallpox, diphtheria, tuberculosis, typhoid, human immunodeficiency virus.

Discussion of the evolution of these stages is outside the scope of this article. Suffice it to say that microbes at any given stage can travel to higher stages, given commensurate environmental conditions.

Conclusion

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease, monkeypox and other EIs and REIs have taught us a couple of lessons: human intervention inside biological diversity needs to be reigned in; preventive and management technology needs to catch up with mutating microbes, the unsuspecting healthcare workforce throughout the globe needs to be ever more cohesive to pre-empt pathogenic evolution of viruses, bacteria, and parasites. Expedited drugs and vaccine manufacture in the case of SARS-CoV-2 helped humans get back on track. We have already made strides in unveiling monkeypox genesis and progression. The future is likely to bring us similar challenges. In this regard, Ali Zumla (Professor of infectious disease and international health at University College London Medical School) is co-director of Pan-African Network For Rapid Research, Response, Relief and Preparedness for Infectious Disease Epidemics (PANDORA-ID-NET), a cross-continental strategy to deal with EIs and REIs[13].

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Paraganglioma: A Difficult and Threatening Ordeal of Pregnancy

VS Gulwe*, JM Kharche*, UR Malu**, SP Shirale***, ST Gupta**

Abstract

Paraganglioma (PGL) in pregnancy is an extremely rare condition, and its diagnosis is often difficult because the clinical symptoms can mimic those of pre-eclampsi a gestational hypertension, and gestational diabetes. Here we report the case of a 24-year-old female primigravida – known case of hypothyroidism who presented with labile hypertension, resting tachycardia and hyperglycaemia with proteinuria at 27-week gestation. We suspected that she might have gestational diabetes along with hypertension and a catecholamine secreting tumour (CST) as her renal Doppler was suggestive of an extra-adrenal mass at left lumbar region which was confirmed on MRI of abdomen and pelvis, and serum catecholamine levels were found to be significantly increased. She underwent laparoscopic mass removal and the pathology confirmed PGL. When typical paroxysmal hypertension and resting tachycardia is accompanied by headache, palpitation, and sweating during the gestational period, an adrenal or extra-adrenal tumour should be suspected.

Key words: Catecholamine secreting tumours (CST), Paraganglioma (PGL), Pheochromocytoma (PCC), plasma free metanephrin and normetanephrin, preeclampsia.

Introduction

Pre-eclampsia/eclampsia is one of the leading causes of maternal mortality. Worldwide 50,000 maternal deaths occur every year, occurring at a rate of 1.5/1,00,000 live births[1,2]. Pre-eclampsia can be confused with many other clinical diseases including acute fatty liver, cholestasis of pregnancy, catecholamine-secreting tumors like PCC and PGL[3].

PCC/PGL is a rare type of CST that arises from chromaffin tissues in the adrenal gland and rarely seen during pregnancy (Approximately 7 in 1,00,000) and PCC is more common than PGL[4,5]. 90% of pregnant women have PCC or PGL symptoms just before delivery, which may lead to a delay in diagnosis and increased health risks for both the foetus and the mother[6(B2)]. PCC/PGL might be suspected in a patient by observing characteristic manifestation that is 5 H's: Paroxysmal hypertension, Headache, Hyperhidrosis, Hyperglycaemia, and Hypermetabolism[7(G0)].

Case history

A 24-year-old primigravida with 27 weeks of gestation was referred in our hospital with recently detected hypertension [Blood pressure (BP) was 210/110 mm of mercury (Hg)] with hyperglycaemia (Random blood sugar was 140 mg/dl) with proteinuria which was initially misdiagnosed as pre-eclampsia and gestational diabetes.

She gave a history of history of headache, sweating, and intermittent palpitations since the last 2 years which was relieved with medication. She had labile blood pressure (details mentioned in Table I). Due to her persistent symptoms and uncontrolled blood pressure, she was investigated for secondary hypertension in form of a renal Doppler. It was suggestive of a large, well-defined, solid heteroechoic predominantly hypo echoic lesion measuring 62 x 66 x 70 mm (AP x TR x CC) in the left lumbar region anterior to the perirenal fascia suggestive of PCC while other investigation suggestive of proteinuria and hyperglycaemia (only single reading of RBS was 140 mg/gl. All other readings were normal with a normal glycosylated haemoglobin).

Table I:

| Investigations			
24-hours ambulatory blood pressure and pulse rate monitoring reports			
Parameters	Average value	Maximum	Minimum
Systolic blood pressure (mm of Hg)	144	170	123
Diastolic blood pressure (mm of Hg)	103	119	86
Pulse (Beats/minute)	95	118	80

To confirm the diagnosis, her MRI abdomen and pelvis was done without revealing foetus identity, along with plasma free metanephrin and normetanephrin. The MRI report was suggestive of a solid round-to-oval shaped lesion measuring approximately 66 x 68 x 80 mm (AR x TR x CC) with smooth margin noted in retroperitoneum just anterior to the left

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kidney suggestive of neoplastic aetiology (most likely extra-adrenal PGL/PCC Fig. 1, 2, 3) while serum plasma free metanephrin and free normetanephrin values were on the higher side (Table I) so blood parameters and radiological findings were correlated with our diagnosis of PGL.

As the patient was a primigravida, our primary goal was mother and foetus safety with control of blood pressure. Controlling blood pressure was difficult as BP was fluctuating from systolic 210 - 100 mmHg and diastolic was 120 - 80 mmHg. BP was controlled with an alpha-blocker and later on, an addition of a beta-blocker was done. After starting medications, patient was symptomatically better but after 8 days of admission she developed foetal distress in the form of foetoplacetal insufficiency. As the mother's health was deteriorating, termination of pregnancy was planned.
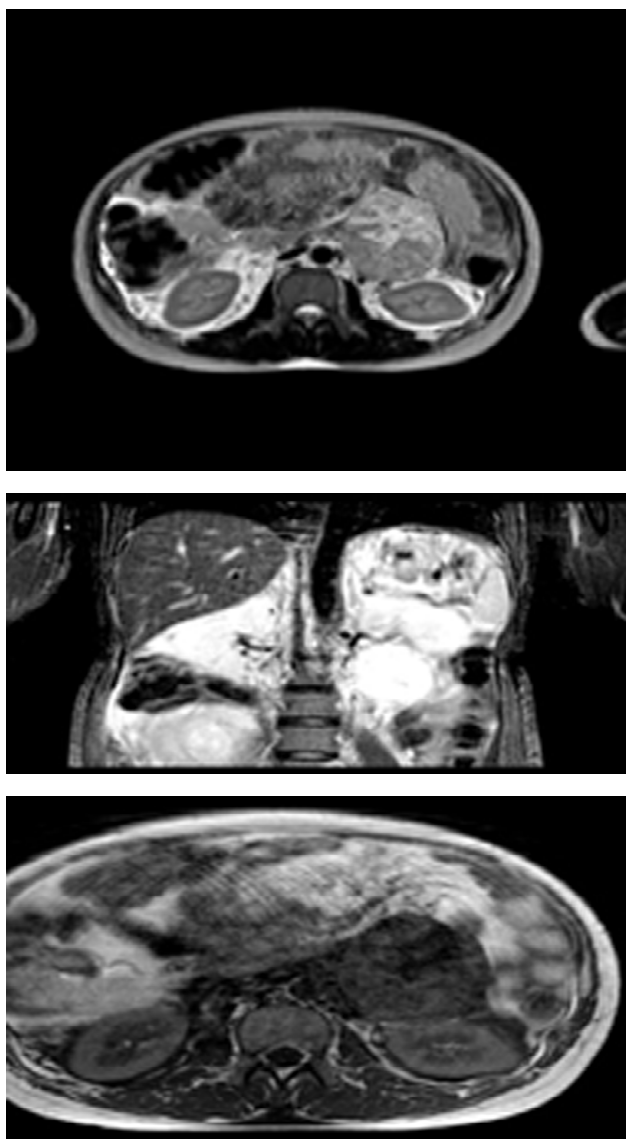
Fig. 1, 2, 3: *MRI abdomen and pelvis.*

We could not revive the foetus which delivered vaginally with the help of magnesium sulphate; but mother's blood pressure was under control with the help of alpha-blocker (Prazosin) and beta-blocker (labetalol) and patient was discharged with medication.

After 1 week of discharge, the patient's reassessment was done. At that time her blood pressure was under control with help of medications, so her DOTA scan was done. It was suggestive of a well-defined mass of 67 x 63 x 80 mm with increased somatostatin receptor expression seen at left lumber region of abdomen (SUV max = 6.6) located at lower pole of left kidney. These findings were suggestive of a neuro-endocrine tumour (NET) – extra adrenal PGL Fig. 4 and 5. After the scan, patient was planned for laparoscopic extra-adrenal mass removal with multidisciplinary approach, mass removed of size 67 x 62 x 27 mm (Fig. 6, 7) and studied histopathologically which was suggestive of PGL(Fig. 8, 9).

It was a multidisciplinary approach; after removal of the mass, the patient was kept in the intensive care unit for 2 days for observation. Post-operatively, patient had one episode of rise in blood pressure (170/100 mm of Hg) along with tachycardia which was we controlled with a beta-blocker and the patient discharged without any medication.

Table II:

Lab. parameters of the patient		
Parameters	Patient values	Normal values
Plasma free metanephrine	380 pg/ml	< 65
Plasma free normetanephrin	7196 pg/ml	< 196
Total proteins	5.7 gm/dl	6.3 - 8.2 gm/dl
TSH	9.8	
Serum cortisol	22.17 ug/ml	
Urine albumin	3+	
24-hour Urinary protein	4839 mg/24 hr	20 - 140

Discussion

According to the 2017 World Health Organisation (WHO) classification, adrenal CST is divided into two categories: intra-adrenal PGL, which is more commonly referred as PCC and extra-adrenal PGL. Because the two tumour types cannot be distinguished based on histological characteristics, anatomical location is utilised to separate them[8,9].

Extra-adrenal PGL can develop from either the sympathetic or parasympathetic paraganglia chain. Generally sympathetic PGL is a catecholamine secreting functional tumour and primarily seen in the abdominal and thorax
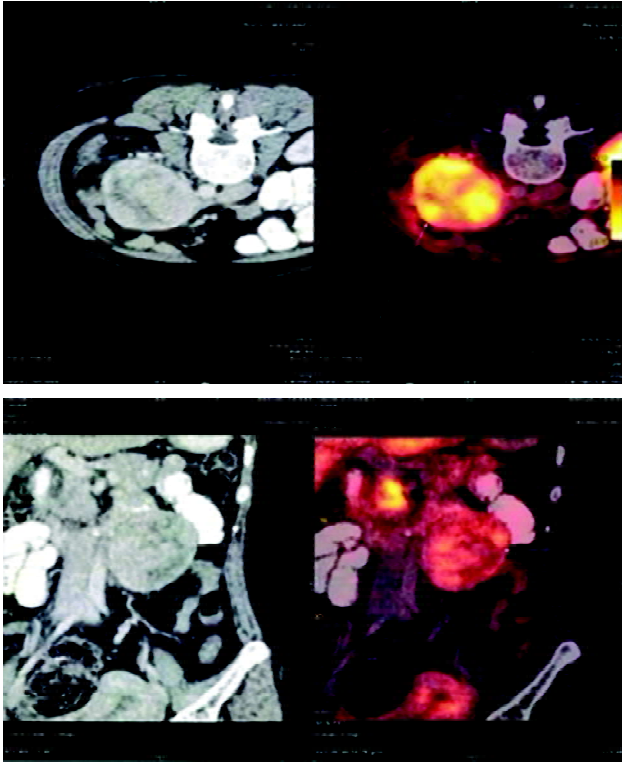
Fig. 4, 5: *Gallium-68 DOTANOC PET SCAN.*
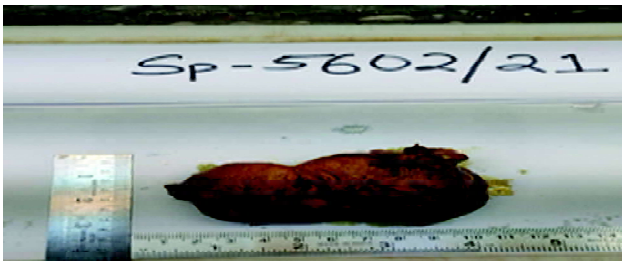
Fig. 6, 7: *Gross picture of Paraganglioma.*

