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IRON PROFILE PARAMETERS STATUS IN SICKLE CELL DISEASE PATIENTS: A CASE CONTROL STUDY FROM CHHATTISGARH INDIA

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ABSTRACT Sickle cell disease subjects are at risk of imbalance in iron and related parameters due to hemolytic pathophysiology and bone marrow stress. Frequent blood transfusion further causes the imbalance to escalate. We tried to assess various hematological parameters in Sickle cell disease subjects in state of Chhattisgarh and compare the parameters in Sickle cell trait and normal subjects. **Materials and methods:** 200 subjects each with sickle cell disease (SCD), Sickle cell trait (SCT) and Normal healthy control subjects (C) as per High performance liquid chromatography and electrophoresis reports. All the subjects were assessed with hematological and iron profile parameters. These parameters were compared between groups. Results: All the hematological parameters were found to be significantly different between study groups. On post hoc analysis, Hb and RBC were found to be significantly lower in study groups SS as compared to AA and AS, while WBC was found to be significantly higher in study groups SS as compared to AA and AS. Frequency of low S.iron levels, high S. ferritin levels high TIBC levels and low transferrin saturation levels was significantly higher in SS group. Conclusion: Serum transferrin is significantly reduced while serum ferritin is significantly increased in sickle cell disease subjects. This indicates high saturation of iron in sickle cell disease subjects. In the backdrop of frequent blood transfusion required in SCD subjects, this finding must be kept in mind while administrating blood transfusion.

KEYWORDS : Sickle cell Disease, Sickle cell trait, Ferritin, Transferrin, Iron.

INTRODUCTION

Sickle cell disease (SCD), characterized by presence of abnormal structural variant of haemoglobin (Hb) named as sickle haemoglobin (HbS), is the first genetic disease with known molecular basis. HbS is a structural variant of Hb where Glutamic acid, an amino acid at position no. 6 of beta globin chain of Hb, gets replaced by Valine. The reason being, change of nucleotide, Adenine to Thymine (GAG replaced by GTG) of codon b of the beta-globin gene which is located on short arm of chromosome number 11. Sickle Hb gets polymerized at low oxygen tension and causes deformation of the RBC from discoid shape to sickle like form. (Akinbami, 2013) About 50% of SCD subjects in world populations are found in India. (Akinbami, 2013)

Sickle cell Anaemia (SCA) characterized by red blood cells (RBCs) deformity result in chronic haemolytic anaemia, infarctive crisis, frequent infections and risks of serious complications.(Andrews, 2013) In the homozygous state of the disease, both the β-globin genes are mutated (HbSS) and the individuals suffer from life-long severe haemolytic anaemia, attacks of pain crisis,chronic organ system damage and marked decrease in life expectancy. In heterozygous (HbAS) condition, both normal and mutated hemoglobin are produced. These individuals do not experience symptoms, are generally healthy and are said to be sickle cell traits (SCT). (Khodiar, 2016)

Iron deficiency may occur in patients with SCDs as a SCA patient is not immune to environmental factors that precipitate IDA. Such factors include poor nutrition and parasitic infestations and varying bacterial infections, which may disturb iron metabolism. In addition, excessive urinary iron loss, poor absorption, and metabolism of iron owing to multiple mucosal/submucosal infarcts, and progressive multiple organ damage/failure make SCA patients highly susceptible to IDA. Alternatively, iron-overload due to repeated transfusion is known to cause hemochromatosis leading to serious damage to body tissues. Therefore, maintaining an appropriate level of iron in the body of sickle patients is essential. (Koury, 2004)

Inthis study we tried to assess various hemtological and iron profile parameters in SCD, SCT and control subjects and compare them.

