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HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN CLINICAL SPECTRUM OF INDIAN SICKLE CELL DISEASE PATIENTS

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ABSTRACT

Sickle cell disease (SCD) is a hemoglobinopathy prevalent in Chhattisgarh and other states of central India. In Chhattisgarh, SCD patients and carriers comprise ~9.35% of the population. Objective behind this study was to determine hematological and biochemical parameters along with serum electrolytes in SCD patients. Fifty subjects were included from each of four categories viz., SCD patients in steady state, in vaso-occlusive crisis state, SCD carriers and healthy controls. Hematological, biochemical parameters and serum electrolytes were measured. Statistical analysis was done to find significantly different parameters among four categories (P value ≤0.05). Hemoglobin, erythrocyte count, red cell distribution width, hematocrit, total leukocyte count, serum bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase, serum sodium and potassium were found to be significantly different amongst four groups. Platelet count was decreased during vaso-occlusive crisis (VOC) though not significantly. Serum Sodium and potassium were significantly decreased during vaso-occlusive crisis. Implications of these findings were discussed.

KEYWORD: Sickle cell disease, vaso-occlusive crisis, electrolytes, clinical laboratory parameters

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INTRODUCTION

Sickle cell disease (SCD) is caused by a mutation leading to replacement of hydrophilic glutamic acid residue with hydrophobic valine residue at the 6th position of β globin chain, which causes polymerization of hemoglobin molecules under hypoxic milieu, resulting in rigid sickled erythrocytes.[1] In heterozygous state, the individual is asymptomatic and is referred as sickle cell carrier (HbAS). In homozygous state, referred as SCD (HbSS), sickled erythrocytes have difficulty in passing through the small blood vessels and block them resulting in well-known hemolytic and vaso-occlusive complications characteristic of SCD.[2] In India, SCD is common in central and southern states with distribution in Chhattisgarh, Odisha, Madhya Pradesh, Andhra Pradesh, Maharashtra, Karnataka, Kerala, Tamil Nadu and Gujarat and is considered as the second most common hemoglobinopathy after thalassemia.[3] In Chhattisgarh, SCD patients and carriers comprise about 9.35% of population.[4]Patients with SCD suffer repeated vaso-occlusive events indicated by ischemia and inflammation, in which erythrocytes, leukocytes and platelets play a key role.[5] In spite of the fact that red cell sickling is more prominent during vaso-occlusive crisis (VOC), continuous sickling does take place at a lower rate in the steady state. As a consequence, a considerable proportion of sickled cells is always present in the circulation of SCD patients even in steady state.[6] Various factors affect sickling of erythrocytes including acidic pH that promote water loss through K-Cl co-transport.[7] Repeated sickling and desickling changes the membrane elasticity and permeability that leads to electrolyte imbalance. Erythrocytes which are more prone to sickling tend to become irreversibly sickled cells (ISC).[8] These ISCs are readily phagocytized in spleen affecting blood parameters like hemoglobin, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) and serum bilirubin, liver enzymes viz., aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) and electrolytes etc.[9, 10] Some studies on clinical laboratory parameters including hematological and biochemical parameters in SCD patients have been done in past, but those were limited by non-inclusion of specific phenotypes like steady and VOC states, small range of age groups and small sample size.[11, 12] On this background, this study was aimed to determine the hematological indices and biochemical parameters along with less commonly determined variables such as serum electrolytes in Indian SCD patients.

MATERIALS AND METHODS

(i) Recruitment of subjects

The study was performed at Sickle Cell Institute Chhattisgarh (SCIC), Raipur, India during the period of July 2014 - June 2015. The protocol for the study was approved by Institutional Ethics Committee of SCIC, Raipur and informed consent were taken from all the subjects for participating in this study. Subjects were classified into four categories viz., SCD patients in steady state (HbSS-SS), SCD patients in vaso-occlusive crisis state (HbSS-VOC), SCD carriers (HbAS) and healthy controls (HbAA). Fifty subjects were included from each category. The diagnosis of the of HbSS, HbAS and HbAA individuals was based on results of solubility test, followed by hemoglobin electrophoresis at pH 8.6 on cellulose acetate paper and high performance liquid chromatography (HPLC) using Hb Variant (Bio-Rad). HbSS-SS subjects in were defined as normally active SCD patients, not having fever and skeletal/abdominal pain at presentation and for ≥ 4 weeks after last VOC.[13] Similarly HbSS-VOC subjects were defined as SCD patients having skeletal and/or abdominal pain without clinical or radiological evidence of osteomyelitis or surgical abdomen.[14] Subjects were aged between 8-55 years. Subjects were excluded if undergoing hydroxyurea and/or immunosuppressant therapy, having renal or hepatic insufficiency and blood disorder other than SCD. Subjects having pregnancy or a history of blood transfusion within preceding 3 months were also excluded.

