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Blood Reduced Glutathione and Serum Transferrin Levels in Sickle Cell Anaemia

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ABSTRACT: Blood reduced glutathione (GSH) level was measured in 50 sickle cell anaemia (SCA) patients with haemoglobin SS and compared with 20 control subjects with haemoglobin AA and AS. Other parameters compared include haemoglobin, packed cell volume (PCV), serum iron, total iron binding capacity (TIBC) and transferrin. The effect of riboflavin supplementation was also studied in 12 of the SCA subjects. Haemoglobin, PCV, serum iron, TIBC, as well as percentage saturation of transferrin values were all significantly lower ($P<0.05$) in the SCA patients than in the control subjects. However the mean erythrocyte haemoglobin concentration (MEHC) value of 34.7 ± 4.0% recorded for the SCA group did not suggest an anaemia that required iron therapy. There was a significant positive correlation between TIBC and serum iron levels in both the control and the SCA group, ($r = 0.48$, $P<0.001$, and $r = 0.53$, $P<0.05$, respectively). Transferrin saturation was significantly associated with haemoglobin level in the control but not in the SCA group($r = 0.479$.$P<0.05$, $n = 20$ and $r = 0.011$, $P>0.05$, $n = 50$ respectively). However, GSH level was positively related to haemoglobin in both groups but only significantly so in the SCA patients, ($r = 0.462$, $P<0.05$, $n = 50$), while MEHC was also significantly associated with GSH in only the SCA group, ($r = 0.591$, $P<0.05$). The iron status but not the haemoglobin level of the SCA patients was significantly improved by the riboflavin supplementation. The GSH level increased only slightly in the supplemented but decreased significantly in the supplemented group during the study period. The reduced GSH and transferrin levels in the SCA group compromised their antioxidant status, increased oxidant stress in these patients contributing in part to the haemolytic anaemia associated with this disease. The protective function of GSH in red blood cell, the factors accounting for increased oxidant stress in SCA patients and the consequent decrease in blood GSH level observed in these patients are discussed. A closer look at the possibility of raising haemoglobin concentration and blood GSH through administration of riboflavin in the management of SCA is recommended.

Key Words: Sickle cell anaemia; Reduced glutathione; Iron status; Riboflavin; Haematological parameters.

Introduction

Although oxygen is essential to human life and about 95% of the oxygen we breathe in is used for energy production, a small percentage is used to make reactive oxygen species, (ROS), including hydrogen peroxide (H_2O_2), Halliwell, 1994).

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In the presence of metal ions such as copper and iron, H_2O_2 produces hydroxyl radicals, which can attack cells, Aruoma (1994); alter deoxyribonucleic acid (DNA), p[roteins and membrane phospholipids, (Gate *et al*, 1999). According to White *et al* (1978), erythrocytes are exposed to a higher concentration of oxygen and are more susceptible to damage by oxygen than most cells. Unstable haemoglobins such as those present in sickle cell anaemia (SCA) patients undergo spontaneous oxidation *in vivo* leading to Heinz-body production, (Lui *et al*, 1996), and shortened life span. However, proteins, including plasma protein transferrin, iron storage protein ferritin and copper transporting protein cerruloplasmin are very useful for sequestering iron and copper respectively in safe forms, (Halliwell 1990, Aruoma, 1994). Another means of preventing oxidative damage to erythrocytes is provided by reduced glutathione (GSH). GSH helps to detoxify H_2O_2 in the presence of glutathione peroxidase. It also prevents oxidation of thiol groups in haemoglobin with eventual reduction in Heinz body production (Lachant *et al*, 1983). During these reactions GSH is converted to oxidized glutathione (GSSG). Riboflavin in its coenzyme form (flavin adenine dinucleotide, FAD) is needed for the regeneration of GSH through the action of glutathione reductase, (Varma, 1983). Blood GSH levels have been reported to be low in haemolytic anaemia and Ajayi and James, (1984) have reported increased glutathione reductase activity due to oral riboflavin supplementation. Sickle cell anaemia is a haemolytic disease with about 20% of the African population carrying the gene (Oluboyede *et al*, 1981). This paper reports on blood GSH and serum transferrin levels in SCA patients in Nigeria and the relationship found between these two parameters and haematological indices. The effect of oral supplementation of riboflavin (6mg daily for eight weeks) on the blood GSH level and iron status of patients with SCA is also reported.

