Total Antioxidants Status and Some Hematological Values in Sickle Cell Disease Patients in Steady State

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Congenital hemoglobin mutations may alter the delicate balance of free-radical generation and antioxidant defense systems in the red cell. Oxidative stress may thus play a role in the pathophysiology of the clinical manifestations of the disease. We assessed the total antioxidant status in steady-state sickle cell anemia (SCA) patients and related it to certain hematological parameters and their recent clinical history.

Forty (25 males/15 females) adult SCA patients and 30 age-matched controls were studied. All patients and control subjects had total antioxidant status (TAS), hematocrit, white blood cells, platelets and reticulocyte count done.

The results showed that TAS levels were about 50% lower in the SCA patients compared with the controls. Among the SCA patients, 57.1% of those with TAS levels <1.00 mmol/L had bone pain crisis >3 times in the past year, compared with 16% in those with TAS levels >1.00 mmol/L. Total leukocyte count and platelets were also significantly higher in the SCA patients than controls.

Our data support the growing evidence that oxidative stress has a role to play in the pathophysiology of SCA and intervention aimed at increasing the antioxidant capacity of these patients may be beneficial.

Key word: sickle cell anemia

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INTRODUCTION

The molecular basis of the prototypical genetic disease sickle cell anemia (SCA) is that valine is substituted for glutamic acid at the sixth position of the hemoglobin chain. This ostensibly minor change in structure results in reduced solubility and stability of the mutant gene product, hemoglobin S (HbS). The pathogenesis of SCA evolves mainly from the polymerization of HbS causing chronic hemolytic anemia and vaso-occlusive phenomena. These underlie the significant morbidity and mortality associated with the disease.[1]

SCA is primarily a disorder of red blood cells (RBCs), which are a significant source of free radicals in biological systems. RBCs have a rich oxygen supply and are densely packed with redox-active hemoglobin residues. The bonding interaction between heme iron and oxygen in oxygenated hemoglobin is associated with an electron transfer.[2] In response, there is an integrated network of the antioxidant system, consisting of both enzymes and low-molecular-weight compounds, which help to mitigate oxidative stress and injury to the red cell and tissues in general. Congenital hemoglobin mutations may alter this balance and create a pro-oxidant reactive milieu. This may be contributory to the pathophysiology of the abnormalities that underlie the clinical course of SCA.

In this study we assessed the serum total antioxidant status (TAS) in a group of SCA patients in steady state and related it to their clinical history.

MATERIALS AND METHODS

Forty known SCA patients (25 males/15 females) in steady state were randomly selected from the routine hematology clinic of the University College Hospital, Ibadan. After obtaining informed consent, a structured questionnaire was administered to all participants. Blood samples were then taken for full blood count (FBC), reticulocyte count and TAS. The samples for FBC and reticulocyte count were taken into EDTA bottles. Analysis was according to standard hematological procedures. FBC evaluation included hematocrit, and white cell, differential and platelet counts. Samples for TAS were drawn to plain bottles. They were allowed to clot; serum was separated and stored at -20°C. TAS was assessed within two weeks of collection.

Determination of TAS was performed using Total Antioxidant Status kit, cat no. NX2332 (Randox, UK). The kit assesses the integrated antioxidant system, which encompasses all the biological components in serum with

antioxidant activity. The principle of the test is as follows: incubation of ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6 sulfonic acid)] with a peroxidase (metmyoglobin) results in the production radical cation ABTS+. This species is blue-green in color and can be detected at 600 nm. Antioxidants in the added sample cause inhibition of this color production to a degree that is proportional to their concentration. The procedure was carried out as specified by the manufacturer in the technical literature insert. Absorbance was measured with a spectrophotometer (CE 594, Cecil Instruments, Cambridge, UK.)

Thirty age-matched, confirmed-HbA subjects (19 males/11 females) served as controls. They were from the same racial stock and socioeconomic background, and were treated similarly.

STATISTICS

Results are expressed as mean (±SD). Significance testing was done using Student's t test, and the Pearson's correlation coefficient was used to establish correlation. Significance was considered at 20% probability ($p<0.05$).

RESULTS

The mean total antioxidants status level was significantly lower among patients with SCA compared with controls ($p<0.001$). The mean value in the SCA group was less than half that for the control group (Table 1). TAS was <1.00 mmol/L in 21 (52.2%) in the SCA group, whereas none of the control group had a value <1.00 mmol/L.

Amongst the SCA patients, those with TAS <1.00 mmol/L (57.1%) had >3 vaso-occlusive crises in the past year, but only 16% among those with TAS >1.00 mmol/L experienced the same frequency of vaso-occlusive phenomena (Table 2). The mean TAS of the 25 SCA patients who had >3 vaso-occlusive phenomena in the last year was significantly lower than that of the 15 SCA who had it less frequently (0.65 mmol/L vs. 1.18 mmol/L, $p<0.001$).

The mean TAS for patients who had not received a blood transfusion in their lifetime was 1.05 ± 0.99 mmol/L; while for those who had received >3 units, the level was 1.02 ± 0.01 mmol/L.

Total leukocyte and platelet counts were significantly higher in the SCA patients than in controls ($p<0.003$ and 0.05, respectively). The absolute counts of neutrophils, lymphocyte, eosinophils and monocytes were all significantly higher in SCA patients than in controls ($p<0.001$, 0.03, 0.002 and 0.001, respectively).

There was no significant correlation between TASes in the SCA patients studied with age and all the hematological parameters.