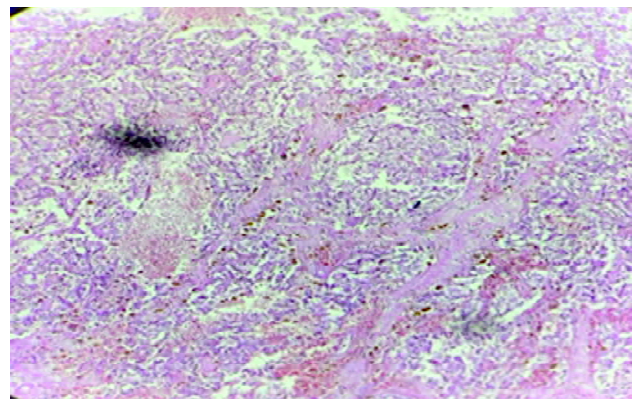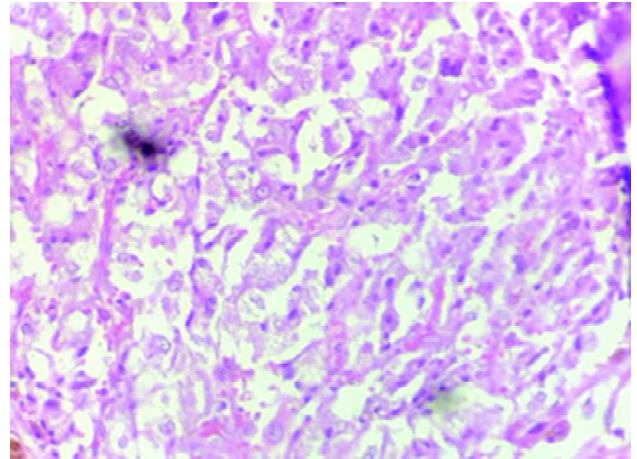Fig. 8, 9: *Histopathological examination of tumour.*

area[10]. While PGL deriving from the parasympathetic chain are catecholamine non-secreting tumours and located in neck and skull base[11]. In this case report we detected PGL in the abdominal area. Many-a-times the diagnosis for CST gets delayed in pregnancy as it is difficult to differentiate signs and symptoms of other common disorders which are observed during pregnancy, i.e., hyperemesis gravidarum, gestational induced hypertension, pre-eclampsia, eclampsia, and gestational diabetes mellitus[12,13].

In this case, the patient presented with confusing clinical symptoms and laboratory findings in which PGL mimics pre-eclampsia or gestational hypertension and gestational diabetes. Severe hypertension along with proteinuria go in favour of severe pre-eclampsia, although resting tachycardia and severe sweating are not typical signs of this disease. Generally, pre-eclampsia occurs in the second trimester after 20 weeks of gestation as compared to CSTs which can show symptoms and signs at any phase of gestation[14]. The fact that this patient only had the first paroxysmal episode of hypertension which was labile in nature associated with resting tachycardia in the third trimester, led us to look for PCC/PGL as a differential diagnosis. However, previously asymptomatic tumour can show symptoms for the first time at a late gestational age due to increased abdominal pressure due to foetal movement, uterine enlargement, uterine contraction, labour, physical and emotional stress, although catecholamines do not cross placenta but utero-placental insufficiency occurs due to paroxysmal reduction and

increment of blood pressure that may lead to intrauterine hypoxia[15].

When diagnosis of CSTs is suspected from clinical history and physical examination, immediate biochemical markers should be performed for confirmation of diagnosis. The essential test for diagnosis of CSTs is confirmation of excessive catecholamine secretions[16]. Most sensitive tests for diagnosis of PCC and PGL are measurement of plasma free metanephrin, normetanephrin or urinary fractionated metanephrin; but evidences suggest that plasma free metanephrin and nor metanephrin are better than urinary parameters for diagnosis of pheochromocytoma[17]. MRI without Gadolinium is the diagnostic imaging test of choice in pregnancy in a suspected case of PCC/PGL as it provides good visualisation of abdomen and pelvis without radiation[18], but a golden test for diagnosis of PCC/PGL is MIBG (Metaidobenzylguanidine) scan but not recommended in pregnancy due to its potential undesirable effect on foetus[19].

Compared to recent nucleotide (DOTA PET, FDOPA PET and FDG PET) scans, MIBG and MRI scan are less sensitive[20-22]. Among nucleotide scan, DOTA PET SCAN is more sensitive compared to others[23].

The management of CST in pregnancy involves blood pressure control and avoidance of labile blood pressure and it requires proper equilibrium between vasodilatation and vasoconstriction to avoid foetal demise. Although surgical removal of tumour is the definitive treatment, medical management also important[24].

Phenoxybenzamine, a non-specific, long-lasting alpha-adrenergic antagonist is the drug of choice even though it crosses the placenta. Fair neonatal outcomes after phenoxybenzamine treatment in pregnancy have been reported[25,26]. Neonatal respiratory distress and hypotension have been documented in some cases whose mothers were treated with phenoxybenzamine. It is therefore suggested that neonates should be monitored after delivery whose mothers were taking pheoxybenzamine for treatment[27]. Maternal tachycardia observed during use of phenoxybenzamine is due to noradrenaline release from presynaptic nerve. While hypotension documented due to its prolonged half-life and irreversible blockade of alpha-adrenoceptors[25]. Alternatives to phenoxybenzamine include other alpha-adrenergic antagonists such as prazosin, and doxazosin. These agents produce less tachycardia with shorter duration of action when compared with phenoxybenzamine, which allow them in dose titration and decreased evidence of post-operative hypotension[15,28]. In our case, the patient's blood pressure was under control on alpha-blockers and beta-blockers before surgery and all antihypertensive drugs were stopped after surgery. Methyldopa which is commonly used for hypertension during pregnancy may worsen the symptoms of CST; hence this drug should be avoided[29].

Traditionally, it has been suggested that vaginal delivery should be avoided in pregnant women with PGL/PCC[30], as there is a high-risk of hypertensive crisis during active labour but some cases are noted in literature of successful vaginal delivery without maternal and foetal mortality[31-35]. Unfortunately, in our case, foetus could not survive which was delivered by vaginally without damaging mother's health, as magnesium sulphate inhibits secretions of catecholamine.

In cases where the CST diagnosis is established during the third trimester, the laparoscopic approach may be difficult due to the enlarged uterus. Therefore, medical treatment is commenced with observation until sufficient foetal maturity is achieved. Delivery is then planned during final trimester, with concurrent or delayed adrenalectomy[5,14]. In our case, the mass was removed via laparoscopic approach after 1 week of delivery. After surgical removal of CST, careful post-surgical vital monitoring is required as the patient may land into hypovolumic shock due to sudden fall in catecholamine levels after removal of CST[36]. But in our case it was managed properly and the patient was discharged without any medication for hypertension.

Conclusion

Although PGL is a rare cause of hypertension in pregnancy, it should be considered in the differential diagnosis in the pregnant female who presents with atypical hypertension and symptoms. A multidisciplinary team approach is important for the management of pregnancy and PGL for better outcome of patient.

Renal Doppler

Grade II medical renal disease with large well defined solid hypo echoic lesion measuring 6.2 x 6.6 x 6.1(APXTRXCC) in left lumbar region anterior to perirenal fascia suggestive of neoplastic aetiology likely extra adrenal PCC.

Section study shows a well circumscribed tumour arranged in lobular and zellballen pattern separated by fibrous septa. Individual tumour cells are round to polygonal with abundant granular cytoplasm, round to oval nucleus with stippled chromatin. Mitoses (approximately 10/10 Hpf) noted. Spindle shaped sustentacular cells with slender nuclei are also seen. There is no capsular/vascular invasion. Area of haemorrhage and congested blood vessels noted.

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Metronidazole-induced Reversible Cerebellar Syndrome in Two Brothers (Neo Postulation for Drug-Cerebellar Toxicity)

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Abstract

Reversible cerebellar syndrome caused by metronidazole observed in two brothers of a family who received the drug for enteritis. The metronidazole toxicity seems not related to cumulative dose or duration related phenomenon. Postulations suggested in literature for the development of cerebellar toxicity are discussed. A newer hypothesis is discussed and described based on analysis carried out on molecular docking, drug metronidazole 3D structure, and its binding affinity with structural gene, potassium voltage gated channel interacting protein (KCNIP4). Visualization is done with the ball and stick model of metronidazole with KCNIP4 gene using CHIMERA software.

Key words: Metronidazole, cerebellar toxicity, molecular docking, KCNIP4 gene, 3D structure.

Introduction

Metronidazole, a bactericidal and anti-protozoal antibiotic is widely used in clinical practice for trichomonal, amoebic infections, hepatic encephalopathy, *H. pylori* infections, *Clostridium difficile*, etc. Though the drug is safe, but peripheral/CNS toxicity has been reported uncommonly irrespective of the dose and duration of its consumption. Most of the adverse effects are reported early in the course, and disappear within weeks of discontinuation of the therapy. Flair MRI T2W scan findings revealing hyperintensities within dentate nuclei of cerebellum are characteristically described with reversible cerebellar syndrome[1]. The proclivity to understand the mechanisms of action of metronidazole-induced neurotoxicity, its adverse effects with cerebellar involvement, described earlier by many workers remains poorly understood[2,3]. Dose and duration of the drug in development of toxicity is also debatable. A newer formulated mechanism is proposed as regards the development of cerebellar toxicity and symptoms reversibility on drug withdrawal. The study encased utilising binding affinity of the drug and features of structural gene KCNIP4 docking with the drug[4,5]. For the purpose CHIMERA software was used.