MATERIALS AND METHODS

This hospital based, observational case control study was conducted in the Department of Biochemistry, Pt. J.N.M. Medical College and DR. B.R.A.M. Hospital, Raipur, C.G. among patients who attended the Sickle Cell OPD, Department of Biochemistry, Pt. J. N. M. Medical College and Inpatient ward of Paediatrics department, Pt. J. N. M. Medical College. The study was approved by the institutional ethical committee of Pt. J. N. M. Medical College. All the guidelines of Declaration of Helsinki were followed during the study. Study recruited 200 patients with SCD and 200 SCT patients with and 200 age and gender matched normal healthy controls. Patients of sickle cell anemia (HbSS) and sickle cell trait (HbAS), confirmed on Hb electrophoresis / HPLC were included in the study, Patients who were on hydroxyurea treatment and had recent blood transfusion in last 6 months were excluded from study. Other exclusion criteria were, patients with hemoglobinopathies other than SCD and SCT. Patients on iron supplementation therapy (including iron-fortified vitamins), iron chelation therapy and anticoagulant therapy. Patients with history of any active bleeding disorders like bleeding piles, peptic ulcer disease etc., or, any coagulation disorder, or, history of any major trauma, surgery, or blood loss in last 3 months, pregnancy, lactation, history of menstrual irregularities such as menorrhagia, and patients on oral contraceptives, patients with hepatitis, hemochromatosis, hemosiderosis, acute inflammation (respiratory infection), abscess, immunization, chronic inflammation or malignancy were also excluded.

After obtaining informed written consent, the study recruits were subjected to detailed clinical history and thorough examination. Any investigations available with them was also assessed. About 4 ml venous blood sample was collected with asceptic precautions.1.0 ml of the blood was transferred into EDTA vials and was usedfor determination of complete blood count. Remaining blood sample was allowed to clot and serum was separated. And was used for estimation of iron parameters. Serum Iron and TIBC was measured by Ferrozine/Magnesium Carbonate method using fully automated biochemistry I-Lab 650i (Werfen© Germany)autoanalyzer.Serum Ferritinwas measured by Electrochemiluminiscence (Roche cobas© e411, Roche Diagnostics, Mannheim, Germany) Percentage Transferrin Saturation was calculated as: 100 X (serum Iron/TIBC) Complete blood cell count: By an Cobas m 511© integrated 5 part

hematology analyser (Roche Diagnostics, Mannheim, Germany).

Statistical analysis was done using Microsoft Excel and IBM software SPSS v16.0. The analysis of the results was done by Chi-square test,ANOVA.A'p' value of <0.05 was considered significant.

RESULTS

The study group consisted of total 600 subjects (200 HbSS, 200 HbAS, 200 HbAA). Comparison of various hematological parameters between study groups was performed using ANOVA. All the parameters were found to be significantly different between study groups. On post hoc analysis, Hb and RBC were found to be significantly lower in study groups SS as compared to AA and AS(p = <0.0001 and < 0.0001 respectively)while WBC was found to be significantly higher in study groups SS as compared to AA and AS (p = <0.0001). (table 1)

Table: 1 Comparison of various hematological parameters between study groups

Parameters	Group	Mean	S.D.	S.E.	F	p value
Hb (%)	AA	13.23	2.33	0.16	247.77	<0. 0001
	AS	12.58[a]	1.87	0.13		
	SS	8.93[a,b]	2.02	0.14		
	Total	11.58	2.81	0.11		
RBC (10^6/mm^3)	AA	5.26	0.89	0.06	139.03	<0.0001
	AS	5.37	0.93	0.07		
	SS	3.93[a,b]	1.06	0.07		
	Total	4.85	1.16	0.05		
MCV (fl)	AA	84.32	8.79	0.62	11.23	<0.0001
	AS	81.82[a]	8.38	0.59		
	SS	80.26[a]	8.78	0.62		
	Total	82.13	8.80	0.36		
MCH (pg)	AA	25.45	3.85	0.27	4.76	0.009
	AS	24.28[a]	4.12	0.29		
	SS	24.83	3.47	0.25		
	Total	24.85	3.85	0.16		
MCHC (gm/dl)	AA	30.11	2.82	0.20	11.31	<0.0001
	AS	28.94[a]	2.80	0.20		
	SS	28.94[a]	2.95	0.21		
	Total	29.33	2.91	0.12		
WBC (10^3/mm^3)	AA	7.10	2.17	0.15	55.09	<0.0001
	AS	7.77	2.40	0.17		
	SS	10.67[a,b]	5.36	0.38		
	Total	8.51	3.93	0.16		
PLT (10^5/mm^3)	AA	2.62	0.92	0.07	9.542	<0.0001
	AS	2.74[a]	0.85	0.06		
	SS	3.01[a,b]	0.95	0.07		
	Total	2.79	0.92	0.04		

