PRO-OXIDANT AND ANTI-OXIDANT STATUS IN PATIENTS OF SICKLE CELL ANAEMIA

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ABSTRACT

The role of oxidant damage to red cells in sickle cell anaemia has been of interest in recent years. Although, available reports suggest that sickle cell erythrocytes are susceptible to endogenous free radical mediated oxidant damage there remains discrepancy in the status of antioxidant enzymes and antioxidant vitamins in these patients. In view of this, 107 cases of sickle cell anaemia (36 'SS' and 71 'AS' pattern - as confirmed by haemoglobin electrophoresis) were subjected to analysis of malondialdehyde, ascorbic acid, superoxide dismutase and albumin. The results were compared with 54 age and sex matched healthy controls. The results indicate a marked increase in lipid peroxidation and superoxide dismutase levels in both 'SS' and 'AS' types of sickle cell anaemia as compared to controls. Although no difference was observed in the levels of albumin in these groups, the levels of ascorbic acid were significantly depleted in sickle cell anaemia patients. The results are indicative of enhanced lipid peroxidation along with imbalance in the pro-oxidant and antioxidant status in patients of sickle cell anaemia.

KEY WORDS

Pro-oxidant, antioxidant enzymes, antioxidant vitamins, sickle cell anaemia.

INTRODUCTION

Sickle cell anaemia (SCA) is one of the commonest inherited disorders in man. It is prevalent in Central Africa, Eastern Countries, Mediterranean region and parts of India.

In India SCA is highly prevalent in certain communities in Central India like Mahars, Teli's and Kunbi's in areas around Nagpur (1). Mukherjee MB (2) found that the prevalence of sickle cell trait among Mahars in Nagpur was 22.2%.

Sickle cell anaemia results from a point mutation in the genetic code such that a single amino acid (glutamic acid) is replaced by another (valine) in the globin chain of haemoglobin (Hb). This substitution transforms normal adult haemoglobin (HbA) into sickle haemoglobin (HbS). If an individual is homozygous for the sickle cell mutation (HbSS) almost all the haemoglobin is sickle haemoglobin. In the heterozygous individual

(HbAS) upto 40% of the haemoglobin is sickle haemoglobin. Under conditions of low oxygen tension HbS molecules undergo aggregation and polymerize and the red cells acquire a 'sickle' or 'holly leaf' shape. Sickling of red cells leads to two major consequences, a chronic haemolytic anaemia and occlusion of small blood vessels resulting in ischemic tissue damage.

Sickle cell anaemia is a condition in which there is observed oxidative stress in the body. There are reports available which indicate that sickle cell erythrocytes produce twice as much superoxide, hydrogen peroxide and hydroxyl radical as compared to normal healthy controls (3). There are also reports which indicate significantly enhanced lipid peroxidation in sickle cell anaemia as compared to controls. But there are contradictory reports as far as the status of the anti - oxidants is concerned in these patients specially that of superoxide dismutase and ascorbic acid (vitamin C).

The prevalence of sickle cell anaemia is alarmingly high in Vidarbha region. Very few studies have been carried on the role of free radicals and status of anti - oxidants in SCA patients in this region. Moreover no study has been carried out to compare the pro-oxidant and anti-oxidant status in

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homozygous and heterozygous SCA patients. It was with this view in mind that it was thought pertinent to assess and evaluate the pro-oxidant and anti-oxidant status in homozygous as well as heterozygous patients and to possibly identify whether these parameters could be in any way associated with the patho-physiology of the disease.

MATERIAL AND METHODS

The present study was carried out on 107 cases of sickle cell anaemia attending the Regional Haemoglobinopathy Detection Centre (RHDC) at Indira Gandhi Medical College, Nagpur. All cases of sickle cell anaemia [(either HbSS (Homozygous) or HbAS (Heterozygous)] were confirmed by haemoglobin electrophoresis. Out of the 107 cases, 71 were heterozygous (40 males and 31 females) and 36 were homozygous (20 males and 16 females).

A total number of 54 healthy, age and sex matched controls were also chosen for the study. Homozygous cases belonged to the age group 11-30 years while heterozygous cases and controls were between the age group of 11-40 years. All were free from acute illness like URTI / fever etc. None had history of concomitant illness like Rheumatic Heart Disease / Diabetes mellitus / Hypertension or others. Patients of SCA in acute crises were excluded from the study. Homozygous patients with history of blood transfusion within past 3 months were excluded from the study. All the cases and controls chosen for the study gave their written consent for participating in the study after being explained the nature of the study.

Haemolysed samples were excluded from the study. The blood samples were collected in an EDTA vial for estimation of superoxide dismutase [based on the reaction between superoxide radicals and 2-4-iodophenyl-3-4-nitrophenol-5-phenyltetrazolium chloride (INT), (4)], ascorbic acid [using phosphotungstic acid as colouring agent, (5)], malondialdehyde [based on the fact that lipid peroxides condense with 1-methyl -2-phenyl indole under acidic conditions resulting in the formation of chromophore - [Randox Laboratories, UK] , haemoglobin [by acid haematin method (6)] and albumin [by the bromocresol green dye method, (7)]. Data was analyzed by applying student 't' test.