Subjects and Methods

A totalof 70 volunteers took part in this study after giving informed consent. The SCA patients (n=50) aged between 18-43 years were attending the University College Hospital, Ibadan for their periodic check up. They were on routine drugs of folic acid and antimalarial tablets. The controls consisted of individuals aged 19-43 years of age. They were made up of 13 subjects with haemoglobin AA and 7 with AS. An initial base line blood sample (10ml) was collected from all the volunteers after, which 12 of the SCA patients were administered with 6mg riboflavin tablets (given twice daily as 3mg riboflavin tablets) for 8 weeks. Another blood sample was collected from the 12 supplemented SCA patients as well as 12 of the unsupplemented SCA patients at the end of the study period. An aliquot of each blood sample was collected into a tube containing EDTA and used for the assay of haemoglobin, Dacie and Lewis (1984), packed cell volume (PCV), Green J.H. (1980), and reduced glutathione Beutler *et al*, (1963). The remaining blood sample was collected in iron-free tubes for serum separation and the serum was analysed for serum iron and TIBC, Cook *et al* (1985). Transferrin saturation values were calculated from the ratio of serum iron to TIBC. The mean erythrocyte haemoglobin concentration (MEHC) was calculated from the following equation:

$$\text{MEHC (\%)} = \frac{\text{haemoglobin(g\%) x 100}}{\text{haematocrit (\%)}}$$

(Davidson *et al*, 1975).

Statistical Analysis

Student's "t" test was used to compare the means from the groups, while Pearson's correlation coefficient was calculated to establish any association between the variables. A p-value <0.05 was taken to be statistically significant.

Results

Table 1 shows the blood GSH, haematological and iron status indices of the participants. Forty-three out of the 50 SCA patients, (86%) had haemoglobin levels less than 10g/100ml, while over 90% of them showed PCV values below 30%. Although none of the participants had an MEHC value less than 30%, nonetheless, haemoglobin, PCV, and MECH values were all significantly reduced in the SCA patients. The serum iron, TIBC and transferrin levels that were also lower in the SCA group are also presented in the same table. The difference in the serum iron values were not significant. The TBC and serum iron levels correlated positively in both the control and the SCA group, ($r=0.48$, $P<0.05$, $n+20$ and $r=0.53$, $P<0.05$, $n=50$ respectively).

Association was also sought between parameters with antioxidant properties namely transferrin and GSH, with those related to erythrocytes *viz* haemoglobin PCV and MEHC. Transferrin correlated positively with PCV, haemoglobin and MEHC the control group. However, the most significant association in the control group was between transferrin and haemoglobin, $r=0.479$, $P<0.05$ (Table 2). In the SCA group, GSH was a more important determinant of MEHC, and haemoglobin level ($r+0.591$ $P<0.001$ and $r=0.417$ $P<0.001$ respectively).

Table 3 shows that serum iron concentration improved after riboflavin intake. The GSH level, transferrin and haemoglobin levels did not change significantly nonetheless, the level of GSH was significantly reduced in the unsupplemented group, $P<0.05$.

Table 1: Mean values of measured and derived parameters

	SCA group(n=50)	Control group(n=20)	P-value
Haemoglobin(g/100ml)	8.3(1.19)*	14.8(1.12)	<0.001
PCV)%)	23.0(7.4)	40.3(5.2)	<0.001
GSH(µg/ml)	96.5(32.6)	136.3(27.9)	<0.001
MEHC(%)	34.7(4.0)	36.9(3.0)	<0.05
Serum iron (µg/ml)	93.6(28.3)	101.3(14.6)	ns
TIBC(µg/100ml)	386.7(71.8)	273.2(90.0)	<0.05
Transferrin Saturation	24.6(7.1)	37.7(5.9)	<0.05

- Mean (± S.D.), MEHC = mean erythrocyte haemoglobin concentration derived thus:

MEHC = haemoglobin (g%) x 100, ns = not significant
PCV(%)

Discussion

The findings of this study support earlier observations that haemoglobin and PCV levels are lower in SCA patients, compared to individuals not having the disease (Serjeant and Serjeant, 1982, Ballas *et al*, 1982). But contrary to the report of Oluboyede *et al*, (1981), the serum iron level was not significantly different from the control group, an observation confirmed by the MEHC value calculated for the SCA group. According to Davidson *et al*, (1978), an MEHC value <30% meant that the red cells were lacking in haemoglobin and was indicative of anaemia that required an iron therapy. In this study, although the SCA

group had significantly reduced levels of haemoglobin and PCV, theiur mean MEHC value was not less than 30%.

Table 2: Pearson's Correlation coefficients between indices with antioxidant properties (GSH and transferrin) and haematological parameters.

	PCV (%)	Haemoglobin(g%)	MEHC(%)
(a) GSH(µg/ml)	0.082	0.288	0.344
Transferrin	0.285	0.479*	0.016
(b) GSH (µg/ml)	0.266	0.462*	0.591*
Transferrin	0.032	-0.011	-0.048

(a) represents Control group n=20 (b) represents sickle cell anaemia group n=50
PCV =packed cell volume, GSH = reduced glutathione, MEHC = mean erythrocyte haemoglobin concentration, derived thus:

$$\text{MEHC} = \frac{\text{haemoglobin(g\%)} \times 100}{\text{PCV(\%)}}$$

*indicates a significant correlation between the parameters being compared at $P<0.05$.

