A study of serum iron level and oxidative stress in the steady state of sickle cell of anaemia

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Abstract

Background: Sickle cell anaemia (SCA) is a hereditary disorder, associated with severe haemolytic anaemia, periodical vasooclussive pain, and premature death. Prevalence of SCA is alarmingly high in north Maharashtra comprising districts of Nandurbar, Dhule, Jalgaon and Nashik. Non heme iron and depleted erythrocytic glutathione are associated with generation of free radicals leading to oxidative stress. Oxidative stress may enhance haemolysis by damaging erythrocytic membrane.

Aim and Objectives: To evaluate serum iron, erythrocytic MDA and erythrocytic Glutathione in relation with possible markers of oxidative stress in the steady state of SCA individuals.

Methods: A total of 90 subjects were recruited in the study including 30 sickle cell trait (HbAS), 30 sickle cell disease (HbSS) and 30 healthy controls. In all subjects serum iron and bilirubin, erythrocytic Malondialdehyde (MDA) and glutathione (GSH) concentration were investigated simultaneously.

Results: Serum iron level found significantly ($p<0.001$) lowered in sickle cell trait (HbAS) while significantly ($p<0.001$) elevated in sickle cell disease (HbSS) compared to control. Serum bilirubin and Erythrocytic MDA found significantly ($p<0.001$) elevated in HbAS and HbSS. Erythrocytic glutathione was found significantly ($p<0.001$) depleted in both HbAS and HbSS in comparison of control. There is positive correlation between erythrocytic MDA and serum iron.

Conclusion: Our study suggests, elevated serum iron and bilirubin is result of excessive hemolysis. Depleted Erythrocytic glutathione and elevated malondialdehyde reveals hyphened oxidative stress, further which could be sickling and hemolytic propagator aspect in sickle cell anaemia.

Keywords: Iron, Glutathione, Malondialdehyde, Sickling, Oxidative stress

Introduction

Sickle cell anemia (SCA) is a genetic disorder characterized by severs hemolytic anemia, repeated vaso-occlussive crisis, high morbidity, and mortality. The prevalence of sickle cell anaemia is alarmingly high in the districts of Nandurbar, Dhule, Jalgaon, Gadchiroli, and Nagpur in Maharashtra. In the peripheral areas of Nandurbar and Dhule districts sickling disorder is seen predominantly in the Pawara and Bhil communities of the tribal population.[1]

SCA results from gene encoding for substitution of valine for glutamic acid at the 6th position in the β-globin chain of haemoglobin (Hb), resulting in an abnormal globin $β^S$. This results in the transformation of normal haemoglobin HbAA ($α_2β_2$) to 'sickle haemoglobin' HbS ($α_2β S_2$). Upon deoxygenation, HbS undergoes aggregation and polymerization, thus changing the discoidal erythrocyte into a crescent or sickle shape.[1] In sickle cell anemia variable degree of haemolysis and intermittent vaso-occlusion leads to chronic organ damage involving the spleen, brain, bone and the penis. The consequences of the haemolysis include chronic anaemia, jaundice, predisposition to an aplastic crisis, and cholelithiasis. Delayed growth, dactilytis, acute chest syndrome, stroke, priapism and leg ulceration are common in sickle cell anaemia.

Iron deficiency was reported in sickle cell disease.[2,3,4] Iron deficiency anaemia was found to be more common in sickle cell anaemia patients as compared to sickle cell trait.[5] Nutritional inadequacies and excessive urinary iron loss may cause iron deficiency anaemia in sickle cell disease patients.[6] However, iron deficiency is not expected to be present in hereditary anaemias like thalassemias and sickle cell anaemia since there is a premature destruction of erythrocytes, which contributes towards iron stores and reutilization.[7] It has been also hypothesized that iron deficiency might be beneficial to the sickle cell patient by reducing the percentage of sickle cells, thus reducing painful crisis.[8]

Erythrocytes become more vulnerable during the sickling, the denatured haemoglobin releases iron, which may produce free radicals through Fenton's reaction. These free radicals target the erythrocyte membrane by initiating lipid peroxidation, which may be involved in the progression of sickling, thereby converting the reversible sickle cells (RSC) into irreversible sickle cells (ISC), thus leading to occlusion and haemolytic consequences.[9] It was also proposed that high levels of non heme iron found in erythrocyte membrane due to the deficiency of GSH.[10] The sickle erythrocytes generate approximately two times more amounts than usual of superoxide, peroxide, and

hydroxyl radicals.[11] Thus, oxidative stress may play a major role in the pathogenesis of sickle cell anaemia by enhancing the sickling phenomena.

