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Biochemistry

The Effect of Antioxidant Supplementation on the Oxidant and Antioxidant Status in Sickle Cell Anaemia

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ABSTRACT

Background: Sickle cell anaemia is a hereditary disorder, associated with severe haemolytic anaemia, periodical vaso-occlusive pain and premature death. Oxidative stress is one of the factors that may enhance the rate of haemolysis by damaging the erythrocyte membrane by lipid peroxidation.

Aim: The present study was carried out to investigate the oxidant and antioxidant status in sickle cell individuals and the effect of antioxidant supplementation on oxidative stress.

Material and Method: A total of 90 subjects participated in the study, including 30 heterozygous (HbAS) and 30 homozygous (HbSS) sickle cell patients and 30 age and sex matched healthy controls. Oxidative stress was evaluated by measuring the levels of serum malondialdehyde (MDA), plasma protein carbonyl , serum nitric oxide (NO), the erythrocytic activity of superoxide dismutase (SOD) and catalase and the total antioxidant capacity (TAC) of plasma before and one month after of antioxidant supplementation.

Results: The baseline levels of MDA, protein carbonyl, NO and the activity of SOD were significantly ($p<0.001$) elevated in the HbSS and HbAS groups as compared to those of the controls. The baseline level of the activity of catalase and the TAC of plasma were significantly ($p<0.001$) decreased in the HbSS and HbAS groups as compared to those in the controls. After the supplementation of the antioxidants, we found a significant ($p<0.001$) decrease in the levels of MDA, protein carbonyl, NO and in the activity of SOD, while there was a significant ($p<0.001$) increase in the level of activity of catalase and in the TAC of plasma in both the groups of sickle cell patients.

Conclusion: The values of both the oxidants and the antioxidants did not meet that of the controls, thus suggesting a spontaneous generation of free radicals that consumed the antioxidants. Therefore, antioxidant supplementation is essential in sickle cell individuals in the steady state as well as in illness, to prevent the oxidative damage to the erythrocytes.

Key Words: Oxidative stress, Sickle cell anaemia, Superoxide Dismutase, TAC of plasma, Nitric Oxide

INTRODUCTION

Sickle cell anaemia (SCA) results from a point mutation in the genetic code, GAG→GTG, which causes the 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 ($\alpha_2\beta_2$) to 'sickle haemoglobin' HbS ($\alpha_2\beta^S{}_2$). Upon deoxygenation, HbS undergoes aggregation and polymerisation, thus changing the discoidal erythrocyte into a crescent or sickle shape [1].

The prevalence of sickle cell anaemia is alarmingly high in the districts of Nandurbar and Dhule in Maharashtra. The sickling disorder is seen predominantly in the Pawra and Bhil communities of the tribal population [1]. A 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.

Oxidative stress is an imbalanced redox status which is caused due to the over-production of oxidants and the depletion of antioxidants. Oxidative stress may play a major role in the pathogenesis of sickle cell anaemia by enhancing the sickling phenomena. Various studies have shown an enhanced production of the reactive oxygen species (ROS) and a decreased antioxidant status in SCA.

The sickle erythrocytes generate approximately two times more amounts than usual of superoxide, peroxide and hydroxyl radicals [2]. The erythrocytes become more vulnerable during the sickling,the iron released and 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 [3].

With the above view, the present study was aimed to measure the levels of MDA, protein carbonyl, NO, the activity of SOD and catalase and TAC of plasma before and after one month of the supplementation of antioxidant to evaluate its effect on oxidative stress in the sickle cell anaemia individuals.

MATERIAL AND METHODS

The present study was carried out in the Department of Biochemistry, ACPM Medical College, Dhule and the Medicare Hospital, Nandurbar, Maharashtra. Prior to the start of the study, local ethical clearance was obtained. 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 and pregnancy.

After obtaining a written consent from all the subjects who were included in the study, 5 ml of blood was withdrawn aseptically from the antecubital vein from each subject. 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, plasma and serum respectively. The serum lipid peroxide product, malondialdehyde (MDA) was measured by a thiobarbituric reaction described by K Satoh [4]. Plasma protein carbonyl was measured by using a (dinitrophenyl hydrazine) DNPH–guanidine complex according to the method of Levine et al[5]. Serum nitric oxide (NO) was evaluated by the cadmium granule reaction which was described by Cortas and Wakid [6]. The activity of erythrocytic SOD was determined by the inhibition of the reduction of riboflavin according to Winterbourne's method [7]. The activity of erythrocytic catalase was measured by the method which was described by L Goth [8]. The total antioxidant capacity of plasma was determined by the ferric reducing ability of plasma (FRAP) assay[9]. The assessment of the above parameters was conducted before the antioxidant supplementation and on the 30th day of the antioxidant supplementation in the form of an antioxidant tablet which was composed of predominantly antioxidant vitamins and trace elements. The statistical analysis was carried out by using the SPSS (Statistical Package for Social Sciences) statistical software, version 16.0 for Windows. The paired and unpaired Student's t tests were applied for the significance and the results were expressed in mean values with SD. P values which were <0.05 were considered as a significant difference.