Table 3: Effect of riboflavin supplementation on haemoglobin, GSH, transferrin and serum iron SCA patients.

	Supplemented	Group (n=12)	Unsupplemented	Group (n=12)
	Before	After	Before	After
Haemoglobin(gm/ 100ml)	10.0 (1.2)*	10.8 (1.5)	8.9 (1.2)	9.1 (1.1)
GSH(µg/ml)	94.4 (41.0)	100.3 (39.7)	80.9 (26.8)	46.9 (16.8)
Serum iron(µg/100ml)	106.4 (3.78)	142.4 (54.1)	94.4 (22.5)	88.2 (18.6)
Transferrin	36.2 (14.7)	48.2 (17.9)	29.0 (9.2)	26.7 (11.0)

*Values represent the mean (± SD)

Transferrin and GSH are considered antioxidant agents that would protect the cells against oxidant stress, (Halliwell 1994, 1997). Therefore, the lower levels of transferrin and GSH reported for SCA patients in this study would imply a reduced ability of SCA erythrocytes to be protected against free

radicals produced in the body. Additionally, in view of the lower GSH level, coupled with similar iron levels as that observed in the control group, the SCA patient could be expected to be under increased oxidant stress because iron can increase the reactivity of oxygen and H_2O_2 (Halliwell, 1994). This study has also revealed that while haemoglobin level was dependant on transferrin in the control group, haemoglobin was dependent on GSH in the SCA group. Thus the lower GSH level in the SCA group might partly explain the low haemoglobin level in this group. One can also infer from the association between MEHC and GSH that as the level of blood GSH increases, the possibility of an individual developing anaemia that would need iron therapy decreases particularly in SCA patients (see Table 2). From various reports on GSH level ion SCA patients, different methods have been used and the level of GSH has been reported differently. However, in all cases, lower GSH levels have been reported for SCA patients. Wetterstroem *et al* (1984) reported that in SCA patients, the GSH level was 84% of the value for the control group while Lachant *et al* (1983) reported 89%. In this study, the mean GSH value of 96.5µg/ml recorded for the SCA patients was 69% of that recorded for the control group (136.3 µg/ml). It is important to note here that in the report of Wetterstroem *et al* (1984), SCA patients who were Glucose-6-phosphate dehydrogenase, (G-6-PD) deficient were excluded. In this study however, neither the patients nor the control group were screened for G-6-PD deficiency. The inclusion of G-6-PD deficient individuals could have reduced the mean blood GSH level especially in the SCA group since the incidence of G-6-PD deficiency is reported to be high among SCA patients (Lewis *et al*, 1966). G-6PD is needed to maintain the content of GSH in red cells, GSH being necessary to prevent haemolysis (Rang and Dale, 1993). Sickled erythrocytes are also reported to have low catalase activity and high superoxide dismutase activity, Das (1979), as well as low serum vitamin E concentration Essien (1995), George *et al* (20001). All these factors would enhance GSH consumption and probably account for the low GSH level observed in the SCA group, and the significant decrease in GSH level in the SCA group not supplemented with riboflavin observed in thgis study. FAD, which has ribloflavin as its cofactor, is needed by the glutathione reductase for the regeneration of GSH from GSSG produced after free radicals such as hydroxyl ion and H_2O_2 have been metabolised by the combined action of GSH and glutathione peroxidase (Varma, 1983).

Although, ROS formed in the human body as part of normal metabolic reactions, endogenous antioxidant defences are inadequate to scavenge them completely, (Halliwell, 1996). Thus, the consumption of diet-derived antioxidants or supplements that may help boost the antioxidant defense should be encouraged. The idea of using riboflavin to enhance blood GSH level is not new. Schendel and Gordon (1975) reported significant increase in GSH level in subjects supplemented 10mg riboflavin daily for 7 days. In this study, it is possible that the riboflavin supplementation affected only one of the variables (GSH), that contributes to the antioxidant defence system in SCA explaining why the GSH increase in the supplemented group was not significant. However, the significant decrease of GSH level in the unsupplemented group suggests a role for riboflavin.

Since genetic counselling is not generally practised in this country, it is possible that the incidence of SCA would increase with population growth. It is therefore being suggested that SCA patients should be discouraged from indiscriminate use of vitamin tablets containing iron unless the patient has been diagnosed to be iron deficient. This would prevent iron overload among SCA patients in Nigeria where self-medication is commonly practised. In addition, a closer look at the riboflavin status of SCA patients could be beneficial. Riboflavin supplementation as part of the management of SCA may be important in diminishing the cumulative oxidative damage in sickled erythrocytes.

From this study we conclude that reduced GSH and transferrin levels in SCA patients compromises their antioxidants status, and possibly place them under increased oxidant stress, contributing partly to the haemolytic anaemia associated with the disease.

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