DISCUSSION

Unlike earlier studies that measured isolated antioxidants, the present study utilized a method that assesses all the biological components of serum with antioxidant activity. This has obvious advantages. The separate measurement of all the different antioxidants is time consuming, labor intensive, costly, requires complicated techniques and the interactions of these antioxidants in serum will be difficult to assess.[3,4] Added to this will be the accumulation of the separate errors of each measurement. Various methods exist for the measurement of total antioxidant capacity of biological fluids. These include the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®)-equivalent antioxidant capacity of plasma (TEAC) assay, the ferric-reducing ability of plasma (FRAP) assay, and oxygen radical absorbance capacity (ORAC) assay.[4] A TEAC-based assay was used in this study for several reasons. It is available in commercial kits by Randox, which are amenable to routine use. The ORAC assay requires a fluorescence detector[5] that may not be available in most laboratories in resource countries such as ours. The FRAP assay measures only nonprotein antioxidants, excluding albumin, the main antioxidant of serum and glutathione, the most important cellular antioxidant.[4]

Consistent with the known biology of SCA, the present study demonstrates a 50% reduction in the total antioxidant capacity of serum of patients with SCA in the steady state compared with healthy HbA controls. HbS-containing red cells auto-oxidize faster, generating a greater extent of superoxide, hydrogen peroxide, hy-

Table 1. Total antioxidant status and some hematological parameters in sickle cell anemia

	SCA Patients (mean + SD)	Controls (mean + SD)	P Value
Age (years)	22.5 ± 5.1	22.0 ± 3.9	>0.05
Hematocrit (%)	22.3 ± 4.1	39.4 ± 3.7	<0.001
Total Leukocyte count (x10⁹)	8.6 ± 3.1	6.9 ± 1.5	<0.003
Platelet count (x10⁹)	355 ± 145	318 ± 69	<0.05
Absolute neutrophil count (x10⁹)	4.8 ±1.8	3.9 ± 0.9	<0.01
Absolute lymphocyte count (x10⁹)	3.2 ± 1.3	2.7 ± 0.7	<0.03
Absolute eosinophil count (x109)	0.18 ± 0.07	0.09 ± 0.07	<0.002
Absolute monocyte count (x10⁹)	0.8 ± 0.49	0.02 ± 0.05	<0.001
Total antioxidant status (mmol/L)	0.98 ± 0.51	1.8 ± 0.43	<0.001

droxyl radicals and lipid oxidation products when compared with HbA-containing red cells.[6] The antioxidant defense capacity in SCA is also reduced. Both the enzymatic (glutathione peroxidase and catalase) and the low-molecular-weight (carotene and vitamin E) antioxidants have been shown to have decreased activity and concentrations in plasma.[7-10] These place SCA patients at increased risk of oxidative stress and injury.

Increasing evidence indicates a role for oxidative stress in the pathophysiology of episodic vascular occlusion as seen in SCA. The enhanced generation of free radicals in SCA creates a reactive milieu that can inactivate the nitric oxide (NO)-mediated vascular relaxation and yield secondary reactive species that may further impair vascular function. Another consequence of decreased NO and accelerated formation of reactive oxygen species is the increased expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule (ICAM-1).[11] Erythrocytes from sickle cell patients display enhanced binding to endothelial cells via interactions between erythrocyte 4 1-integrins and endothelial cell VCAM-1.[12] The decreased vascular relaxation and increased endothelial adherence are contributory to the vaso-occlusive phenomena.[13] Examining the relationship among the frequencies of the most common vaso-occlusive crisis, bone pain crisis and TAS, we observed that the mean TAS of the 25 patients who had >3 vaso-occlusive episodes in the preceding year was about half of those who experienced less than that. This indicates that a pro-oxidant environment may be conducive for vaso-occlusives.

Our study did not reveal any statistically significant correlations between the TAS of the SCA patients with the hematological parameters studied. However, we observed a negative correlation between TAS and the reticulocyte counts. This would suggest that antioxidants may have some protective role against red-cell destruction in these patients. It has been demonstrated the accumulation of malonyldialdehyde, a product of fatty-acid oxidation, disturbs the organization of phospholipids in the human erythrocyte membrane bilayer.[14] This may be associated with a greater likelihood of destruction by the reticuloendothelial system. A negative correlation was also observed with the total leukocyte count in these patients. This agrees with the findings of Claster: that activated neutrophils can contribute to oxidative stress and injury.[15]

Table 2. Relationship of total antioxidant status levels (TAS) and the frequency of bone pain crisis

Frequency of Crises	TAS <1.0 mmol/L	TAS >1.0 mmol/L
≤3	42.9%	84%
3	57.1%	16%

p=0.007

The increasing evidence of the role of reactive oxygen species in the pathobiology of SCA suggests new therapies for the disease and gives new insights into the mechanism of action of some currently accepted therapies. Ohnishi and Ohnishi propose a cocktail of antioxidants to alleviate the frequency and severity of anemia and crisis in SCA patients.[16] However, two small prospective studies examining the effect of oral vitamin-E supplementation on clinical outcomes in sickle cell disease failed to show any effect on the frequency of vaso-occlusive crisis.[17,18] Hydroxyurea has been shown to be beneficial in the management of SCA. It results in lower rates of vaso-occlusive crises and increased time between crises amongst positive effects.[19] Recent studies suggest that the beneficial effects of the drug may partly be due to its ability to increase the generation of NO in the presence of hydrogen peroxide and a heme-containing protein like hemoglobin.[20] Administration of hydroxyurea to rats led to a dose-dependent increase in nitrosyl hemoglobin, which is consistent with increased nitric oxide generation.[21] Although Stolze and Nohl have also demonstrated that the drug can increase free-radical formation,[22] no serious adverse effects of the drug were reported in a multicenter trial.[19]

CONCLUSION

Our study agrees with current evidence that oxidative stress has an important role to play in the pathophysiology of the deleterious vaso-occlusive crises observed in SCA patients. It thus strengthens the case for the use of agents that increase the total antioxidant capacity of these patients with a view to improving their clinical course.

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