(ii) Sample collection

Five ml of venous blood was collected from each subject through phlebotomy. Out of which, 3 ml was collected in commercially available ethylenediaminetetraacetic acid (EDTA) vial and remaining 2 ml in the plain tube. Hematological indices like hemoglobin, erythrocyte count, MCV, MCH, MCHC, RDW, HCT, total leukocyte count (TLC), and platelet count were measured in Auto Hematology Analyzer BC-3000 Plus (Mindray). Biochemical analyses were performed on ILAB 600 Clinical chemistry analyzer, (Werfen). Similarly, serum sodium (Na^+) and serum potassium (K^+) were measured in ILyte analyzer (Werfen).

(iii) Statistical Analysis

Statistical analysis was done on SPSS 16 (IBM) platform. Mean ± S.D., median, range and percentage were used to express data. Kolmogorov-Smirnov analysis was used to study the distribution of data. Chi-square test and Fischer's exact test were used to compare frequency distributions in various groups. To compare data between groups ANOVA and Kruskal-Wallis test were used. Both the tests were followed by post-hoc analysis using the Tukey HSD test. The sample size was calculated considering the power of the study to be 0.8 and α error to be 0.05 using data available in Chhattisgarh population from previous studies. P value ≤0.05 was considered to be significant.

RESULTS

1. Hematological parameters in SCD

Profile of hematological parameters observed in this study is given in Table 1. Hemoglobin and erythrocyte count were found to be significantly reduced in HbSS-VOC as compared to HbSS-SS, HbAS and HbAA

subjects. Similar significant differences were also found among HbSS-SS with HbAS and HbAA subjects respectively. Interestingly hemoglobin and erythrocyte counts were significantly lower in HbAS as compared to HbAA subjects. MCV was found to be significantly higher among HbSS-SS as compared to HbAA subjects. MCHC showed significant decrease in HbSS-VOC and HbSS-SS subjects as compared to HbAS and HbAA subjects. RDW was found to be significantly increased in HbSS-VOC as compared to HbSS-SS, HbAS and HbAA subjects. RDW was also found to be significantly increased in HbSS-SS and HbAS as compared to HbAA subjects. TLC was found to be significantly higher HbSS-VOC as compared to HBSS-SS, HbAS and HbAA subjects. Similarly TLC was found to be significantly higher among HbSS-SS as compared to HbAA subjects. Hematocrit was found to be significantly reduced in HbSS-VOC as compared to HbSS-SS, HbAS and HbAA subjects. Similar significant differences were also found among HbSS-SS with HbAS and HbAA subjects respectively. Interestingly hematocrit was significantly lower in HbAS as compared to HbAA subjects. Platelet counts were found to be significantly lower in HbSS-SS in comparison to HbAS subjects.

Table 1
Hematological parameters in different study groups

Characteristic	HbSS-VOC (8-42)	HbSS-SS (8-45)	HbAS (10-55)	HbAA (21-55)	p Value
Hb (gm/dl)	7.348 ± 1.86 (3.2-11.4)[a,b,c]	9.72 ± 2.56 (6.2-18.8)[b,c]	11.89 ± 2.51 (3.5-15.8)[c]	13.53 ± 1.86 (10-17.2)	**<0.0001**
RBC count (million cells per microliter)	2.59 ± 0.668 (1-4.19)[a,b,c]	3.106 ± 0.977 (2-8.25)[b, c]	3.853 ± 0.918 (1.9-6.15)[c]	4.626 ± 0.715 (3.4-6.27)	**<.0001**
MCV (fl)	83.8 ± 13 (46.2-109)	88.05 ± 11.9 (11.9-121)[c]	85.3 ± 10.4 (55.9-111)	83.23 ± 9.35 (61.4-102)	0.14
MCHC (g/dL)	35.48 ± 8.44 (28.7-78)[b,c]	34.44 ± 3.54 (25.7-48)[b,c]	35.18 ± 3.39 (27.9-43)	35.61 ± 3.41 (30.5-46.5)	0.6
MCH (pg/cell)	28.96 ± 4.25 (19.5-38.8)	30.35 ± 4.58 (19.9-38.5)	29.93 ± 4.42 (19.2-43)	29.44 ± 3.92 (19.5-37.1)	0.4
RDW (in %)	19.54 ± 5.33 (12.7-41.5)[a,b,c]	17.06 ± 3.45 (12.5-27.5)[c]	16.8 ± 3.86 (11.4-26)[c]	14.23 ± 1.59 (11.9-20.1)	**<.0001**
TLC (cells/ mm^3)	14.34 ± 8.87 (3.4-45.9)[a,b,c]	10.74 ± 4.07 (5.2-24.6)[c]	8.722 ± 3.52 (3.3-20.8)	7.74 ± 1.74 (4.2-12.8)	**<.0001**
Hematocrit (%)	22.02 ± 5.41 (11.2-32.8)[a,b,c]	27.94 ± 7.11 (17.7-55.9)[b,c]	33.88 ± 7.51 (15-55.5)[c]	37.99 ± 6.05 (20.4-49.8)	**<.0001**
platelet count (1000per microliter (µL))	263.9 ± 152 (43-611)	293.3 ± 136 (118-693)[b]	237.1 ± 103 (71-595)	253.5 ± 68.2 (125-457)	0.12