Case 1

A 24-year-old male patient before presenting to this hospital was hospitalised in a private nursing home for enteritis; he was given tablet metronidazole 400 mg three times daily for 3 days. He was transferred to the present hospital with features of slurred speech, head nodding, ataxic gate,

dysmetria. The cerebellar involvement was more on the right side than left. On examination, he was found to have head nodding, dysarthric speech, unsteady gate, dysmetria and slow horizontal nystagmus. Patient had bilateral involvement with predominant involvement of the right side. Patient was alert and oriented to senses. Vibration sensations were intact. Rhomberg's sign was negative. Blood counts and haemogram were within normal limits. NMO antibodies (IgG and IgM) were negative. MRI findings on bilateral symmetric T2 FLAIR showed hyperintensities in the dentate nuclei of cerebellum and central medulla, features considered typical of drug toxicity (Fig. 2).
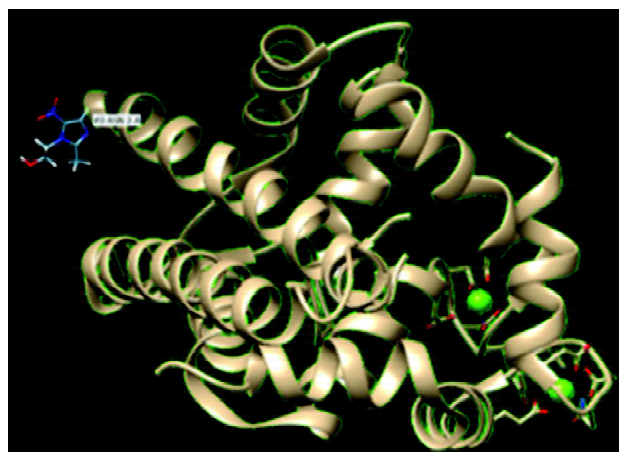
Fig. 1: *The figure shows ball and stick model of drug metronidazole with KCNIP4 gene, visualised with 3D structure and CHIMERA Software. The drug is having bond with leucine and aspartic acid on position 1 and 2 of KCNIP4 gene.*

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Realizing the patient is having metronidazole cerebellar syndrome, the drug was discontinued. Soon after 3 days of discontinuation of therapy, the patient gained his stance. The symptoms had gradually improved. Patient was completely alright after 15 days. Second MRI was not considered as patient had a near complete improvement.
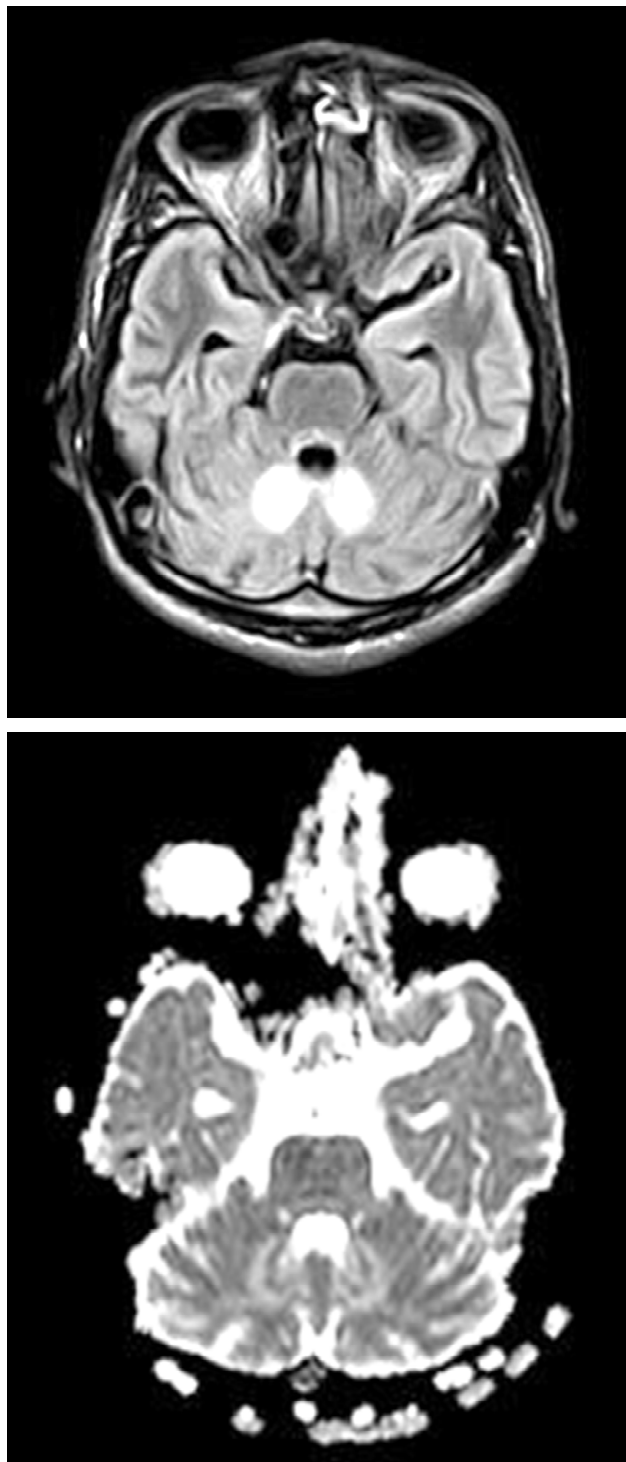
The cumulative dose of metronidazole consumed in 3 days was approximately 4.5 grams.

Case 2

A 22-year-male patient, brother of Case 1 who presented
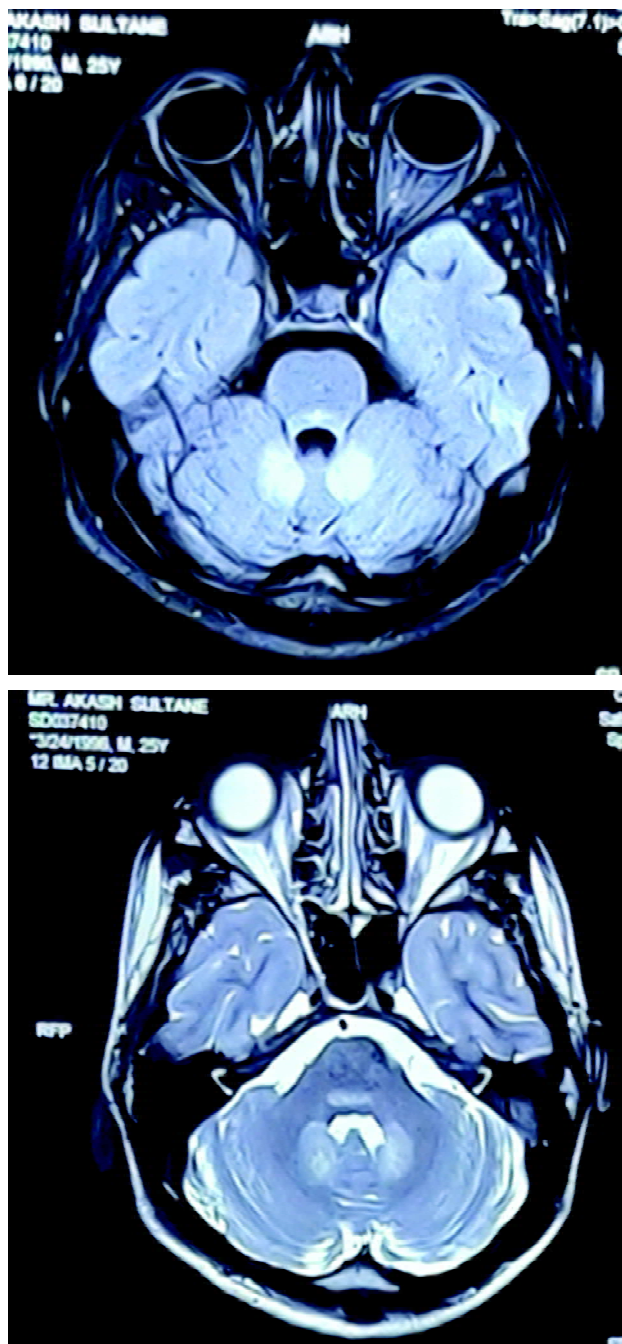
Fig. 2: FLAIR and T2WI of MRI brain showing Hyperintense Signal intensity is noted in bilateral dentate nuclei and posterior part of pons.

Fig. 3: FLAIR and T2WI of MRI brain showing Hyperintense Signal intensity is noted in bilateral dentate nuclei and posterior part of pons.

with features of cerebellar syndrome. The history revealed consumption of tablet metronidazole 500 mg three times daily for 4 days. He was transferred to this hospital for ataxia, slurred speech, and tremors of both hands. On neurologic examination, he was alert, well oriented, deep reflexes were intact, sensations including posterior column were within normal limits. Plantar reflexes were flexor, Rhomberg's sign was negative. Speech was dysarthric, minimal horizontal nystagmus on lateral gaze was noted; there was no vertical nystagmus. Pupils were reactive to light.

Cerebellar testing revealed finger-to-nose testing, dysdiadochokinesia, heal to shin manoeuver were bilaterally affected. Pendular jerk was more evident on the left side.

MRI brain revealed symmetrical altered signals, noted in bilateral dentate nuclei of cerebellum, central medulla and pons appearing hyper on T2W image and FLAIR showing restriction in cerebellar lesion(Fig. 3). Features were suggestive of drug neuro toxicity. The drug was discontinued; clinical improvement was noted after 5 days of discontinuation; and complete recovery after 20 days. Patient refused for a follow-up MRI.

The cumulative dose of metronidazole was approximately 6 grams which he consumed in 4 days.

Discussion

Metronidazole is a 5-Nitroamidazole drug having potent bactericidal activity against widely covered anaerobic bacterial and protozoal infections. The drug is often well tolerated. Most frequently occurring adverse effects are gastrointestinal. Central neurotoxicity is uncommon but events are serious as they develop encephalopathy, seizures, altered mental status and cerebellar syndrome. The possible mechanisms of metronidazole drug hypothesis include:-

1. Binding of metronidazole to neural RNA that inhibits protein synthesis.

2. Modulation of inhibitory neurotransmitter gamma aminobutyric acid receptor within vestibular and cerebellar mitochondrial dysfunction.

3. Others have postulated axonal swelling, vasogenic and cytotoxicity leading to localised oedema detected on MRI[2]. The findings are opposed to ischaemia and demyelination.

4. As the drug is structurally similar to thiazole, a precursor of thiamine, it could lead to reduction in thiamine absorption by acting as a thiamine analogue[6].

We presently encountered two cases in male brothers aged 24 and 22 years, who developed features of cerebellar syndrome with dysarthria, dysmetria, ataxic gaite and nystagmus three days after receiving metronidazole drug with a cumulative dose of 3.6 grams to each for enteritis under hospitalisation. The symptoms regressed soon and we noticed total recovery within 15 days of discontinuation fo the drug in Case no. 1, the elder brother; and it took 20 days for the younger brother for achieving total recovery. The available literature describes the cumulative dose with varying range from 25 to 1,080 grams of the drug and resolution of symptoms in 1 - 3 months after cessation of the medication[3].

In this study, the drug metronidazole's 3D structure was used, for assessing the binding affinity of the drug with structural gene KCNIP4. This gene is a potassium voltage gated channel interacting protein-4 that encodes a member of a family of voltage gated potassium channel interacting proteins. The binding affinity of the gene KCNIP4 is blocked by the drug at the end/edge of aspartic acid and leucine at position 1 and 2 at the heads not permitting any other molecule or the compound to bind this region. This inhibitory reaction and binding mechanism results to effect the energy production for cell maintenance and normal regulation. The binding position which are at 1 and 2 of leucine and aspartic acid bind through hydrogen ion bonds which are considered weak bonds in cell regulatory mechanism. When the drug consumption is stopped, the binding bonds of the drug with the gene end and remain distracted as the bonds are weaker. The gene then functions in a normal way. The neurologic symptoms hence abate. We feel deeply ingrained in offering a possible new tentative explanation/ mechanism for development of reversible drug-cerebellar toxicity.

Whether the studied gene bears any familial functional significance could not be assessed. The Case 1, 24 years and Case 2, 22 years are brothers of the same family, deny history of familial, genetic, or any allergic disorders. Both the brothers suffered with cerebellar ataxia post-metronidazole therapy for enteritis. They recovered from the symptoms soon after omission of the drug. Our above-mentioned hypothesis is found to be novel as has not been described by any other researcher. We tried to highlight the binding position where the drug under discussion alter/ induce the reactionary mechanism by blocking the binding sites (position 1 and 2 of aspartic acid and leucine). Otherwise, the same sites could bind the different molecules to produce energy for cell functioning. KCNIP4 gene was extracted from Gene card database after screening 50 plus genes involved in Genetic testing in ataxia[4].

The newer proposed outcome of drug with KCNIP4, a potassium voltage gated channel interacting gene, needs

further analysis on CHIMERA Software details with cases of similar type.