In this view, the present study was aimed to measure the levels of serum iron, erythrocytic MDA and glutathione in the steady state of sickle cell anaemia individuals to find out any relation between serum iron with oxidative stress in SCA.

Material and Methods

The present study was carried out in the Department of Biochemistry, ACPM Medical College, Dhule and the Medicare Hospital, Nandurbar, Maharashtra. Institutional ethical permission was obtained prior to start the study. A total population of 90 subjects were enrolled in the study, including 30 (15 males and 15 females) heterozygous (HbAS) and 30 (15 males and 15 females) homozygous (HbSS) sickle cell patients and 30 age and sex matched (15 males and 15 females) healthy controls (HbAA) on the basis of the solubility test and the HPLC analysis of blood. The subjects were excluded from the study by using a criteria of age <15 years, other than the HbAS and the HbSS pattern, the past three month's history of crisis, blood transfusion, treatment with hydroxyurea, antioxidant drugs and pregnancy.

Written information along with oral explanation was given in local language regarding the nature and impact of study. After obtaining a written consent from subjects who were recruited in the study, 5 ml of blood was withdrawn aseptically from the antecubital vein. From this, approximately 2 ml of blood in an EDTA (0.47 mol/L K3-EDTA) container and 3 ml blood in a plain container were drawn. The samples were centrifuged at 3000 rpm for 10 min to separate RBCs, and serum. Serum iron estimated with kit based on the reduction of ferrozine under acidic condition, while serum bilirubin was measured by kit based on reaction with diazo reagent. Erythrocytic MDA measured by method Kei Satoh where erythrocytic lysate was treated with thiobarbituric acid in boiling water-bath forming pink colour.[12] Erythrocyte GSH was measured following the method of Beutler based on the ability of the −SH group to reduce 5,5-dithiobis,2-nitrobenzoic acid (DTNB) and form a yellow coloured anionic product.[13] The statistical analysis was carried out by using the SPSS (Statistical Package for Social Sciences) statistical software, version 16.0 for Windows. Paired and unpaired Student's t tests were applied for the significance and the results were expressed in mean values with SD. <0.05 P values were considered as a significant difference. Pearson's correlation used for correlation analysis.

Results

Table 1: Distribution of subjects

	Control (HbAA)	Sickle cell heterozygous (HbAS)	Sickle cell homozygous (HbSS)
Total numbers	30	30	30
Age	15-50	15-50	15-50
Male	15	15	15
Female	15	15	15

Table 2: Showing values of haemoglobin, serum iron, serum bilirubin, erythrocytic MDA, glutathione, serum iron in control (HbAA), sickle cell heterozygous (HbAS) and sickle cell homozygous (HbSS)

Type of parameter	Control (HbAA)	Sickle cell heterozygous (HbAS)	Sickle cell homozygous (HbSS)	P value HbAA Vs HbAS	P value HbAS Vs HbSS
Hb (gm/dl)	12.26±1.24	7.78±1.04	7.2±1.54	<0.001	<0.01
Serum Bilirubin (mg/dl)	0.6±0.2	2.22±0.54	5.03±1.12	<0.001	<0.001
Serum iron µg/dl	115.22±9.58	109.1±23.94	142.4±9.8	<0.001	<0.001
Erythrocytic MDA (nmol/gmHb)	2.74±0.94	16.09±7.22	25.66±11.2	<0.001	<0.001
Erythrocytic glutathione µmol/l	8.3±0.44	6.47±0.17	6.12±0.29	<0.001	<0.001

Table 2 shows significantly (p<0.001) decreased haemoglobin in sickle cell disease (HbSS) compared to sickle trait (HbAS) and controls (HbAA). Significantly (p<0.001) increased serum iron level is noted in sickle cell disease group compared to sickle cell trait and control group. Serum bilirubin is elevated significantly (p<0.001) in sickle cell disease compared to sickle trait and control. Erythrocytic MDA level significantly increased (p<0.001) in sickle cell disease and sickle trait compared to control. Erythrocytic glutathione level is depleted significantly (P<0.001) in sickle trait and sickle cell disease group compared to the control group. There was a positive correlation (r = 0.54) between serum iron and erythrocytic MDA, while a negative correlation (r = - 0.25) was found between serum iron level and erythrocytic glutathione in sickle cell disease (HbSS) subjects.

Discussion

Iron is precious element for human life, however its excess may make worrisome at cellular level. In our study, we found depleted serum iron level in sickle cell trait (HbAS) group while elevated in sickle cell disease (HbSS) group compared to healthy controls.