a p value < 0.05 vs HbSS-SS
b p value < 0.05 vs HbAS
c p value< 0.05 vs HbAA

2. Biochemical parameters in SCD

Profile of biochemical parameters observed in this study is given in Table 2. Total bilirubin was found to be significantly increased in HbSS-VOC as compared to HbSS-SS, HbAS and HbAA subjects. Total bilirubin was also found to be significantly increased in HbSS-SS as compared to both HbAS and HbAA subjects. Direct bilirubin was also found to be significantly increased in HbSS-VOC as compared to HbSS-SS, HbAS and HbAA subjects. AST was found to be significantly increased in HbSS-VOC and HbSS-SS as compared to HbAS and HbAA subjects. Similarly, ALT was also found to be significantly increased in HbSS-VOC as compared to HbAS and HbAA subjects. But it was found to be significantly increased in HbSS-SS as compared to HbAA subjects only. ALP was found to be significantly increased in HbSS-VOC as compared to HbSS-SS, HbAS and HbAA subjects. ALP levels in HbSS-SS were found to be significantly increased as compared to HbAA subjects only. Serum Na^+ was found to be significantly decreased in HbSS-VOC as compared to HbSS-SS subjects. On the contrary, serum K^+ was found to be significantly decreased in HbSS-VOC as compared to HbSS-SS, HbAS and HbAA subjects.

Table 2
Biochemical parameters in different study groups

Characteristic	HbSS-VOC (8-42)	HbSS-SS (8-45)	HbAS (10-55)	HbAA (21-55)	SS crisis (8-42)
(T) Bilirubin (mg/dL)	2.89 ± 2.94 (.4-19.1)[a,b,c]	2.135 ± 1.54 (0.04-6.2)[b,c]	1.018 ± 0.588 (0.2-2.6)	0.62 ± 0.237 (0.2-1.1)	**<.0001**
(D) Bilirubin (mg/dL)	0.966 ± 2.16 (.1-10.8)[a,b,c]	0.33 ± 0.154 (0.1-0.7)	0.224 ± 0.117 (0.1-0.5)	0.168 ± 0.06 (0.1-0.4)	**0.0008**
AST (U/L)	53.6 ± 33.2 (4-161)[b,c]	47.04 ± 39.5 (5-210)[b,c]	27.34 ± 13.4 (3-63)	23.36 ± 10.6 (6-69)	**<.0001**
ALT (U/L)	47.68 ± 40.8 (6-180)[b,c]	37.62 ± 21.2 (6-96)[c]	26.34 ± 18.8 (8-97)	22.88 ± 16.2 (8-108)	**<.0001**
ALP (U/L)	136.7 ± 59.9 (17-312)[a,b,c]	98.84 ± 32.3 (42-173)[c]	87.32 ± 28.2 (46-176)	73.54 ± 26.8 (27-169)	**<.0001**
S. sodium (mmol/L)	138 ± 5.66 (128-156)[a]	140.5 ± 2.84 (136-149)	139.9 ± 3.97 (134-151)	139.7 ± 5.05 (121-150)	**0.03**
S. Potassium (mmol/L)	3.82 ± 0.444 (2.5-4.9)[a,b,c]	4.11 ± 0.191 (3.8-4.6)	4.092 ± 0.402 (3.6-5.1)	4.066 ± 0.392 (3.5-4.9)	**0.00019**