Conclusion

Metronidazole-induced cerebellar dysfunction is an uncommon occurrence though the drug is safe. However, the adverse reactions are reported early in the course and disappear within weeks of discontinuation of the therapy. The mechanism described earlier by many workers remains poorly understood. The present study describes yet another tentative explanation/mechanism based on data based screening, analysis carried-out on molecular docking, the drug's 3D structure and its binding affinity with potassium-gated voltage channel interacting protein KCNIP4 gene.

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Sickle Cell Syndrome Presenting with Proptosis of Left Eye: Due to Orbital Infarction and Haematoma

Abhijit Mishra*, Kripasindhu Gantait**, Kalimujjaman Molla*, Umakanta Mahapatra***

Abstract

Sickle cell syndrome is an important cause of haemolytic anaemia, which occurs due to haemoglobinopathy. Patients with sickle cell syndrome suffer from haemolytic anaemia, with haematocrit ranging from 15 to 30% and significant reticulocytosis, but most common clinical manifestation is recurrent painful vaso-occlusive crises. It can involve many sites like bones, lungs, brain, spleen, penis, retina causing manifestations like painful bone crises, acute chest syndrome, cerebrovascular accident, sequestration crises, priapism, and retinal haemorrhage respectively. Orbital infarction is a very rare vaso-occlusive crisis in sickle cell syndrome. Proptosis may develop because of orbital infarction itself or due to orbital haematoma, which may result from infarction. Here we are presenting the case of a 16-year-old boy first time being diagnosed as sickle cell anaemia from his clinical presentation of proptosis due to orbital infarction and haematoma, a rare vaso-occlusive crisis.

Introduction

Sickle Cell Disease (SCD) is the most commonly inherited haemoglobinopathy worldwide, which is caused by a mutation in the beta globin gene that changes the sixth amino acid from glutamic acid to valine[1]. HbS molecules undergo polymerisation when deoxygenated. Sickle cell disease is associated with reticulocytosis, hyperbilirubinaemia, and the presence of irreversibly sickled cells, but the most common clinical manifestation is different 'vaso-occlusive crises', which occur intermittently mainly as a result of dehydration. Prominent manifestations include episodes of ischaemic pain, (i.e., painful crises) and ischaemic malfunction or frank infarction in the spleen, central nervous system, bones, joints, liver, kidneys, and lungs. Proptosis due to orbital infarction and haematoma is a very rare finding of sickle cell syndrome. Here we are presenting the case of a 16-year-old boy first time being diagnosed as sickle cell anaemia from his clinical presentation of proptosis, which was caused by orbital infarction and haematoma.

Case report

A 16-year-old boy presented with swelling of his left eye since the last 5 days and fever, headache with vertigo since 2 days (Fig. 1). Proptosis was not associated with any ocular pain. Ocular complaints were of insidious onset and progressive in nature. Fever was not associated with any chills and rigors and temperature went up to 102⁰ F. There was neither any significant past history of chronic illness, nor any history of blood transfusion. No significant family history was also present. On admission, he was febrile with normal vitals. Patient was detected to have severe pallor and sternal tenderness without icterus, hepatosplenomegaly and lymphadenopathy. At first, orbital

Fig. 1: *A 16-year-old boy presenting with proptosis of left eye.*

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cellulitis or periorbital abscess were thought of as differential diagnosis. Ophthalmoscopic examination revealed no abnormality in the fundus, and intraocular pressure was 25 mm of Hg in the left eye. Left eyelid was inflamed. Inferior Oblique and Superior Rectus movements were restricted. The pupils were equal and reacting to light.

Basic investigations on admission revealed Hb - 3.3 gm/dl, TLC - 19,800/cmm (N44, L52, M1, E2, B0), platelets - 81,000/cmm. Reticulocyte count - 1.33. Peripheral blood smear examination disclosed sickle cells with marked anisopoikilocytosis (Fig. 2). Fragmented RBCs, Pencil cell, Tear drop cells were also observed in fair numbers. Sickling test was positive. Inflammatory markers were raised: CRP - 50.48 mg/dl, serum ferritin - > 1,650 ng/dl. Serum creatinine and LFT were within normal limits. Bleeding time and Clotting time values were 1 min 30 secs and 3 min 15 secs respectively. Conservative management was started with injection ceftriaxone, tab paracetamol. Tab acetazolamide, and timolol maleate eye drops were also prescribed to lower the intra-ocular pressure.
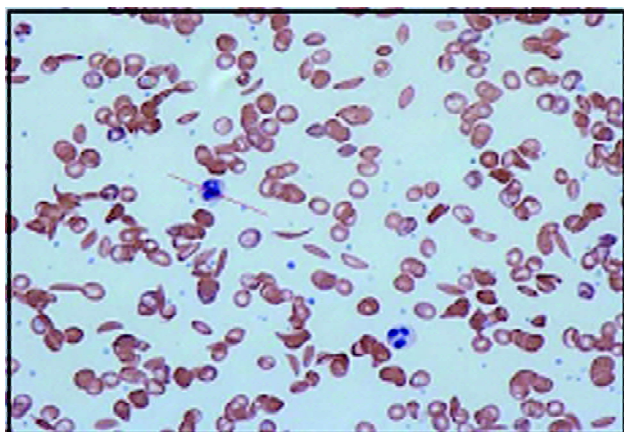
Fig. 2: *Peripheral blood smear showing sickle cells along with marked anisopoikilocytosis.*

Subsequently, HPLC for thalassaemia screening was accomplished and it expressed HbS - 72.5%, HbAo - 11.9%, HbA2 - 5.1%, HbF - 10.9%. MRI scan of brain and orbit revealed a well-defined, variegated, intense lesion in the extra-conal space of left orbit in its superior aspect, which was likely to represent orbital infract with resolving haematoma (Fig. 3). Patient also received three units of PRBC for restoration of haemoglobin level. 5 days after admission, patient developed severe pain in both knee joints and lower back region. These symptoms were alleviated with maintenance of proper hydration, administration of tramadol as analgesic, and prescription of hydroxyurea. 8 days after admission, proptosis began to decrease. After 2 weeks, at his first follow-up visit, we noticed almost complete recovery of ocular signs and

Fig. 3: *T_2-weighted MRI showing orbital infarct with resolving haematoma (Arrow).*

Fig. 4: *Improvement of proptosis after 2 weeks of illness with conservative management.*

symptoms (Fig. 4).

Discussion

Ocular involvement in sickle cell disease is characterised by retinopathy, anterior segment ischaemia, glaucoma, and angioid streaks, and has been well documented[2]. In comparison, orbital bones infarction is less well recognised. Sidman in 1990 published an article which described a child with sickle cell disease with bilateral orbital abscess and frontal bone infarction[3]. In 1997 Curran et al. reported a case of orbital compression syndrome in a patient with

sickle beta-thalassaemia[4]. Ganesh *et al* in 2001 published an article which reveals five cases of orbital involvement in sickle cell disease. Four out of five cases presented with periorbital swelling, among which proptosis was found in two cases. In all four cases, CT and/or MRI of orbit showed a mass adjacent to the orbital wall. In two cases the mass was identified as a haematoma. Orbital wall infarction was demonstrated in three cases[5]. A case study was published in 2018 by Alghamdi, showing recurrent orbital bone subperiosteal haematoma in a patient of sickle cell disease[6].

The course of sickle cell anaemia is punctuated by a variety of painful 'vaso-occlusive crises'. It can involve many sites like bones, lungs, brain, spleen, penis, retina, orbit causing manifestations like painful bone crises, acute chest syndrome, cerebrovascular accident, sequestration crises, priapism, retinal haemorrhage and orbital compression syndrome[7,8], 13 respectively. Orbital infarction is a relatively rare manifestation of sickle cell syndrome. Orbital infarction presents with acute periorbital pain and swelling in conjunction with other features of the painful crisis. The inflammatory response generated by infarcted bone can rapidly spread to the orbit resulting in orbital pain and proptosis. A unique feature of orbital bone infarction is the formation of haematoma, which may aggravate proptosis. The occurrence of orbital haematoma is thought to be related to local vessel wall necrosis and subsequent extravasation of blood. Literature review showed that a few cases have been reported so far. Orbital infarction may also be considered as vaso-occlusive crisis. Management is same like management of other crises: vigorous but careful hydration, thorough evaluation for underlying cause, and aggressive analgesia. To conclude, orbital infarction and haematoma, although rare should be suspected in all cases of sickle cell syndrome presenting with proptosis.

Conclusion

We report a case of orbital infarction with resolving haematoma in a patient with sickle cell anaemia. Sickle cell disease is associated with anaemia and jaundice, but the most common clinical manifestation is different 'vaso-occlusive crises'. Orbital infarction is a very rare vaso-occlusive crisis of sickle cell syndrome. Proptosis in this case was due to orbital infarction along with haematoma in the extra-conal space. So, orbital infarction along with haematoma should be suspected in all cases of sickle cell syndrome patients presenting with proptosis. Most of the cases of orbital involvement do not require any special treatment, but respond well with conservative management like maintenance of proper hydration, administration of analgesic and prescription of hydroxyurea.

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A Rare Case of Minimal Change Disease in a Patient of Myasthenia Gravis

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Abstract

Myasthenia gravis is a chronic autoimmune neuromuscular junction disorder characterised by weakness and fatiguability. It is associated with thymoma in 10% of cases. Paraneoplastic glomerulonephritis is a rare clinical presentation of the same. We report the case of a 27-year-old male who presented with complaints of fluctuating weakness and diplopia, who was diagnosed as seropositive myasthenia gravis with B2 variant of thymoma. 9 months after the disease, he developed anasarca with nephrotic range proteinuria and deranged renal function tests and was found to have minimal change disease.

Key words: Myasthenia gravis, thymoma, minimal change disease.

Introduction

Myasthenia gravis is a common autoimmune neuromuscular junction disorder but its association with proteinuria is a rare entity. Minimal change disease is a rare paraneoplastic manifestation of thymoma. Both myasthenia gravis and minimal change disease are related to the dysfunction of T-lymphocytes. We report here a case of myasthenia gravis who developed minimal change disease after thymectomy.

Case report

A 27-year-old male with no comorbidities, presented with complaints of fluctuating weakness in bilateral upper and lower limbs for 3 months, and diplopia for 1 month – with aggravation during the night; and gradually progressed to a level when he had difficulty chewing hard food, and even getting up from bed during evening time. On examination, the patient was conscious, oriented, and vitally stable. There was no pallor, icterus, pedal oedema, skin rash. Nervous system examination was suggestive of ptosis in both eyes with bilateral medial rectus palsy which improved on ice pack test. There was weakness in all 4 limbs on repeated activity, with demonstrable improvement on rest. Higher mental functions, other cranial nerves, and sensory system examination was normal. Other systemic examination showed no abnormality.

On investigation, patient had a haemoglobin of 15.7 gm/dl, TLC of 13,000 cells/mm^3, platelet count of 2,00,000 cells/mm^3; muscle enzyme CPK was 68 U/L, CK-MB was 2 U/L and LDH was 667 U/L; thyroid profile was normal; ANA was negative and myositis profile was also negative. In the background of fluctuating weakness, Acetylcholine Receptor antibody levels were checked which turned out to be 22.14 nmol/l (normal: < 0.40). Diagnosis of myasthenia was confirmed. CECT chest was planned, which was suggestive of 3.9 x 10.0 x 5.4 cm, horizontally placed cystic density with heterogeneous contents and peripheral enhancement, likely a Cystic thymoma (Fig. 1). PET scan done to rule-out metastasis, turned out to be normal. The patient was started on IV steroids and pyridostigmine. Thymectomy was done and biopsy was suggestive of B2 variant of thymoma (Fig. 2).

