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Research Article

Malondialdehyde and Haptoglobin among Sickle Cell Disease Children in Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria

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Abstract

Objectives: Sickle Cell Disease (SCD) is global public health problem. The disease is characterized by free radical associated oxidative stress. This study investigated the malondialdehyde (MDA) and haptoglobin (Hp) levels among children with SCD in Sokoto, Nigeria.

Methods: This study investigated the serum malondialdehyde (MDA) and haptoglobin (Hp) levels among 60 children with SCD. The subjects for this study were categorized into two groups; those in the steady state A (n = 30) and those presenting with vaso- occlusive crisis (VOC) B (n = 30). Twenty-two age-matched non- SCA children served as control (C).

Results: The Hp was significantly lower among the sickle cells subjects compared to controls (p = 0.00) while the MDA level was significantly higher among the SCD subjects compared to controls (p = 0.00). The result showed a statistically significant difference between the MDA and Hp levels of group A versus C (p = 0.000 and 0.011) and B versus C groups (p = 0.000). We did not observe a statistically significant difference between A versus B group (p > 0.05). The effect of age on the MDA and Hp among sickle cell disease children was compared. Age did not have a statistically significant effect on the MDA and Hp levels of the sickle cell subjects (p=0.191 and 0.520) respectively. There was no statistically significant difference in the MDA and Haptoglobin level among sickle cell disease subjects based on gender (p = 0.948 and 0.423) respectively. Ethnicity, maternal occupational group and income level had no statistically significant effect on the MDA and Haptoglobin level among sickle cell disease subjects (p > 0.05).

Conclusion: The finding from this study indicates that SCD subjects tend to have lower values of Hp and higher values of MDA compared to controls. Strategies using antioxidants and therapeutic haptoglobin to protect against plasma lipid oxidation by cell-free haemoglobin may reduce the deleterious effects of lysis-associated cell-free Hb seen in SCD.

Keywords: *Malondialdehyde; Haptoglobin; Children; SCD; UDUTH; Sokoto; Nigeria*

Introduction

Sickle cell disease (SCD) is a genetic disorder that involve the haemoglobin in the erythrocytes. It is a genetic disease of global public health significance. A significant number of children are born yearly with SCD in Nigeria [1-2]. Current estimate shows that about 25% of adult Nigerians have sickle cell trait and 3% have SCD [3] (Adekile *et al.,* 2016). A previous report by Jiya *et al.* [4] indicated that 12.5% of patients presenting to the Paediatric Department in the University Teaching Hospital in Sokoto have SCD. SCD is responsible for a significant 20% of neonatal death [5-6]. The WHO voted SCD as a challenge of significant public health consequence [7].

The clinical consequences can be divided into 4 groups: haemolysis and haematological complications, vaso-occlusion, infection, and organ dysfunction [6]. The complications seen among SCD patients is major cause of mortality and morbidity associated with this disease. These complications can have a significant negative consequence on the quality of life (QOL) among these patients [8]. The oxidative damage commonly seen in sickle RBCs due to the unstable nature of red cells contain HbS is the main cause of the complications associated with this disease resulting in the formation of free radicals' generation.

The oxidative damage commonly seen in sickled erythrocytes predisposes the RBCs to haemolysis. The haemolysis of the red cell results in the release of free radical haemoglobin contained in the cytoplasm and generation of oxidative products. This free haemoglobin causes a reduction in body's antioxidant defense mechanisms [9].

The active phase protein Haptoglobin (Hp) is produced in the liver and released in plasma. Hp production also takes place produced in other secondary tissues including the arterial vessels, brain, intestine, kidney, lung, skin and spleen [10-12]. The normal values of Hp ranges from 0.3- 3mg/ml but tend to increase significantly in the presence of systemic inflammation. Plasma haptoglobin scavenge free haemoglobin and heme and have been shown to be depleted in haemolytic states such as SCD [13]. Haptoglobin is a plasma protein with the highest binding affinities for haemoglobin (Hb) and heme [14]. It renders Hb heme relatively non-reactive [15-16]. It inhibits Hb- and heme-mediated micro vascularity in SCD mice [17]. Plasma haptoglobin level are often depleted in SCD patients and mice due to chronic intravascular haemolysis [18-19].

