Original Research Article

The association of circulating soluble adhesive factor and markers of oxidative stress in patients of sickle cell anaemia

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Abstract

Background:SCD is caused by a variant of the β-globin gene called sickle haemoglobin (HbS).It is one of the major condition leading to place an increasing demand on health services of India. Many prognostic and diagnostic markers have been studied so far for sickle cell disease. **Objectives:** To find out the relationship between circulating adhesive molecule and oxidative stress (MDA, SOD and Catalase) in sickle cell disease patients with HbAS and HbSS pattern. **Material and methods**: 50 pateints with HbSS pattern, 50 patients with HbAS pattern diagnosed by Hb Elecrophoresis were selected along with 50 healthy subjects with HbAA pattern (controls) were included in the study. E- selectin estimated by sandwitch ELISA and MDA, SOD and Catalase were estimated by specrophotometric chemical methods. Pearson correlation was used assess the correlation .**Results:**. There was significant positive correlation of MDA with E-selectin in HbAS subjects also it was found that there was significant negative correlation of SOD with E-selectin in HbAS subjects and significant negative correlation of Catalase with E-selectin in HbSS subjects.**Conclusion:** The study suggests that an excess of ROS certainly have implications in SCD pathophysiology, the assessment of oxidative stress in these patients may provide significant information regarding the use of current medications and may lead to new therapeutic strategies. Additional studies are needed to test the probable mechanisms involved in this complex network of markers and their role in SCD pathogenesis.

Keywords: SCD (sickle cell disease), E- selectin, MDA (malonaldehyde), SOD (superoxide dismutase), Catalase.

Introduction

The most common inherited blood disorders include hemophilia, von Willebrand disease, thrombophilia, thalassemia and sickle cell anemia. Out of which Sickle cell gene is found amongst different tribal groups mainly of central and southern parts of India, which varies from 5 to 34% of their population.[1] A to T transversion in the 6th codon of the human β-globin gene this point mutation is the molecular basis for sickle cell disease which changes a polar glutamic acid residue to a non-polar valine in the β- globin polypeptide and thus drastically decreases the solubility of this sickle haemoglobin.[2] In patients with sickle cell disease (SCD), due to instability and insolubility of HbS, the polymerization of the deoxy HbS makes the RBCs nondeformable to traverse the microcirculation. Result of which patients with SCD suffer repeated vasoocclusive events characterized by ischemia-reperfusion injury and inflammation.[3] Thrombotic events, including strokes, avascular necrosis, and pulmonary emboli, are seen commony in SCD. All of these changes increase the expression of endothelial cell-adhesion molecules and the synthesis of inflammatory cytokines, causing leucocytosis.[4] E-selectin mediates the adhesion of neutrophils to activated vascular endothelium and may function as a tissue-specific homing receptor for T cell subsets.[5] Oxidative stress is described as an imbalance between oxidants/free radicals and antioxidants it contribute to vaso-occlusion with ischaemia/ reperfusion injury and haemolytic anaemia.[6] Oxidative stress can promote adherence of sickled blood cell to the endothelium, while the supplementation of antioxidants can reduce the expression of adhesion molecules. The interaction between sickle red blood cells and endothelial cells is associated with a threefold increase in oxidative stress.[7] Monitoring oxidative stress involves different parameters associated to pro-oxidant and antioxidant biomarkers. The most important defence mechanisms against ROS include enzymatic SOD,catalase,glutathione peroxidase(GPx), peroxiredoxin (Prx)) as well as non-enzymatic systems reduced glutathione (GSH), ubiquinols, uric acid, vitamins C and E, flavonoids, carotenoids.[8] Based on the background objectives of the study are to investigate the the relation of soluble E-Selectin and oxidative stress marker like MDA, SOD and Catalase in patients of sickle cell disease and compare them in three groups HbAS , HbSS and HbAA (controls).

Material and Method

For this study We randomly selected 100 adult belonging to age group of 15 – 40 years sickle cell patients from the sickle cell clinic run in the Acharya Vinoba Bhave Rural Hospital and Jawaharlal Nehru Medical College, Sawangi (Meghe) Wardha in the year 2013. They were diagnose by haemoglobin electrophoresis as HbAS- 50 and HbSS – 50. 50 age sex matched healthy subjects having sickling test negative and having Hemoglobin electrophoresis pattern as 'AA' included as controls in the study. Haemoglobin electrophoresis was done by cellulose acetate paper. E selectin was done by using sandwitch ELISA kit. Oxidative markers were estimated by spectrophotometric chemical methods respectively MDA by Buege and Aust, 1978, [9] Catalase by Aebi 1984 [10] and SOD by Marklund and Marklund,1974[11] To assess the degree of association between the variables studied, the Pearson correlation

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was used. The software used in the analysis was SPSS 17.0 version. The results were expressed as mean ± standard deviation (Mean ± SD). The results were represented in the form of tables and p< 0.05 was considered to be significant. The study protocol was approved by Institutional Ethical Committee. (Ref.No.DMIMS (DU)/IEC/ 2012-13/829).