After 9 months of treatment, he noticed swelling over his face and gradually all over his body over the course of 15 days (Fig. 3, 4). It was associated with frothing in urine and gradually a reduction in urine output to around 400 ml/24 hrs. Blood pressure was in the normal range.
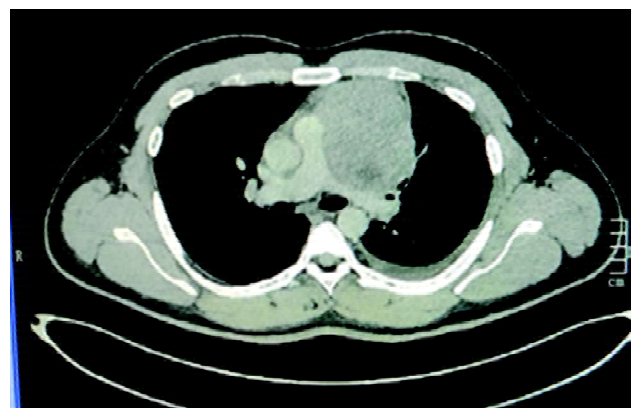
Fig. 1: *CECT chest demonstrating a mass in superior mediastinum with heterogenous content and peripheral enhancement suggestive of cystic thymoma.*

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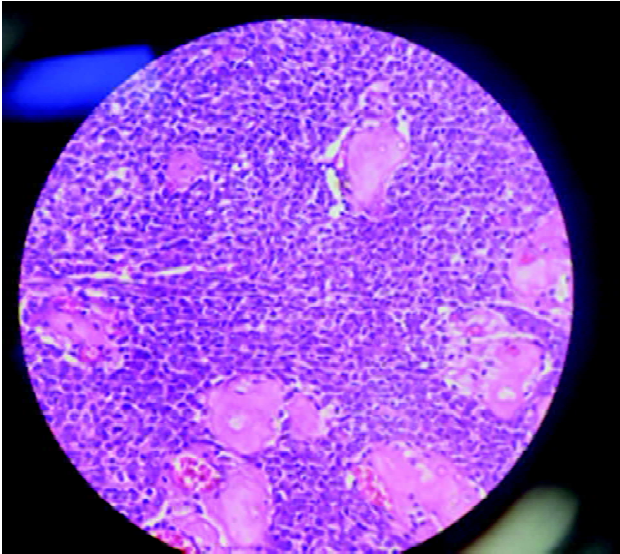
Fig. 2: *Thymic biopsy (Haematixylin-eosin, 10 x magnification): Cyst separated by fibrous septae in background of normal thymic tissue.*

Fig. 3: *Facial puffiness.*

Fig. 4: *Swelling in both legs extending up to thigh.*
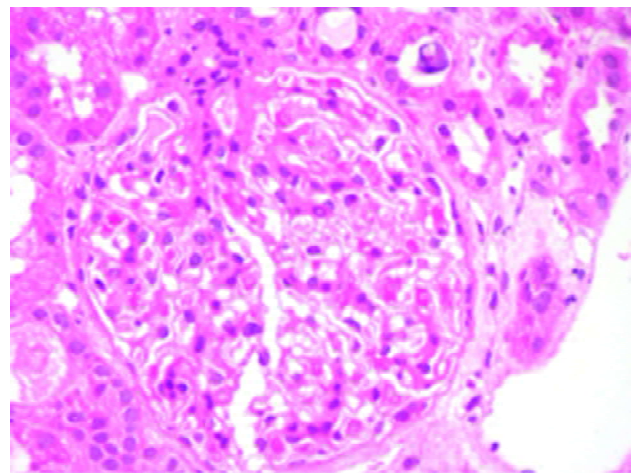
Fig. 5: *Kidney biopsy (Haematoxylin-eosin 10 x magnification): Normal glomeruli.*

Investigations were suggestive of proteinuria with a 24 hrs urine protein of 7g/24 hrs (nephrotic Range). There were no casts or haematuria. Urea was 40 mg/dl, creatinine was 2.2 mg/dl, Na^+/K^+ was 133/4.2 mEq/l, C3, C4 levels were normal, ANA was negative, and ultrasonography revealed normal kidneys. Renal biopsy was done which was suggestive of minimal change disease (Fig. 5, 6).

The patient was started on pulse steroids and ACE-inhibitors, after which there was decrease in anasarca and proteinuria. Patient was discharged on oral steroids. However, the patient returned 1 month later with generalised weakness, therefore the patient was switched to tacrolimus and steroid was gradually tapered after which there was some gain in function.

Discussion

Myasthenia gravis is a neuromuscular disorder
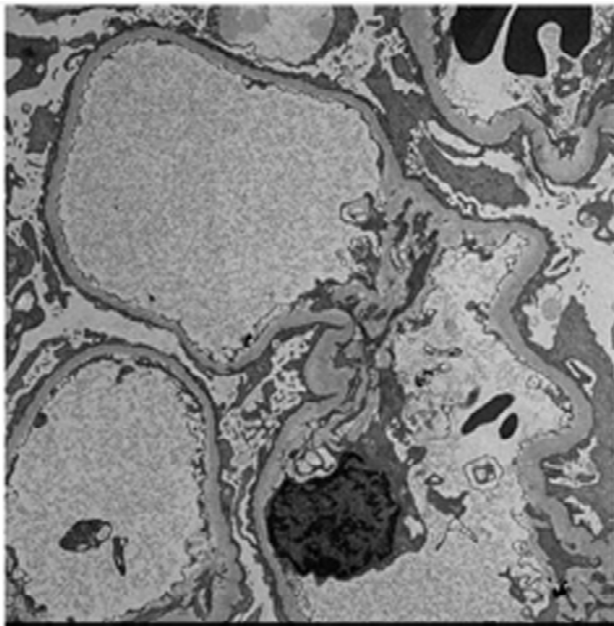
Fig. 6: *Kidney biopsy (electron microscopy): Extensive effacement of foot processes and absence of electron dense deposits.*

characterised by fluctuating weakness of ocular, bulbar, limbs, and rarely respiratory muscles[1]. These symptoms occur as a result of autoantibodies against acetylcholine receptors at neuromuscular junction causing synaptic rundown[2]. Association between minimal change disease and myasthenia gravis is a rare entity. Minimal change disease is a podocytopathy due to T-cell dysregulation and presents with anasarca and nephrotic-range proteinuria. It has a predilection in children and its occurrence in adults is rare.

There may be association between minimal change disease and thymoma. Thymectomy can act as a precipitating factor for minimal change disease as it is followed by changes in lymphocyte function[3].

Our case was a myasthenia gravis patient who underwent thymectomy following which he developed nephrotic range proteinuria and turned out to be minimal change disease on renal biopsy within a year of surgery, whereas the occurrence of minimal change disease in myasthenia gravis is generally a late presentation[4]. The treatment of the same is challenging as high dose steroids are the mainstay of management for minimal change disease; however, the same dose may cause worsening of myasthenia gravis[5] which was quite apparent in our patient and he had to be switched to tacrolimus.

Conclusion

In a patient with myasthenia gravis and thymoma, minimal change disease may occur in the course of illness secondary to T-cell dysfunction post-thymectomy. Therefore, clinician may keep this association in mind. Steroids must be titrated such that it does not worsen the myasthenia.

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Successfully Treated Accidental Thallium Poisoning

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Abstract

Thallium (Tl) once commonly used as a rodenticide, has been banned due to its toxicity, but unfortunately, is still available in India. In this case report we investigated two female patients from the same family, 68 years and 45 years of age, who were hospitalised for abdominal pain, pins-and-needles sensations in lower limbs and alopecia for 10 days. The diagnosis of thallium toxicity was confirmed based on high blood and urine thallium levels. Patients were cured with Prussian blue and haemodialysis.

Introduction

Thallium is a heavy metal which is odourless, tasteless, and water soluble; making it one of the ideal homicidal substances. Thallium toxicity is rarely reported due to a global ban on use of thallium; however, it is still reported in India due to its illegal use. It can be absorbed into the body by any route, i.e., ingestion, inhalation, and skin contact[1]. Clinical manifestations vary with dose and duration of exposure. Majority of patients present with alopecia, skin rashes and neurological manifestations such as quickly progressive and severely painful peripheral neuropathy, mental confusion and lethargy. Combination of these signs and symptoms is knoun as the thallium triad.

Case report

Two female patients belonging to the same family, residents of Aligarh, Uttar Pradesh, aged 45 years (patient 1) and 68 years (patient 2), presented with complaints of abdominal pain with nausea and vomiting for 10 days, pins-and-needles prick sensations and burning in both feet for 7 days and acute loss of hair from scalp for 5 days. They gave history of recent travel to their hometown 5 days prior to onset of symptoms, where they consumed wheat flour which was contaminated with unknown tablet used to keep rodents away. There was no history of fever, diarrhoea, rash, visual abnormality, oral ulceration, any medication intake, altered sensorium, abnormal behaviour, palpitation, bleeding, or breathlessness. On examination, patients were vitally stable, there was alopecia throughout the scalp and madarosis. Nervous system examination was suggestive of diminished ankle reflex and impaired vibration sense in both lower limbs below the knee joint. The other general examination was normal and systemic examination was also normal. Haemogram revealed a Hb of 12.5 g/dl, TLC of 6,000 cells/mm³ and platelet count of 2.5 lakh cells/mm³

for patient 1 and Hb of 10.1 g/dl, TLC of 6,100 cells/mm³ and platelet count of 2.2 lakh cells/mm³. Kidney and liver function tests were normal. Urine examination was normal; ANA was negative; vitamin B12 levels, folate levels, thyroid function test were normal; radiological investigations were normal. Considering the temporal and geographical association of clinical manifestations, a high suspicion of heavy metal toxicity was kept and thallium levels on day 2 of admission were found to be high in both serum and urine in both patients (patient 1 serum Tl: 335 µg/l, and urine Tl - 2,422 µg/l; patient 2 serum Tl: 136 µg/l, and urine

Fig. 1: *45-year-old female with non-scarring alopecia.*

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Fig. 2: 68-year-old female with non-scarring alopecia.

Tl - 2,320 µg/l). Patients were managed with i.v. fluids, gastric lavage, activated charcoal, forced alkaline diuresis with furosemide, i.v. potassium chloride, and Prussian blue. Haemodialysis was initiated. A total of 6 cycles of haemodialysis were given until serum Tl reached <0.1 µg/l. Patients were discharged and followed up. Both patients showed good recovery and alopecia reversed over the next 3 months.

Discussion

Thallium is a bluish-white metal that turns grey on exposure to air. It is used in the glass and dye industry, depilatory creams, fireworks, metal alloys, insecticides, and rodenticides in the form of thallium sulphate, as a radioisotope (Tl-201) in cardiac perfusion scan[2]. It is commonly used in Chinese herbal products. Thallium sulphate was used for the treatment of diseases and conditions including syphilis, gonorrhoea, gout, and dysentery in the past. The fatal dose of thallium is 1gm (> 8 mg/kg), serum levels of more than 15 mg/l are immediately fatal to health and the fatal period is usually 24 - 36 hours. Death can result in less than 48 hours if a large dose is taken. Cause of death is generally respiratory failure due to motor neuropathy, renal or hepatic failure. Thallium has three toxokinetic phases, i.e., intravascular distribution phase for first 4 hours, central nervous system distribution from 4 to 48 hours and elimination phase after 24 hours (renal and fecal excretion)[3].

Early manifestations (24 hrs) include acute abdominal pain and vomiting, headache, paraesthesia, confusion, hallucination, retrobulbar neuritis, ophthalmoplegia, tachycardia; and rarely hepatic, renal and bone marrow failure. Intermediate symptoms (24 hrs to 2 weeks) include quickly progressive severely painful peripheral neuropathy, mental confusion and lethargy and cardiac dysrhythmias. Late manifestations include Mee's lines, acneform rash, peripheral neuropathy, choreoathetosis, tremors, ophthalmoplegia, keratitis, cataract, and cardiomyopathy. Once thallium gets absorbed, only haemodialysis can remove it from the body[4].