RESULTS AND DISCUSSION

[Tables/Fig-1 and 2] show significantly (<0.001) elevated baseline levels of serum MDA, plasma protein carbonyl and serum NO, elevated baseline levels of the erythrocytic activity of SOD and significantly (p<0.001) decreased baseline levels of the TAC of plasma and the activity of catalase in the heterozygous and homozygous sickle cell subjects as compared to those in the controls. After one month of antioxidant supplementation, we noted a significant decrease in the levels of MDA, NO and protein carbonyl and in the activity of SOD as compared to the baseline levels. On the other hand, the levels of the TAC of plasma and the activity of catalase were significantly increased on the 30th day of the antioxidant supplementation.

Earlier studies have reported increased levels of the lipid peroxidation product, MDA in the sickle cell subjects as compared to the controls, which was in accordance with our finding [2,10,11]. The HbS RBC membranes were exposed to increased amounts of the endogenous oxidant. Haemoglobin-free iron acts as Fenton's reagent and produces superoxide, peroxide and hydroxyl radicals, which may further initiate membrane lipid peroxidation [12]. Superoxide/peroxide driven hydroxyl radical ($OH^{\bullet}$) generation is facilitated by membrane-bound hemichrome (HC), a denatured ferric haemoglobin (Hb) which is found in excessive amounts and is bound to the HbS RBC membranes [13]. This enhanced oxidative stress may be a contributing factor in the pathogenesis of sickle cell anaemia.

There are conflicting reports regarding the activity of SOD. Schacter et al (1988) reported the decreased activity of SOD in the homozygous subjects as compared to the heterozygous subjects and the controls [14,15], Das and Nair (1980) and Titus et al (2004) have shown an elevated activity of SOD in the homozygous as well as the heterozygous subjects [10,11], which may be due to

Parameters	Controls (n=30) mean±SD	Heterozygous (n=30)			
		Baseline mean±SD	'p' value	On 30th day mean±SD	'p' value
Serum lipid peroxide (MDA) nmoles/ml	0.84±0.32	2.58±0.42	p<0.001	1.66±0.24	p<0.001
Serum Nitric Oxide µmol/l	32.11±6.49	63.41±16.75	p<0.001	40.38±11.17	p<0.001
Plasma Protein Carbonylnmoles/mg protein	0.52±0.14	1.38±0.32	p<0.001	0.81±0.27	p<0.005
Erythrocytic SOD U/g Hb	1307±119	2256±419	p<0.001	1609±235	p<0.001
Erythrocytic Catalase KU/g Hb	144.79±17.84	79.01±10.21	p<0.001	106.65±10.83	p<0.005
TAC of plasma mmol/l	2.11±0.32	0.71±0.16	p<0.001	0.88±0.17	p<0.05

[Table/Fig-1]: Levels of MDA, NO, Protein Carbonyl, SOD, Catalase activity and TAC of plasma in Controls (HbAA) and Heterozygous Sickle Cell (HbAS) Patients.
Values are expressed in mean with standard deviation (mean±SD); n = number of subjects.

Parameters	Controls (n=30) Mean±SD	Homozygous (n=30)			
		Baseline Mean±SD	'p' value	On 30th day Mean±SD	'p' value
Serum lipid peroxide (MDA) nmoles/ml	0.84±0.32	4.13±0.48	p<0.001	3.12±0.61	p<0.001
Serum Nitric Oxide µmol/l	32.11±6.49	82.88±33.18	p<0.001	69.61±22..81	p<0.005
Plasma protein Carbonyl nmoles/mg protein	0.52±0.14	2.74±0.51	p<0.001	2.32±0.43	p<0.01
Erythrocytic SOD U/g Hb	1307±119	3440±629	p<0.001	2923±695	p<0.001
Erythrocytic Catalase KU/g Hb	144.79±17.84	64.786±10.15	p<0.001	73.08±7.58	p<0.005
TAC of plasma mmol/L	2.11±0.32	0.504±0.19	p<0.001	0.706±0.18	p<0.05