RESULTS AND DISCUSSION

As seen in Table I, significantly higher levels of malondialdehyde were observed in both homozygous as well as heterozygous sickle cell anaemia patients as compared to controls. Earlier reports (8), (9), (10) also indicate similar changes. The increased lipid peroxidation observed in SCA

Tabel 1. Levels of malondialdehyde, superoxide dismutase, vitamin C, haemoglobin and albumin in total cases and control

Parameters	HbSS	HbAS	Controls
MDA (m mol /L)	4.15* (± 0.30)	2.52* (± 0.33)	1.83 (±0.22)
SOD (U/ml)	323.83* (± 20.04)	255.87* (±27.66)	199.14 (± 21.11)
SOD (U/gHb)	4989.48* (±159.50)	2123.77* (±334.63)	1391.87 (±58.34)
Vitamin C (mg %)	0.44# (±0.04)	0.64# (± 0.04)	0.93 (±0.08)
Haemoglobin (gm %)	6.5# (±0.44)	12.31# (±1.56)	14.22 (±1.10)
Albumin (gm %)	4.05** (±0.33)	3.99** (± 0.35)	4.09 (±0.46)
* = Significantly higher (P<0.001) as compared to controls. ** = not significantly different from controls. # = Significantly lower (P<0.001) as compared to controls.			

patients can be attributable to the increased autoxidation and iron decompartmentalisation seen in these patients (11). The accelerated HbS auto-oxidation results in accelerated superoxide production resulting in the formation of pathological amounts of hydrogen peroxide due to superoxide dismutation. The hydrogen peroxide, which is formed, produces hydroxyl radicals ($\cdot\text{OH}$) in the presence of iron.

The accelerated oxygen radical production can have serious adverse effects on cell membrane protein and lipid resulting in thiol oxidation and lipid peroxidation. Lipid peroxidation can affect the activities of certain enzymes, like the $\text{Na}^+ - \text{K}^+$ ATPase (12) and Ca^{++} ATPase (13), in the sickle cell. The deformability of sickle RBC's is diminished by exposure to malondialdehyde causing increased rigidity, dehydration and decreased deformability of RBC's (14). Thus an excessive accumulation of oxidant damage in sickle cells could contribute to the accelerated membrane senescence of these cells. Rice-Evans C *et al.* (15) measured the oxidative damage to sickle erythrocytes and found that the endogenous free radical mediated oxidative damage correlated with the proportion of irreversibly sickled cells (ISCS). It is well known that the

proportion of irreversibly sickled cells are responsible for the features of sickle cell anaemia like haemolysis and microvascular occlusion.

Contradictory reports are available regarding the levels of anti-oxidant enzymes in sickle cell anaemia patients. While Das SK (16) reported significantly increased levels of superoxide dismutase (SOD) in homozygous sickle cell anaemia patients, and Beretta L *et al.* (17) found significantly increased SOD levels in heterozygous SCA subjects, Schacter LP *et al.* (18) reported contradictory findings. Beretta L *et al.* (17) reported significantly higher GSH - Px levels in sickle erythrocytes. Das and Nair (16) and Beretta L *et al.* (17) reported significantly lower levels of catalase in SCA patients. As shown in Table I, the levels of the enzyme superoxide dismutase (SOD) were significantly increased in both homozygous as well as heterozygous patients of sickle cell anaemia. The increased levels of SOD, as found in this study, could be able to dismutate the increased flux of superoxide ions, exposing the sickle erythrocytes to high levels of hydrogen peroxide. The weakly membrane bound GSH - Px enzyme, could prevent lipid peroxidation of the sickle cell membrane. However, reduced catalase activity, reported in earlier studies, will not be able to protect the cytosolic components from the toxicity of hydrogen peroxide.

The situation is further compounded by the low levels of certain anti-oxidant vitamins seen in these patients. Table I depicts that the levels of vitamin C were significantly lower in homozygous as well as heterozygous SCA patients. This findings are in agreement with the findings of Jain S (19) who found that homozygous SCA patients had

significantly lower levels of vitamin C. However Adelekan *et al.* (20) did not find any significant difference in the levels of vitamin C between heterozygous SCA patients and controls. The role of vitamin C as an anti-oxidant is well known. Chou PT, Khan AU (21) proposed that while ascorbic acid may act synergistically with vitamin E, the anti-oxidant effect may be due to quenching of singlet oxygen in the aqueous medium.

Other authors have reported lower levels of plasma and RBC membrane vitamin E in SCA patients as compared to controls. Therapy with vitamins C and E in patients of sickle cell anaemia may be considered as sickle cell patients tend to be deficient in them. There is paucity of literature as far as supplementation of vitamin C in SCA patients is concerned. Small trials of vitamin C supplementation have been carried out (22).

Albumin exerts its anti-oxidant effect in the body by binding copper ions and heme tightly and iron ions weakly. It thus plays a role in preventing copper and iron from participating in lipid peroxidation. However as shown in table I, there was no significant difference in the levels of albumin in the homozygous patients or heterozygous SCA subjects as compared to controls.

The results of this study indicate that there is no significant effect of age or sex on lipid peroxidation in both SCA groups (Bar diagrams I and II). The haemoglobin levels in both the SCA groups were significantly lower as compared to controls. This indicates that the oxidative damage in sickle cell trait may be strong enough to result in significantly lower haemoglobin levels in both male and female sickle cell trait cases. A modest decrease in the

Bar Diagram I. MDA levels in patients of different age groups with sickle cell anaemia

Bar Diagram II. MDA levels in male and female cases of sickle cell anaemia

hemoglobin level in sickle cell trait has been documented (23). Reports suggest that sickle cell trait may contribute to a patient's illness (24). Thus, the sickle cell trait may not be a "benign" form of sickle cell anaemia as considered earlier.

In conclusion, it is clearly evident that both homozygous as well as heterozygous patients are exposed to enhanced oxidative stress as compared to controls. It is also evident that the anti-oxidant system is imbalanced in these patients and is probably unable to effectively counteract the augmented oxidative stress. It now remains to be seen whether oral supplementation of anti-oxidant vitamins could be of help to these patients.

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