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A Rare Cause of PUO with Aplastic Crisis: The Great Masquerader

Bhatt Shrey Nandkishor*, MPS Chawla**, Sai Kiran*, Keshu Jindal*

Abstract

Kikuchi Disease is also known as Kikuchi Fujimoto Disease or Histiocytic Necrotising Lymphadenitis. Few studies suggest infectious and others suggest autoimmune aetiology. Multi-organ involvement is seen like bone marrow and liver. Lymph node biopsy is an important investigation for diagnosis. Here, we report the case of a 24-year-old female who presented with the complaints of high-grade fever, erythematous pruritic rash and cervical lymphadenopathy associated with aplastic anaemia, who was diagnosed as Kikuchi Disease. She was treated with hydroxychloroquine and followed up for recurrence and progression.

Key words: Kikuchi, histiocytic necrotizing lymphadenitis, aplastic anaemia.

Introduction

Kikuchi Disease is a rare and benign condition of lymphohistiocytic cells of uncertain aetiology[1]. It is characterised by fever, lymphadenopathy, erythematous rash, and leucopenia. The exact aetiology is not known, but based on its clinical presentation, course and histologic changes, it suggests an immune response of T-cells and histiocytes to some inciting agents like EBV, HHV, HIV, Parvovirus B19, Paramyxovirus, Parainfluenza Virus, Yersinia Enterocolitica and Toxoplasma. Of the autoimmune illnesses, Systemic Lupus Erythematosus (SLE) is the most common illness known to develop in connection with Kikuchi Disease. Hydroxychloroquine, steroids and IVIg are used in the treatment of Kikuchi Disease[2]. However, it is a self-limiting illness seen in predominantly young females less than 40 years.

Case

A 24-year-old female, resident of Delhi and native of Nepal with no co-morbidities presented with complaints of fever for 20 days, high grade, continuous associated with chills, rigors and erythematous pruritic rash which appeared on day 3 of illness, resolved spontaneously after 2 days. These complaints were associated with sore throat, generalised bodyache and nausea. Fever was documented as 102° F. On examination, there were pallor and bilateral cervical lymphadenopathy with largest of size 2 x 2 cm. On ENT examination, there was posterior pharyngeal wall congestion with normal tonsils. Rest of the systemic examination was within normal limits. Haemogram showed a haemoglobin of 8.3 g/dl, total leukocyte count 1,200 cells/cumm with DLC of 40/55/2/3, platelet count 0.8 lakh/cumm. Liver Function Tests were deranged with AST/ALT 206/79 U/L with normal total bilirubin. There was progressive fall in haemoglobin, total leucocyte count and platelet count during the hospital stay. Patient was managed as a case of febrile neutropenia with pancytopenia and appropriate antibiotics were administered and aetiological work-up was done. IgM dengue and chikungunya were negative, EBV serology was equivocal. HbsAg, anti HCV, HIV ELISA and CMV DNA PCR were negative. Serology for IgM parvovirus B19 was positive. ANA by immunofluorescence was positive in titres of 1:640 (homogenous pattern). ESR was 74 mm/hr, quantitative CRP 56.8 mg/dl, LDH 940 U/L, C3 and C4 levels were normal. Extended Nuclear Antigen (ENA) and Vasculitis Profile were negative. Radiological investigations did not reveal any abnormality.

Patient continued to run fever; however, cultures remained sterile and antibiotics were upgraded and antifungals were started. Patient underwent bone marrow studies and cervical lymph node biopsy on day 4 of illness. Simultaneously, patient was started on filgrastim 300 microgram daily for 7 days until day 10 of admission following which, cell lines improved to Hb of 9.6, TLC - 10,600 and platelet count - 1.8 lac/cc. Bone marrow biopsy was suggestive of hypocellular marrow with few lymphocytes, plasma cells, and mast cells. There was no atypical cell, parasite or granuloma with an overall impression of aplastic anaemia. Lymph node biopsy suggested diffuse sheet of foamy histiocytes, karyorrhectic debris with dense fibrin deposition, lymphoid collection along with scattered plasma cells and tingible body macrophages. Overall, the features were suggestive of Kikuchi Necrotising Lymphadenitis. Gram's stain, ZN stain and PAS stain were negative.

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Patient was managed as a case of Kikuchi necrotising lymphadenitis with aplastic crisis. Patient was started on tablet Hydroxychloroquine 200 mg BD along with haematinics on day 10 and all other drugs were withdrawn. Patient became afebrile on day 15, lymph nodes decreased in size and haematological parameters improved. Patient was discharged with stable vitals on day 16. 2 weeks after discharge, the lymph node completely regressed and patient continued to remain afebrile. Complete blood count showed a haemoglobin of 11.8 g/dl, TLC - 6,000 cells/cumm and platelet count - 3.2 lakh/mm, and liver function tests normalised. After 6 months, hydroxychloroquine was stopped and patient is being followed up for recurrence and progression.
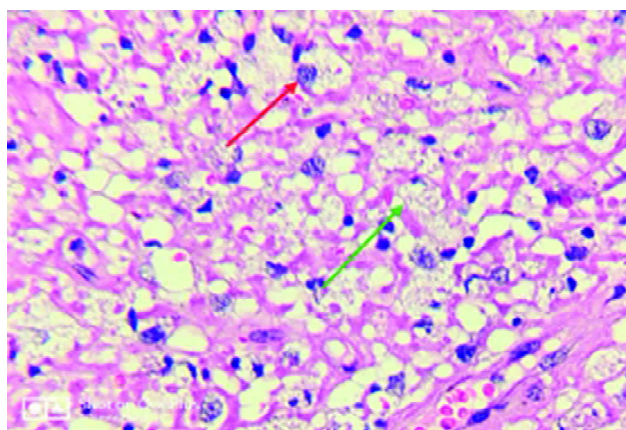
Fig. 1: 40x view: Red and green arrow-large cells with central to eccentric round nucleus with abundant vacuolated cytoplasm – foamy histiocytes.
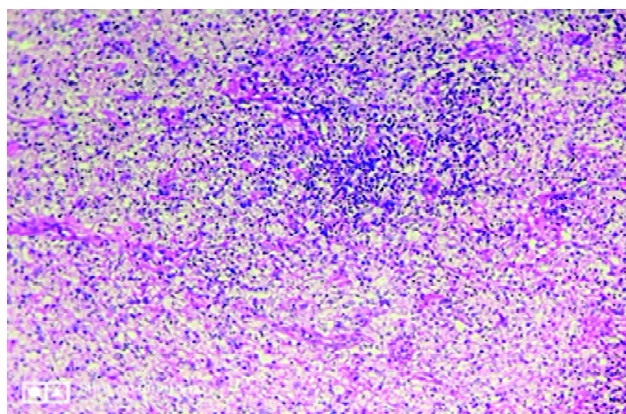
Fig. 2: 10x view: Large round to oval cells with abundant clear cytoplasm – foamy histiocytes.

Discussion

The exact aetiology of Kikuchi disease is not known yet. However, there are a few case reports of various viral agents like HIV, EBV, HTLV-1, Parvovirus B19 being possible aetiological agents, but none have been proven so far[3]. In many scenarios, Kikuchi disease has been seen in association with SLE and a possible autoimmune mechanism has also been proposed[1].

Kikuchi disease is characterised by tender cervical lymphadenopathy in 60 - 90% cases with fever as a common symptom associated with other B symptoms like weight loss, night sweats, fatigue, sore throat, and skin involvement as erythematous, maculopapular, nodular and other cutaneous lupus-like rashes occurs in 40% of cases. There are no strict diagnostic criteria defined till now for this disease. Initial laboratory work-up reveals elevated inflammatory markers, deranged liver function tests in the form of transaminitis. Leukopenia occurs in around 22 - 58% of cases with atypical peripheral lymphocytes. Autoimmune work-up specifically evaluating for SLE and associated antibodies should be done[4].

Histological characteristics of lymph node biopsy in Kikuchi disease remain unique and ultimately clinches the diagnosis[5]. At present there are no guidelines for the treatment of Kikuchi disease. Few studies suggest usage of hydroxychloroquine, steroids, and IVIg alone or in combination[6]. Our case had positive ANA titres and positive Parvovirus B19 serology. However, further work-up for SLE was not fulfilling EULAR/ACR criteria.

Conclusion

Kikuchi disease can be mistaken for tuberculosis, lymphoma, or SLE as it may have varied presentations. Therefore, tissue biopsy must be performed to establish the diagnosis. A proper follow-up should be done as it may be an initial manifestation of SLE.

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Atypical Manifestation of Dengue Fever: A Tale of 2 Cases

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Abstract

Dengue fever is a common tropical illness with varying severity often causing thrombocytopenia and haemorrhagic manifestations. We describe 2 cases with central nervous system manifestations in dengue fever. First is the case of a 20-year-old female who presented with high grade fever, headache, altered sensorium, and seizures. Clinical findings were suggestive of meningoencephalitis. Initial blood investigations of the patient showed thrombocytopenia. The magnetic resonance imaging of the brain showed bilateral thalamic, internal capsule, and occipital lobe hyper-intensities. Based on the preliminary examination and investigations, a diagnosis of acute febrile illness with meningoencephalitis was made. Further investigations showed Dengue NS1 antigen positivity in serum and a raise in anti-dengue IgM antibodies. Following symptomatic treatment and antiepileptic drug usage, the patient made a complete recovery. In another case, a 32-year-old gentleman with a short duration of fever and altered sensorium was diagnosed with dengue fever based on NS1 antigen positivity and IgM antibody positivity. He had poor GCS and MRI brain revealed bilateral thalamic and cerebellar hyper-intensities suggestive of encephalitis. He succumbed to the illness. These 2 cases highlight the importance of knowing the atypical manifestation of dengue fever.

Key words: Dengue fever, meningoencephalitis, seizure.

Introduction

Dengue virus is a RNA virus belonging to flavivirus genus. Rapid raise in urban population and poor public health infrastructures have led to significant increase in the number of dengue cases. A recent approximation indicates 390 million dengue infections per year, out of which around 96 per cent show clinical features[1]. Dengue virus infection can lead to a wide array of clinical symptoms. In its milder form, patients maybe asymptomatic or have undifferentiated viral illness. In severe cases it can lead to haemorrhage, circulatory collapse, and profound shock. Unlike other arboviral diseases, neurological complications due to dengue infection are rare. However, in recent years there has been a surge in neurological manifestation of the infection[2]. We describe two cases of dengue encephalitis to describe this rare presentation of a common disease.

Case report

Case 1

A 20-year-old student presented with high-grade fever and generalised headache of 5 days duration. One day before presentation to the hospital, the patient had an episode of generalised tonic-clonic seizure which lasted for 5 minutes. Since the episode of seizure, the patient had altered sensorium with reduced speech output. She also had diffuse arthralgia involving multiple joints. There was no history of seizures in the past. On examination, she was febrile (101° F), pulse rate of 66 beats per minute, and blood pressure 130/70 mm of Hg. Skin rash, oedema, abdominal distension, and icterus were absent. There was no evidence of cutaneous or mucosal bleeding. On CNS examination, neck stiffness was present and Brudzinski's sign was positive. She was conscious but confused and obeyed simple verbal commands. Speech output was reduced. There were no features suggestive of focal neurological loss. Rest of the systemic examination was unremarkable.