[Table/Fig-2]: Levels of MDA, NO, Protein Carbonyl, SOD, Catalase activity and TAC of plasma in Controls (HbAA) and Homozygous Sickle Cell (HbSS) Patients.
Values are expressed in mean with standard deviation (mean±SD); n = number of subjects.

the variation in the genetic expression. The elevated activity of SOD may imbalance the cellular antioxidant defence, resulting in the accelerated generation of H_2O_2, which is a product of the dismutation of $O^{\bullet}_2$. This effect is significantly exacerbated under conditions in which the H_2O_2 catabolism is altered. An enhanced activity does not decrease the intensity of the oxidative damage, but rather, it increases the concentration of H_2O_2. Further, it may inhibit the activity of erythrocytic catalase, leading to the denaturation of Hb and the formation of Heinz bodies, thus contributing to sickling and haemolytic effects [16].

In the present study, the TAC level was assessed by the FRAP assay, based on the antioxidant power of low molecular weight antioxidants such as vitamins (A,E,C) and trace elements (Zinc, Selenium, Copper). The lower levels of TAC suggested the depletion of these low molecular weight antioxidants. Previous studies have reported significantly decreased levels of tocopherol, retinol, carotenes, ascorbic acid and zinc [17,18,19]. The deficiency of these antioxidants may account for some of the observed manifestations of sickle cell disease, such as an increased susceptibility to infection and haemolysis [19]. The regular supplementation of these antioxidants may ameliorate some of the sickle cell manifestations such as vaso-occlusive crises, acute chest syndrome, recurrent infection and growth retardation [20]. The haemolysis which was observed in the sickle cell anemia subjects correlated with the percentage of the circulating ISCs. The supplementation of the sickle cell patients with vitamin E led to a greater than 50% reduction of the ISCs (from 25% to 11%) [21]. However, it was stated that vitamin E was ineffective as a terminating factor in disorders which were characterized by iron decompartmentalization [2].

Huang et al (2003) carried out a short trial on the supplementation of vitamin E and C in SCA. Specifically, the supplementation with 500 mg of vitamin C per day or 400 IU of vitamin E per day for 2 months, resulted in a reduction in the lipid peroxidation of equal to 10% on the basis of the measured urinary excretion of the 8-iso-$PGF_{2\alpha}$, However, the supplementation with a combination of both vitamin C and vitamin E conferred no additional benefits [22]. Natta et al (1979) investigated the vitamin E levels in sickle cell patients before and after the supplementation of vitamin E. He found a significantly low level of vitamin E in the SCA patients. After the supplementation of 150 IU of vitamin E (dl-α tocopheryl acetate), three times a day for 2 months, the plasma tocopherol levels were found to be similar to the controls [23].

Muskiet et al (1991), have seen the effect of the supplementation of α-tocopherol, vitamin C, zinc, soybean oil and fish oil in thirteen patients with homozygous sickle cell disease. They found a reduction in the urinary zinc and an increase in the plasma vitamin C, plasma cholesterol ester and erythrocyte (RBC) ω-3 fatty acids. The plasma and RBC α-tocopherol levels were found to be increased. They also observed a decrease in the irreversibly sickled cells by 37.5%, decreased RBC protoporphyrin and urinary porphyrins, and an increase in the RBC total fatty acid cholesterol ratio. However, they also observed that the supplements did not change the haemoglobin concentrations, the RBC age (reticulocytes, polyamines), or the number of aplastic and vaso-occlusive crises[24]. Lanchant and Tanaka (1986) observed that Vitamin C corrected their hydrogen peroxide-induced sensitivity for lipid peroxidation and haemolysis in the sickle cell individuals [25].

In the present study, one month of antioxidant supplementation showed a significant elevation in the antioxidant status, with a decrease in the oxidant level, thus suggesting the necessity of antioxidant supplementation in SCA. Elevated oxidative stress may certainly have a role in the pathogenesis of sickle cell anaemia. The antioxidant supplementation may intervene to the chain reaction process of the oxidants and probably delay the sickling effect. However, further studies are needed to see the effect of the increased duration of antioxidant supplementation on oxidative stress and on the health status of the SCA patients. The antioxidant supplementation may be of additional benefit in crises conditions along with the crises treatment that ameliorates the vaso-occlusive and the haemolytic consequences.

CONCLUSION

In the present study, we observed increased oxidative stress in terms of elevated serum MDA, serum NO, plasma Protein carbonyl and erythrocytic SOD activity. However, a decrease in the antioxidant capacity of plasma may be due to the overburden of the ROS. The supplementation of antioxidants shows the improved antioxidant capacity of the plasma as well as a decrease in the oxidant levels. The present study may be helpful for the further treatment policy for sickle cell anaemia in the steady state as well as in the crisis state.

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