a P<0.05 Vs AA, b P<0.05 Vs AS

Comparison of various iron parameters between study groups was performed using ANOVA. All the parameters were found to be significantly different between study groups. Serum Iron and Transferrin saturation were found to be significantly lower in study groups compared to AA and AS (p= < 0.0001 and <0.0001 respectively) while TIBC and Serum Ferritin were found to be significantly higher in study group as compared to AA and AS (p = < 0.0001 and < 0.0001 respectively) (Table 2)

Table 2: Comparison of various iron profile parameters between study groups

Parameters	Groups	Mean	S.D.	S.E.	F	p value
S. Iron (mcg/dl)	AA	153.44	68.53	4.85	65.02	<0.0001
	AS	124.40[a]	82.19	5.81		
	SS	76.61[a,b]	49.36	3.49		
	Total	118.15	74.96	3.06		
TIBC (mcg/dl)	AA	140.30	41.59	2.94	57.48	<0.0001
	AS	134.09	32.76	2.32		
	SS	313.40[a,b]	324.55	22.95		
	Total	195.93	206.99	8.45		
Ferritin (ng/ml)	AA	112.63	64.50	4.56	72.82	<0.0001
	AS	92.01	98.51	6.97		
	SS	390.36[a,b]	463.53	32.78		
	Total	198.33	307.45	12.55		
Transferrin saturation (%)	AA	115.43	52.29	3.70	42.07	<0.0001
	AS	95.24[a]	59.76	4.23		
	SS	62.18[a,b]	62.64	4.46		
	Total	91.09	62.26	2.55		

a P<0.05 Vs AA, b P<0.05 Vs AS

Various iron profile parameters status was compared between study groups. Significant difference was found between groups regarding frequency of iron status. Frequency of low S.iron levels (x2=11.8 p= 0.19) (Fig 1), high S. ferritin levels (x2=111.6 p<0.0001) (Fig 2), high TIBC levels (x2=134.8 p<0.0001) (Fig 3) and low transferrin saturation levels (x2=151.2, p<0.0001) (Fig 4) was significantly higher in SS group.

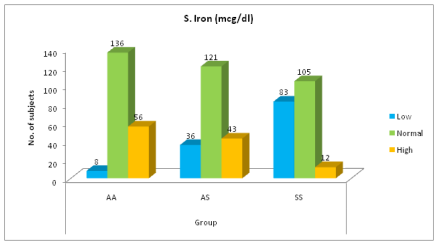

Fig. 1: S. Iron in concentration in different study groups.

AA, AS and SS represent control, sickle cell carrier, sickle cell disease patients respectively.

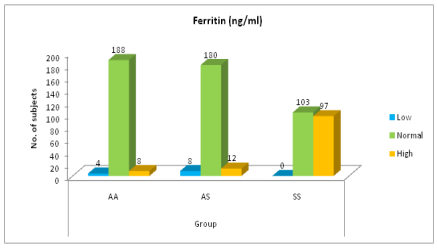

Fig. 2: S. Ferritin concentration in different study groups.

AA, AS and SS represent control, sickle cell carrier, sickle cell disease patients respectively.

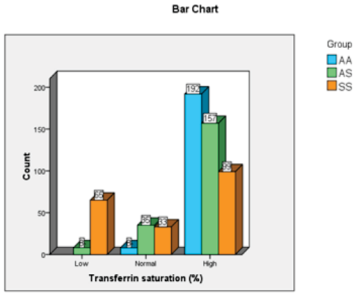

Fig. 3: Transferrin saturation and TIBC in different study groups.

AA, AS and SS represent control, sickle cell carrier, sickle cell disease patients respectively.