The patient's full blood count showed a low platelet count (69 x 10^9/l) with a haemoglobin of 13.6 g/dl and lymphopenia (WBC count of 2 x 10^9/l). Liver function tests were deranged, which showed elevated alanine aminotransferase (444 IU/L) and aspartate aminotransferase (263 IU/L). Renal function tests showed no abnormalities. Dengue NS1 antigen and anti-dengue IgM antibodies were positive in the serum. Peripheral

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smear and QBC for malaria were negative. Being endemic diseases and with similar presentation, leptospirosis and scrub typhus were ruled-out through appropriate tests. Blood cultures were sterile. MRI brain demonstrated symmetrical hyper-intensities involving bilateral thalami, bilateral posterior limb of internal capsules and periventricular white matter of bilateral occipital lobes with associated micro-haemorrhages within the bilateral thalamic lesions. It also showed subtle enhancement of leptomeningeal enhancement involving bilateral parietal lobes (Fig. 1, Fig. 2). Electroencephalogram done showed diffuse electrical dysfunction showing generalised low amplitude discharges which indicated encephalitis. CSF examination was deferred as the patient had thrombocytopenia.

The patient was managed conservatively with antipyretics and intravenous fluids. To prevent further seizures, the patient was started on levetiracetam twice daily. Patient responded well to the treatment and her sensorium started to improve completely by the seventh day, and platelet count rose to 150×10^9/l cells. She was discharged to home and is doing well on one and three-month follow-up without any neurological sequelae.

Fig. 1: MRI T2 FLAIR sequence imaging of brain showing bilateral thalamic hyper-intensity (as pointed by white arrows).

Fig. 2: MRI T1 sequence imaging with contrast showing meningeal enhancement.

Case 2

A 28-year-old male presented with history of high-grade fever for 7 days and altered sensorium for 3 days. He had no other features suggesting involvement of any other organ system. On examination, he was unconscious, pupils were reactive bilaterally, and there was no neck rigidity. Babinski sign was positive and ankle clonus was seen. His blood pressure was 110/70 mm of Hg with pulse rate of 98 beats per min. His Glasgow Coma Scale (GCS) on admission was 4/15. Rest of the systemic examination was unremarkable.

Initial blood investigations revealed a haemoglobin of 20g/dl, platelets of 50×10^9/l and WBC count of 15×10^9/l. Electrolytes were deranged, and showed hypernatraemia (Na - 154 mmol/l). Liver function and renal function tests were within normal limits. QBC for malaria was negative. Dengue NS1 antigen and anti-dengue IgM antibodies were positive in the serum. Through appropriate tests, rickettsial infections and leptospirosis were ruled-out.

CT scan done on admission revealed hyper-densities in bilateral thalamic region and bilateral cerebellar hemispheres. MRI brain showed hyper-intensities in bilateral thalami and cerebellum as shown in Fig. 3 and 4. It also showed subarachnoid haemorrhage, diffuse cerebral

oedema, diffuse cerebellar oedema with tonsillar herniation. MRV revealed no cortical vein thrombosis. CSF examination deferred due to thrombocytopenia and features suggestive of elevated intracranial pressure.
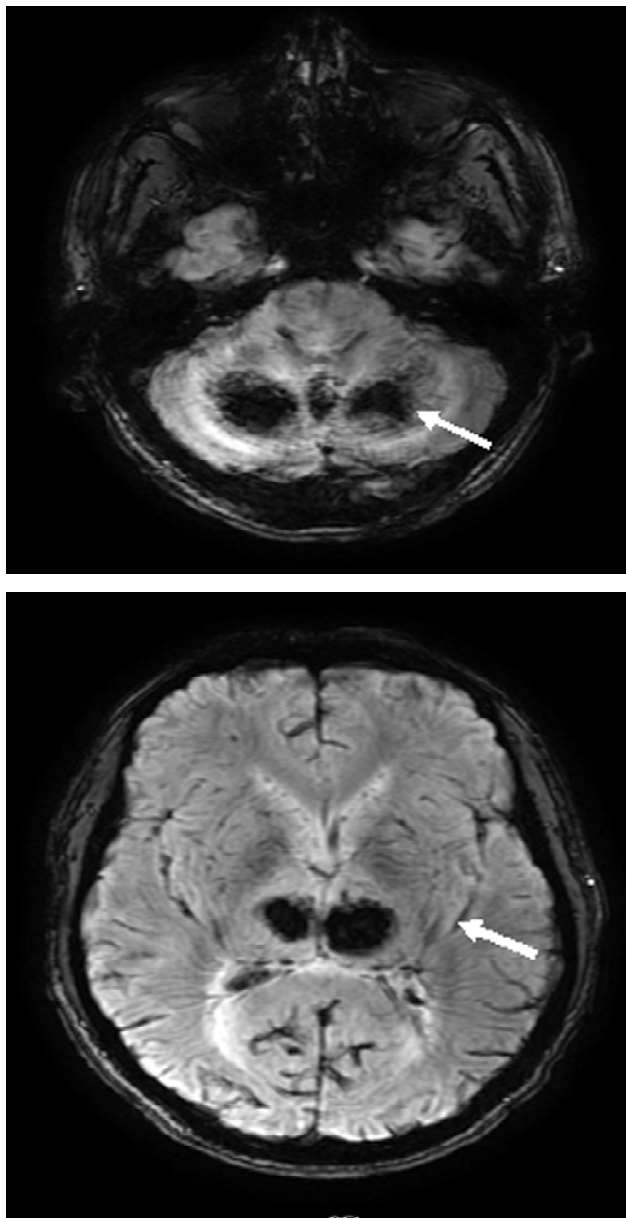
Fig. 3 and 4: MRI 3D SWAN sequence showing haemorrhages in bilateral thalamus and cerebellum (pointed by white arrows).

The patient was treated symptomatically with mannitol to reduce the intracranial pressure and levetiracetam to prevent seizures. Hypernatraemia improved with desmopressin. Despite improvement in sodium levels, the patient's sensorium failed to improve. In view of poor GCS score, the patient was intubated. In spite of the aggressive measures, the patient failed to recover and expired.

Discussion

Dengue viruses are arboviruses belonging to the genus flavivirus. There are four virus serotypes, which are named as DENV-1, DENV-2, DENV-3 and DENV-4. All four serotypes can cause clinical manifestations, which can range from simple dengue fever to severe dengue haemorrhagic fever[3]. Dengue fever usually presents with fever, headache, myalgia, arthralgia, maculopapular rash, leucopenia, and thrombocytopenia. Neurological features in dengue virus infection are uncommon[4].

In recent years, neurological complications of the disease have been increasingly reported. However, the exact incidence rates of neurological manifestation remain unanswered. In a large descriptive study on neurological manifestations of dengue fever by Kulkarni *et al*, the incidence of CNS involvement was 2.64% (154 out of 5,821 patients). Isolated encephalitis was seen in only in 25 out of 5,821 patients (0.4%)[5]. Pathogenesis of the neurological complications of the dengue virus infection is likely to be associated with metabolic alterations, direct invasion of the central nervous system by the virus, and autoimmune reactions. Based on the pathogenesis, CNS complications of the dengue infection can be categorised into[6,7]:-

1. Metabolic disturbances: which includes encephalopathy.

2. Viral invasion: which includes encephalitis, meningitis, myositis, myelitis.

3. Autoimmune disease: which includes Guillian Barre syndrome, optic neuritis, neuromyelitis optica, acute disseminated encephalomyelitis.

Dengue encephalopathy, is a rare but well-recognised entity. Amongst CNS manifestations due to dengue virus, encephalopathy due to metabolic disturbances are common. The possible mechanisms leading to encephalopathy are hepatic failure and shock leading to cerebral hypoperfusion, vascular leak leading to cerebral oedema, electrolytes derangement and intracranial bleeding secondary to low platelet count or coagulopathy. In others, encephalitis leads to encephalopathy. Various studies have demonstrated the ability of dengue virus to cause encephalitis through direct viral neurotropism. Soars *et al*, defined the dengue encephalitis as[8]:-

a. Fever.

b. Clinical features of acute cerebral involvement.

c. Presence of dengue NS1 antigen and/or anti-dengue

IgM antibodies in the serum and/or CSF.

d. Excluding other causes of viral encephalopathy and encephalitis.

Dengue encephalitis cases have been reported by Solomon *et al*, from Vietnam, who diagnosed nine dengue encephalitis cases. Misra *et al* described 11 cases of dengue encephalitis[9]. In similar case series, Kankirawatana *et al* described eight patients[3] and Kularatne *et al* described six patients with dengue encephalitis[10]. No classical clinical features of dengue encephalitis have been described. Common features from the case series studies described above are fever, headache, altered consciousness, and seizures. Out of nine cases described by Solomon *et al*, virus or antibody in CSF was demonstrated only in two patients[4]. Eleven cases described by Misra *et al*, were based on the serological tests and no CSF study was reported[9].

The imaging findings of the dengue encephalitis are variegated[5,11,12]. Imaging findings that are described in the literature include diffuse cerebral oedema, haemorrhages and focal abnormalities in the region of thalamus, globus pallidus, hippocampus and internal capsule. In the MRI, lesions are visualised as hyper-intensities. Lesions are usually localised. Extensive lesions caused due to dengue encephalitis have not been widely reported in the literature. Kamble *et al*, described a case of dengue encephalitis with extensive lesion, which involved bilateral thalami, posterior pons, and midbrain[12].

In the first case, on admission, the patient had history of fever, altered sensorium and generalised tonic-clonic seizures. Blood tests showed presence of dengue NS1 antigen and anti-dengue IgM antibodies. These features pointed towards the diagnosis of dengue encephalopathy. Biochemical parameters were not deranged enough to cause encephalopathy. Subsequent investigations with MRI showed features of encephalitis. Through other appropriate tests we ruled-out other causes of viral encephalitis. MRI brain showed extensive involvement of the brain parenchyma, which showed hyper-intensities in both thalamic, posterior limbs of internal capsules and periventricular region of bilateral occipital lobes. Haemorrhage was noted within the bilateral thalamic lesions.

In the second case, the patient had a history of fever and altered sensorium. CT scan on admission revealed extensive involvement of the brain parenchyma. Serology revealed rise in anti-dengue IgM antibodies and NS1 antigen positivity. Therefore, this case apropos to the case definition of dengue encephalitis. Lumbar puncture was avoided in the patient, as he had a low platelet count and clinical features suggestive of raised ICT. Prognosis in case of dengue encephalitis is often good as described in previous reports and case series. However, mortality may be seen in extensive disease as described above. Management is symptomatic and there is no proven therapy specific for dengue encephalitis. This case series highlights the not so common manifestation of dengue fever.

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HIV-Associated Cutaneous Kaposi's Sarcoma induced by IRIS following Antiretroviral Therapy

Sana Parvez*, Pallaavi Goel*, Mukesh K Sarna**, Puneet Rijhwani***

Introduction

Immune reconstitution inflammatory syndrome (IRIS) is a set of conditions that result from an exuberant response to residual opportunistic pathogens by a newly reconstituted immune system after initiating immune modifying therapeutic treatment or discontinuing the immunosuppressive therapy. It involves a wide range of pathogens, tumours, and some autoimmune diseases. Kaposi sarcoma (KS) is an angio-proliferative tumour capable of affecting the skin, lymph nodes, and viscera. It is a well-known acquired immunodeficiency syndrome (AIDS) defining illness presenting in patients with low CD4 counts and high viral loads, but can also be reactivated in an IRIS-related process[1]. KS remains the most common tumour in individuals infected with human immunodeficiency virus (HIV) and a significant cause of morbidity and mortality.

Case report

A 30-year-old male, diagnosed with HIV two months back, presented to the outpatient department with complaints of multiple reddish patches over his chest, back and shoulders for the last 20 days. He was on highly active antiretroviral therapy (HAART), i.e., Tenofovir 300 mg,

Fig. 1: Showing multiple hyper-pigmented nodular raised lesions, similar lesions were also found on the back and the shoulder.