a p value < 0.05 vs HbSS-SS
b p value < 0.05 vs HbAS
c p value< 0.05 vs HbAA

DISCUSSION

SCD has diverse phenotypic presentations among different individuals, which depend on environmental and genetic factors. Hematological and biochemical laboratory features have been also found to be variable. Generally SCD patients report to health facilities at an early age due to VOC and pain. But SCD carriers are diagnosed mostly incidentally at a later age. This finding is also represented by the age groups of SCD patients and carriers, who participated in the study. Hemolysis, a constant finding in SCD, is depicted by hemoglobin levels and erythrocyte counts observed in this study.[15] Hemoglobin level and erythrocyte counts are similar to those reported by Khan et al. (2010) and Shrikhande et al. (2007) in central India. But these studies were limited by lack of a group of SCD patients in VOC and healthy controls respectively.[12, 15] Values of MCV, MCH and MCHC observed in this study represent anemia of chronic disease and high MCV in a steady state may be due to macrocytosis.[12] Mean MCV of steady state patients was found to lie in normal range for Indo-Arab haplotype.[16] The decrease in MCV during VOC could be due increase in number of dense cells and dehydration. This decrease in MCV may increase MCHC in VOC as seen in this study. The findings of this study were found to be in coherence with Khan et al., Shrikhande et al. and Kar et al.[15,16] Hyes et al. (1985) also reported similar findings in Jamaican population.[17] Marrow stimulation in SCD results in increased rate of erythropoiesis leading to the release of cells under different stages of maturation, causing anisocytosis, which may be aggravated by VOC leading to a further rise in RDW.[18] The initial phase of VOC is known to be have increased RDW and decreased platelet count.[19,20] Webster et al. (1986) and Qurtom et al. (1989) have also reported RDW to be higher in SCD patients compared to SCD carriers and healthy controls[21, 22] but, they had not considered separate VOC and steady state groups in their studies. Current study has shown significantly higher RDW during VOC compared to steady state. Current study has shown significantly higher TLC in VOC compared to steady state. Higher TLC in SCD patients compared to healthy controls were also noted by Meshram et al. (2014) and Awogu et al. (2000) in Maharashtra (India) and Nigerian population respectively, but not specifically during VOC.[11, 23] Higher TLC can be explained on the basis of demargination of intravascular neutrophils, accelerated release of leucocytes from bone marrow and release of inflammatory mediators during VOC.[24] Significantly lower hematocrit in SCD patients in VOC compared to steady state, SCD carriers and healthy controls may be attributed partly to lower erythrocyte count and sticky erythrocyte surface that accelerates rouleaux formation.[25] Other factors may be lysis of ISCs and increased plasma volume.[26] Platelet count was found to be significantly higher in SCD patients in steady state as compared to healthy controls. These findings are coherent with those of Omoti (2005) and Okpala (2002) in African subjects.[27, 28] Platelet count decreases during VOC, which suggests that platelets are consumed at site of vaso-occlusion.[29] But there was no significant difference between platelet count in SCD patients in VOC and steady state. Findings related to platelet function may be further studied in Indian population. Previous studies assessing serum bilirubin levels and liver enzymes viz., AST, ALT and ALP in SCD have found results similar to those of our study, but none of them considered all the clinical phenotypes related to disease except Chuku et al.[30-32] Higher total bilirubin in SCD patients can be attributed to accelerated hemolysis, resulting elevation in bilirubin that exceeds the clearance capacity of the liver.[33] Results also indicate towards increased rate of conjugation of bilirubin in liver

cells during VOC, but not in steady state or SCD carriers. Elevated AST and ALT levels may be related to damage of liver parenchyma due to intra-hepatic sickling or viral hepatitis and transfusion-related iron overload.[34] A significant rise in ALP in SCD patients during VOC and steady state may be contributed by intra-hepatic cholestasis due to widespread sickling within the sinusoids and increased hemolysis.[35] As rise in AST levels is more pronounced than ALT, an extra-hepatic cause may be involved as lysis of ISCs and bone remodeling and growth in younger SCD patients.[36] Low urinary specific gravity and increased Na^+ loss in urine are common finding in SCD patients. High concentration of Na^+ in urine is noted in these patients both in VOC and steady state, although hyponatremia is noted only during VOC.[37] This hyponatremia along with renal cause is also contributed by alteration in membrane cytoskeleton exhibiting changes in membrane permeability of erythrocytes leading to leakage of K^+ from erythrocytes and entry of Na^+ into erythrocytes.[38, 39] Kidneys try to compensate for this loss by losing K^+ for Na^+ in distal convoluted tubule, but it is not enough because of cross destruction of vasarecta.[40] This along with intermittent mineralocorticoid excess may be the cause of significant decrease in K^+ in our study.[41] Most of our findings are in accordance with previous studies done in same population, though no individual studies have previously included all the biochemical and hematological parameters assessed in our work.

CONCLUSION

Thereby, we conclude that SCD phenotype in crisis showed significantly lower hematocrit and significantly higher RDW, serum bilirubin and liver enzymes. Further, levels of electrolytes in serum should not just be seen as a result of erythrocyte membrane defects, but as a complex inter-play of hematological and renal functions in SCD patients. Since the management of VOC primarily consists of maintaining adequate fluid balance, levels of electrolytes should be considered as an objective sign to assess the state of recovery of patients. High dose of Na^+ and K^+ is required for compensation of the losses. Further clinical studies are required for calculating the amount of Na^+ and K^+ to be given per kg body weight. Studies may to be done to assess the effect of supplementing one ion on the level of another. Further studies may be designed to analyze the utility of MCV as a predictive factor for indicating dehydration that may induce VOC.

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