DISCUSSION

It has been estimated that about 50% of the total world population of SCD patients resides in India especially in the central zone.(Khan , 2010)Chhattisgarh is a newly created state of central India and most of the people of this region either belong to the tribal or backward classes. Occurrence of SCD is found to be very high in this state. In this current study, we attempted to show the iron profile status and hematological parameter of SCD and carrier states of patients visiting Sickle cell OPD, Pt.J.N.M.M.C.,Raipur(C.G.) Iron plays a central role in erythropoiesis and many other intracellular processes in all the tissues of the body.(Koury, 2004)Although iron is an important element required by the human body, the control of this necessary but potentially toxic substance is an important aspects of human health anddisease.(Andrew, 2008)Iron deficiency leads to decrease in the amount of red blood cells or haemoglobin. (Stedman's Medical Dictionary, 2006)Iron overload on the other hand leads to toxicity and

cell death via free radical formation and lipid peroxidation. (Schrier, 2011)

There are several reasons to believe that iron deficiency may occur in patients with SCDs as a SCA patient is not immune to environmental factors that precipitate IDA. Such factors include poor nutrition and parasitic infestations and varying bacterial infections, which may disturb iron metabolism. In addition, excessive urinary iron loss, poor absorption, and metabolism of iron owing to multiple mucosal/submucosal infarcts, and progressive multiple organ damage/failure make SCA patients highly susceptible to IDA.(Patel, 2016)

Iron deficiency or overload, complicating SCA, is likely to worsen the clinical state of the disease. (Koury, 2004)Recently, serum ferritin is considered as an effective indicator of the iron status of the body. (Patra, 2012)

In this study, serum iron was significantly lower in sickle cell disease subjects when compared with control subjects and SCT, this agrees with studies conducted by Akinsegun et al. J.A. Olaniyi et al.and Dr. J. P. Warade et al.(Akinsegun 2013; Olaniyi, 2014 and Warade, 2010)

TIBC was significantly increased in sickle cell disease subjects in comparison to control subjects and sickle cell carriers and this goes with the study conducted by Dr.J. P. Warade et al.[13] andChandni Patel et al. (Warade, 2010 and Patel, 2016)

A higher fold increase in ferritin level in sickle cell disease patients has also been noticed in the present study. This may be because chronic haemolysis causes increased absorption of iron from gastrointestinal tract. (Ballas, 2001)This agrees with the study conducted by Mishra et al., Khodiar et al., and Olaniyi et al.(Mishra, 2015; Khodiar, 2016 and Olaniyi, 2014)

Transferrin saturation(%) was significantly lower in sickle cell disease subjects when compared with control subjects and SCT.

In this study, the mean haemoglobin levels were also found to be significantly decreased in sickle cell disease patients as compared to sickle cell carrier and normal subjects and this agrees with studies conducted by Khodiar et al , Mohanty et aland Olaniyi et al.(Khodiar, 2016; Olaniyi, 2014 and Mohanty, 1998)

CONCLUSION

The present study shows that there is a difference in serum iron level, TIBC, transferrin saturation, and serum ferritin level in Sickle Cell Disease and Sickle Cell Carrier patients.

The serum iron level in SCD is found to be 51.1% less as compared to normal subjects and 31.17% less as compared to SCT. TIBC level in SCD is found to be more than twice as compared to normal controls and SCT.

The serum ferritin level is also found to be nearly thrice in SCD as compared to normal Controls and nearly more than thrice as compared to SCT. Transferrin saturation in SCD is found to be 46.14% less as compared to normal subjects and 28.64% less as compared to SCT.

The serum ferritin was the most important marker in the present study and is a measure of available iron stores in the body. It is significantly increased in Sickle Cell Disease patients. This shows patients are iron overloaded and iron supplement should be deferred in them. This iron overload can be prevented by exchange transfusion rather than conventional transfusion. Medical management of iron overload would be chelation therapy, which is indicated when the serum ferritin level > 1000 ng/d.

Conflict of interest : Authors declere that they have no conflict of interest.

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