Lamivudine 300 mg, and Efavirenz 600 mg for the past 2 months. All routine laboratory investigations were done, as listed in Table I. HIV RNA viral load and CD-T4 lymphocyte levels were also tested again after baseline at the time of diagnosis, as listed in Table II. On examination, the patches were hyperpigmented red-coloured raised plaques (Fig. 1), that were painless in nature and not associated with any itching or burning sensation. A large rounded growth was also noticed behind the left ear (Fig. 2A). Gum hypertrophy was observed with no oral thrush on examination of the mouth (Fig. 2B).

Table I: Routine laboratory investigations.

Tests	Results	Normal range
Haemoglobin	10.5 g/dl	14 - 17 g/dl
TLC	5,000 cells/mm^3	4.5 - 11.0 $\times$ 10^3/mm^3
Platelet count	1,00,000/ul	150,000 - 450,000/ul
ESR	25 mm/1st hr	< 15 mm/1st hr
ALT	36 U/L	7 - 55 U/L
AST	34 U/L	8 - 48 U/L
Serum creatinine	0.9 mg/dl	0.74 - 1.35 mg/dl
Serum sodium	141 mEq/l	135 - 145 mEq/l
Serum potassium	3.7 mEq/l	3.5 - 5.5 mEq/l
S. TSH	3.6 mIU/l	0.5 - 5.0 mIU/l
Abdominal sonography	Normal study	
Urine R/E	Clear	
ECG	Normal sinus rhythm	
Chest X-ray	Normal Skiagram	

Table II: Special laboratory investigations.

HIV specific tests	Baseline values	New values
Viral load	> 100,000 copies/ml	11,238 copies/ml
Absolute CD4 count	208 cells/ml	302 cells/ml

All routine investigations were within normal limits. The HIV RNA levels showed a reduction, whereas CD-T4 lymphocyte counts had increased. Hence, an IRIS-related

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pathology was suspected. For further work-up, a skin biopsy was done to rule-out KS as it could be one possibility of IRIS. Histopathological examination revealed extensive vascular proliferation and spindle cells. Immuno-histochemistry for human herpesvirus 8 (HHV 8) was positive. A diagnosis of IRIS associated with cutaneous Kaposi sarcoma was made. CT chest and abdomen were done to rule-out dissemination, both were within normal limits. Hence, HAART was continued as there was no evidence of dissemination on further imaging.
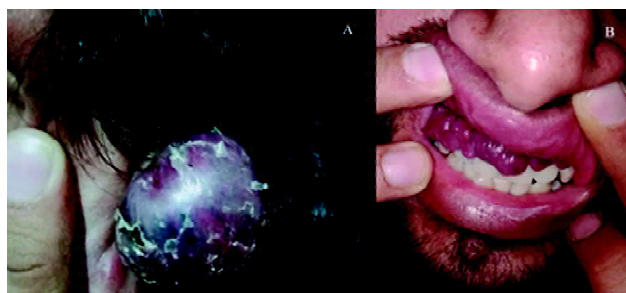
Fig. 2A: *Showing large nodular growth behind the left ear.* **2B:** *Showing gum hypertrophy.*

Discussion

The most common presentation of KS is the HIV-related epidemic type. HIV-KS has been considered an AIDS-defining condition due to its presentation in the setting of severe immunodeficiency with low CD4 T-cell counts and high viral loads. Although there have been case reports that suggested that IRIS-KS occurred in patients with high CD4 counts as well[2]. The pathogenesis of HAART-induced IRIS-KS is characterised by dysregulation of the restored host inflammatory response. HAART causes an increase in CD4+ T-cells and a decrease in HIV viral load which promotes the production of inflammatory cytokines in the host that trigger the expression of HHV-8 gene products into antigens[2]. The production of HHV-8 antigens causes a shift from Th2 (CD4+ T-cell dominant) to Th1 (CD8+ T-cell dominant) immune response, that specifically targets HHV-8 antigen[3]. The strengthened Th2 and Th1 arms of the immune system result in aberrant signalling for excessive inflammation, promotion of angiogenesis, and transformation of endothelial cells by the HHV-8 antigen, all of which contribute to the angio-proliferative manifestations of KS disease. Patients with greater immunodeficiency at the initiation of HAART are at increased risk of developing IRIS, with an incidence reported as high as 25% in patients with a baseline CD4 T-cell count of < 50 cells/mm. Diagnostic criteria for IRIS-induced HIV-KS include a patient on HAART with new, worsening, or recurrent KS lesions in the setting of increased CD4 count greater than or equal to 50 cells/ml or a two-fold increase,

and a decrease in HIV-1 viral load greater than 0.5 log. The time frame for development of KS following initiation of HAART is not clearly defined, although several cases report cutaneous lesions developing within eight to twelve weeks of initiating therapy[2,4,5]. Prognosis in patients with IRIS-associated HIV-KS is promising, particularly in the setting of immunocompetence[6]. The introduction of antiretroviral therapy has led to a decrease in the overall incidence and prevalence of HIV/AIDS-related KS secondary to the recovery of host immune response and reduction of HIV and HHV-8 viral loads. HAART is mainly preventative and therapeutic for clinical HIV-KS, a subset of HIV-seropositive individuals will have onset of new, worsening, or recurrent KS lesions secondary to a paradoxical phenomenon known as immune reconstitution inflammatory syndrome following initiation of antiretroviral therapy[7]. Optimal control of HIV infection by continuing HAART is an integral part of successful therapy, with recommended additional adjunctive local or systemic therapy depending on the extent of the disease. No preventive treatment for KS-IRIS has yet been confirmed. Glucocorticoids are held in reserve for life-threatening cases only, as they may be risky for use in KS-IRIS treatment[8]. Disseminated KS is a rare entity with worst prognosis, though our patient did not have any evidence of dissemination there have been case reports of disseminated KS in HIV patients in India[9]. There have been other case reports about IRIS-KS which further led to complications like Kaposi sarcoma inflammatory cytokine syndrome that further led to poor prognosis and death of the patient[10]. HIV patients who do not receive ART are at a higher risk for progression of KS with a considerable mortality rate. The delay in diagnosis can lead to more opportunistic infections thereby increasing the risk of developing IRIS. Skin lesions in KS can be effortlessly mistaken as haematomas, purpura, angiomas, or naevi. Therefore, it is important to consider HIV-associated cutaneous KS as a differential for any multiple painless reddish skin lesions[11]. IRIS-KS is often not easily identified and may occur in patients who resume HAART after a long gap[8]. There have been many case reports where KS progressed from presenting as skin lesions to further worsening with pulmonary lesions. Such cases had a poorer prognosis[12].

Conclusion

IRIS-associated HIV-KS is a paradoxical immune-inflammatory reaction brought about by improvement in immune status following antiretroviral therapy. In our current era of HAART-controlled HIV disease, dermatologists must remain suspicious of IRIS-associated HIV-KS, regardless of initial CD4+ T-cell count or HIV viral load. Judicious and appropriate screening is recommended for pre-existing KS lesions as well as for evidence of new eruptions following

recovery of the immune system. This condition is best managed with continued disease control on HAART as well as adjunctive local or systemic therapy depending on clinical severity on a case-by-case basis.

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Ring Artefacts in CT Scan: A Red Herring

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Abstract

The computed tomography (CT) scan is an indispensable part of modern medical practice. However, the images are sometimes prone to artefacts, which may confuse the clinician. One such artefact is described here in details along with the diagnostic pitfalls of misinterpretation.

Key words: Neurocysticercosis; ring artefact; detector; radiologist.

Introduction

Computed Tomographic (CT) scan is a very common diagnostic imaging procedure advised by physicians in modern times. This imaging modality is extremely helpful in the detection of a number of pathologies in various organs. Its usefulness has been further demonstrated during the recent Covid-19 pandemic. However, like any other imaging technique, CT scan is also prone to a lot of artefacts[1]. While appearance of such artefacts is fairly common for the radiologists, it is often a source of bafflement for the clinician. But clinicians should also be aware of the radiological appearance of the common artefacts in order to avoid a diagnostic dilemma. We present here one such rare CT scan artefact, which may be a source of considerable confusion for the physician.

The case

A 79-year-old female was admitted with sudden onset unconsciousness following fever. There was no history of convulsion. A non-contrast CT scan done at admission showed multiple ring-like lesions in different parts of the cerebral cortex (Fig. 1). The treating clinician was thinking of neurocysticercosis and a decision was taken to start the patient on anti-helminthic drugs. However, consultation with the radiologist revealed these "lesions" to be nothing more than ring artefacts which were due to some problem with the detector of the CT machine. Meanwhile, the patient's serum sodium levels were found to be 116 mEq/l and correction of that level led to regaining of consciousness. Thus, unnecessary treatment was avoided.

Discussion

There are many different types of artefacts in CT scan imaging, either hardware related or patient related. Common artefacts include motion artefact, noise, pseudo-enhancement, beam hardening and ring artefact[2]. Although

Fig. 1: CT brain of the patient showing ring artefact (Red arrows) in different axial cuts of the brain, simulating neurocysticerosis.

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these vagaries are of interest to the radiologist only, the physician should also have a basic knowledge of their appearances, in order to avoid misinterpretations.

Ring artefact is caused by a miscalibrated or defective detector which creates a bright or dark ring around the centre of rotation[2]. Such artefact can be easily recognised and ignored in imaging of other parts of the body. But in brain CT scan, if of small size, it often simulates pathology like neurocysticercosis or toxoplasmosis. The artefact can be seen either as a full circle or as an arc. *The main clue in detecting this artefact is its presence in the same geometrical location in multiple sequential images[3]*. As seen in our case, the "lesions" were present in multiple sections of the brain, but if observed closely, they were all in the same relative position (Fig. 1). Normal brain pathology would not have this symmetrical appearance.

To eliminate this artefact, the detector needs to be recalibrated or replaced. Sometimes, software-based correction is also possible.

This case is presented with the aim to sensitize clinicians to this rare artefact in a CT scan of the brain. Unless the artefact is recognised, there would be unnecessary prolonged treatment. Another caveat to be learnt from this case is that although clinicians are expected to have a sound skill for the interpretation of CT scan images, it is always a good idea to consult a radiologist for additional inputs.

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Phone (with STD code): .... E-mail: ....

as an Associate Life Member/Life Member/Fellow of the Indian Association of Clinical Medicine.

To the best of our knowledge and belief, the above particulars are correct and we consider him/her a fit and proper person to be admitted as Associate Life Member/Member/Fellow of the 'Association'.

A D.D. No. .... Dated .... drawn on ....

.... for Rs. .... is enclosed herewith.

Signature .... (Proposer)

Name: ....

Fellowship/Membership No.: ....

Signature .... (Seconder)

Name: ....

Fellowship/Membership No.: ....

Subject to approval of the Governing Body, I agree to become Associate Life Member/Member/Fellow, and if admitted, to abide by the Rules and Regulations of the 'Association'.

. . . .
(Signature of the Candidate)

Note by the Hony. General Secretary

- Fellowship Subscription: Rs. 9,000/-
  (Minimum 10 Years Standing after Post-graduate Qualification or 5 years after membership).
- Life Membership Subscription: Rs. 3,000/-
  (Minimum Qualification: Post-graduate Qualification as specified in the Constitution of IACM).
- Associate-Life Membership Subscription: Rs. 3,000/-
  (Diploma, or Post-graduate Qualification not covered as per Constitution of IACM).
- Please attach attested photocopy of Post-graduate Degree/Diploma.
- Cheques shall not be accepted. Demand Draft should be in favour of **"Indian Association of Clinical Medicine"** payable at Agra.

Correspondence Address:
Dr. TP Singh, Hony. Gen. Secretary, IACM, P.G. Department of Medicine, Sarojini Naidu Medical College, Mahatma Gandhi Road, Agra-282 002, U.P.