Results

Mean MDA levels were significantly higher in HbAS (2.77±0.75) as compared to HbAA (1.68± 0.73) which was further significantly higher in HbSS subjects (4.34± 0.93) as compared to HbAS & HbAA. Mean Catalase levels were significantly lower in HbAS subjects (36.04±8.80) as compared to HbAA (48.52± 9.88) which was further significantly lower in HbSS subjects (23.14± 7.047) as compared to HbAS & HbAA. Mean SOD levels were significantly lower in HbAS subjects (2.61±0.60) as compared to HbAA (3.66± 0.61) which was further significantly lower in HbSS subjects (1.44± 0.30) as compared to HbAS & HbAA

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Table 1: Correlation of E-Selectin (ng/dl) with oxidative stress markers in AS pattern

Parameters	Mean	Std. Deviation	N	Correlation 'r'	p-value
E- selectin(ng/dl)	34.95	10.50	50	-	-
MDA(uMol/L)	2.77	0.75	50	0.601	S, p=0.0001
Catalase(U/ml)	36.04	8.80	50	-0.198	NS, p=0.168
SOD (U/ml)	2.61	0.60	50	-0.533	S, p=0.000067

Table 2 :Correlation of E-Selectin (ng/dl) with oxidative stress markers in SS pattern

Parameters	Mean	Std. Deviation	N	Correlation 'r'	p-value
E Selectin(ng/dl)	77.95	8.86	50	-	-
MDA(uMol/L)	4.34	0.93	50	0.023	NS, p=0.874
Catalase(U/ml)	23.14	7.04	50	-0.629	S, p=0.0001
SOD (U/ml)	1.44	0.30	50	-0.175	NS,p=0.224

Discussion

In this study we have tried to compare the relationship between oxidative stress and circulating adhesive factor in sickle cell anemia patients (HbSS) and sickle cell trait (HbAS). In our study results for MDA which is a marker of lipid peroxidation and oxidative stress was found to be significantly higher in HbSS subjects as compared to HbAA and HbAS subjects this finding is consistent with previous studies by Titus J.et al (2004),[13] Manfredini V. et al (2008),[14] Hundekar P.et al (2010),[15] John N. (2010),[16] Emokpae A.M. et al (2010),[17] Adelakun A. et al (2014)[18] and El-Ghamrawy M.K.et al. (2014).[19] In our study it was also observed that MDA levels were significantly high in HbAS group as compared to controls which was similar to the findings of Titus J.et al (2004), [13]John N.(2010),[16] Emokpae A.M.et al (2010),[17] and Hundekar P. et al(2010).[15] This observation could be explained by the abnormal susceptibility of HbS RBC membranes to lipid peroxidation, they might also indicate that HbS RBC membranes are exposed to increased amounts of endogenous oxidant. And the latter may be a contributing factor in sickle disease pathophysiology.[20] The antioxidant system was imbalanced in our study group patients of sickle cell disease as the results suggested that the mean Catalase levels were significantly lower in HbAS and HbSS subjects as compared to HbAA which was in agreement with studies by Manfredini V. et al (2008),[14] Hundekar P.et al (2010),[15] and Emokpae A.M. et al.(2010).[17] The depletion of catalase in our study may be a consequent to oxidative processes and RBC destruction in SCD patients. In our study the mean SOD levels were significantly lower in HbAS subjects as compared to HbAA which was also significantly lower in HbSS subjects as compared to HbAS & HbAA.This finding was supported by the previous studies Schacter L.et al (1988),[21] and Emokpae A. M. et al (2010).[17] However in contrast to our studies Titus J.et al (2004),[13] Manfredini V. et al (2008),[14] Hundekar P.et al (2010),[15] John N.(2010),[16] and Adelakun A. et al(2014)[18] has found that SOD levels were significantly raised in HbSS as well as in heterozygous HbAS subjects. This is an indication that SCA patients produced greater quantities of reactive oxygen species than control HbAS and HbAA. The reason for decreased Catalase and SOD levels in our study subjects can be the antioxidant defense systems in SCA which might be affected and/or is not strong enough to neutralise the excessive production of ROS, chronic oxidative stress is a critical factor in endothelial dysfunction, inflammation and damage to multiple organs.[17] In present study we have found association of elevated levels of E-selectin with Oxidative stress in patients of SCD. There was significant positive correlation of MDA with E-selectin in HbAS subjects (Table-1) however this correlation was not significant in HbSS subjects (Table-2). But it was found that there was significant negative correlation of SOD with E-selectin in HbAS subjects (Table-1) and significant negative correlation of Catalase with E-selectin in HbSS subjects (Table-2) Similar findings showed by Emokpae M.A. and Uadia P.O.(2012) where atherogenic index of plasma was negatively correlated with antioxidant enzymes and positively with MDA.[22] In our study E-selectin levels were significanty associated with MDA levels in HbAS subjects than that of HbSS Subjects, reason might be that diagnosed HbSS patients are mostly on antioxidant treatment and HbAS patients are said to be apparently healthy. But in both cases there is evidence of linear association of oxidative stress with E-selectin as adhesive factor. Such findings in our study can be explained as E-selectin signaling can trigger "inside-out" αMβ2 activation at the leading edge of the neutrophils, whereas the engagement of platelets by activated αMβ2 can trigger "outside-in" signaling in neutrophils, leading to production of reactive oxygen species.[23] There are few limitation of this study it is an observational case control study therefore there is a distinct possibility that a proportion of the patients may not have given a true reflection of their physical and clinical conditions along with their environmental and family background, personal habits and practices or unknown confounding could be a source of bias. There was lack of follow up regarding treatment and nutritional supplementation. The results and findings of this study need to be explored in a much larger trial, with larger sample size, with adjusted confounding variables, with proper follow up and taking patients of all types of congenital Anemias.

Conclusion

In this study both homozygous as well as heterozygous patients were exposed to enhanced oxidative stress as compared to controls. It was also evident that the anti-oxidant system is imbalanced in these patients and is probably unable to effectively counteract the augmented oxidative stress. It is also concluded that there was a significant association of circulating adhesive factor E-selectin with rise in oxidative stress marker MDA and low levels of antioxidants catalase and SOD in patients of sickle cell disease both homozygous HbSS and Traits HbAS. It is therefore advisable to include antioxidant

supplements to the therapies used for the management of sickle cell disease patients in high prevalent areas.

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