Significantly increased serum iron levels reported in homozygous young Jamaican children compared with age and sex matched controls[14] which support our results. Few studies have observed decreased level of iron in homozygous sickle cell patients.[15,16] However, a study reported 4.5% with iron deficiency and 21.3% with iron overload in study population of sickle cell disease.[17]

SCD is an inherited disorder of hemoglobin synthesis characterized by life-long hemolytic anemia, increased erythropoiesis and a chronic inflammatory state with endothelial activation and enhanced red cell and leukocyte adhesion.[18] There is no evidence of iron overload in non-transfused SCD patients, and iron deficiency may be even develop, possibly related to intravascular hemolysis (which constitutes about a third of the hemolysis in SCD), and the resulting excessive urinary losses of iron.[19]

It has been stated that overt iron deficiency may be associated with a marked reduction in the number of sickled erythrocytes in blood smears and a decrease in the levels of serum indirect bilirubin and lactate dehydrogenase.[7] Supporting this, it has been hypothesized that iron deficiency might be beneficial to the sickle cell patient by reducing the percentage of sickle cells, thus reducing painful crisis.[8]

In the present study we noted significantly elevated erythrocytic MDA, might be due to free non heme iron. It is hypothesized that due to decompartmentalized iron, sickle erythrocyte membrane show tenfold response to peroxidation membrane compared to normal.[20] It was also reported that nine fold increased iron was associated with erythrocyte membrane compared normal red cells. This non-heme iron is involved in at least three processes which are potential consequence of this association. First, by acting as a Fenton reagent, membrane associated iron could target the generation of the highly reactive hydroxyl radical directly adjacent to membrane components.[21] Second, iron can catalyze the reinitiation of lipid peroxidation by breaking down the lipid hydroperoxides[22] resulting from the prior peroxidation-terminating action of vitamin E.[23] Third; under certain circumstances iron is capable of actually initiating lipid peroxidation. Heme iron and non heme iron is involved in oxidant damage of tissue[24]. Positive correlation stated between serum iron levels with elevated oxidative stress in SCA patients compared to control[25] which support findings of our study.

Glutathione [γ-glutamylocysteinyloglycin], tripeptide in reduced form (GSH) protects cell from oxidative stress by acting coenzyme antioxidant with glutathione peroxidase.[26] Our study shows significantly depleted glutathione in both sickle cell trait and sickle cell disease groups, as well as inverse correlation between serum iron and erythrocytic glutathione suggesting that increased free radicals are responsible for the depletion of glutathione in erythrocytes. This may be a causative factor for hemolysis which further leads to elevation in serum iron level. It was proposed that high level of non heme iron found in membrane was due to the deficiency of GSH. GSH is able to degrade heme at pH 7.0 which requires oxygen. This was inhibited by superoxide dismutase and catalase implicating involvement of perferryl reactive species in the heme degradation.[10] The amounts of activated oxygen detected would also depend upon the integrity of cellular antioxidant systems. Thus, increased OH$^{\bullet}$ generation by HbS RBC may also reflect their possible deficiency of glutathione.[27]

It was also observed that decreased level of glutathione stating susceptibility of erythrocytes to oxidative osmotic stress sickle cell patients which may enhance in sickling process.[28] Decreased glutathione level was reported in whole blood samples of SCA patients with and without vaso-occlussive crisis[30] which is parallel to our observation. Depressed activity of glutathione reductase was seen in SCA patients causing deficiency of GSH. It is conceivable that depressed erythrocytic glutathione reductase may have accelerating effect on polymerization on deoxy HbS molecule or inhibitory on mechanism that protect erythrocyte from oxidative stress.[30]

In the present study, elevated serum iron is an index of hemolysis and depleted erythrocytic glutathione suggests hyphened oxidative stress in erythrocytes. Overall study shows free iron and depleted glutathione may make erythrocytes more vulnerable during sickling. Antioxidant supplementation can improve the redox status of cells and could prolong transformation of reversible sickle erythrocytes to irreversible sickle erythrocyte. By this complications of sickling can be reduced to a certain extent and living of sickle cell patient maintained healthy.

Conclusion

We found elevated level of iron in sickle cell disease compared to sickle cell trait and control. Positive correlation between serum iron and erythrocytic MDA, inverse correlation between serum iron and erythrocytic glutathione in sickle cell disease reveals elevated oxidative stress in erythrocytes. These could be markers of oxidative stress and aspects sickling phenomena in sickle cell anaemia. Further study on serum iron status and oxidative stress is warranted in larger number of patients in steady state as well as in crisis. The present study also recommends to supplementation of antioxidants that will be helpful in delaying in formation of irreversible sickled erythrocytes and to reduce sickling consequences.

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