Malondialdehyde (MDA) a product of peroxidation that is commonly used as a marker of oxidative stress [20, 21]. The mean MDA levels in serum and saliva in SCD patient has been shown to be higher compared to controls [22].

Although some investigations have been carried out on MDA and haptoglobin levels among SCD in the developed world, there is however lack of data on haptoglobin and MDA level among SCD patients in Sokoto in particular and Nigeria in general. This study will potentially yield evidence-based data that will facilitate the effective management of SCD patients in the area. This study determined the MDA and haptoglobin (Hp) levels among children of African descent with SCD resident in Sokoto, North Western Nigeria.

Materials and Methods

Study area

The cross-sectional study was carried in the Paediatric Department of UDUTH Sokoto and Specialist Hospital Sokoto. The hospitals are tertiary and secondary health facilities respectively located in Sokoto State, in North Western Nigeria. The state is bounded with Zamfara State to the East, Benin Republic to the West, Niger Republic to the North and Kebbi State to the South-East. The state is multi ethnic and tribal with the major being the Hausa and Fulani [4]. The annual growth rate of the state is about 3% while the population was 4.2 million as of 2006 [23].

Sample size calculation

The sample size was determined using the formula (z^2 pq/d^2) [24]

n = minimum sample size

z = standard normal deviation and probability.

p = prevalence to be assessed from previous studies.

q = Calculated proportion of failure (= 1 - P)

d = precision, tolerance limit, the minimum is 0.05.

Therefore n = z^2 pq/d^2

Where Z = 95% (1.96)

P = 3% (0.03) [3].

q = 1 - 0.03 (=0.97)

d = 5% (0.05)

Therefore n = $(1.96)^2$ (0.03) (0.97) / $(0.05)^2$

n = 45

Study population

The study investigated 60 consecutively-recruited children with SCA made up of 30 in VOC and 30 on steady state. The aged range of the subjects was 1 –14 years. A total of 22 age - matched children with haemoglobin AA were observed as controls. The subjects and controls participants were consecutively recruited from UDUTH and Specialist Hospital Sokoto.

Inclusion criteria

Subjects that whose parents/guardians offered verbal informed consent for their ward to participate in the study who were confirmed haemoglobin-SS and aged (1-14 years) were recruited as subjects into the study.

Exclusion criteria

Children who did not meet the inclusion criteria who were >14 years and < 1 year old, has had a recent red cell transfusion in the last 4 months and whose parents or guardian refused to offer verbal informed consent were excluded from participation in the study.

Study design

This case- control study investigated age - matched children who had HbSS (subjects) and HbAA haemoglobin electrophoretic pattern (controls). Socio- demographic information of the participants was obtained by using an interviewer- administered questionnaire. Data

collected included; age, gender and other socio-demographic factors. Laboratory values was obtained by estimating the serum MDA and Haptoglobin.

Ethical considerations

Ethical approval was obtained from the ethical review board of Usmanu Danfodiyo University Teaching Hospital (UDUTH) and Specialist Hospital, Sokoto. We obtained verbal informed consent from the parents or guardians of the subjects prior to the start of the study.

Sampling techniques

Sample collection

Whole blood samples were collected from all participants into plain tubes using strict aseptic techniques. The sample from the plain tubes was allowed to clot naturally. The clotted blood sample was subsequently centrifuged using a bench-top at an optimal speed of 3000 rpm for ten minutes. The sera gotten was appropriately stored at -20^{0}c immediately until ready to be analyzed. The laboratory analysis was carried out at the Haematology Laboratory UDUTH Sokoto, Nigeria. The serum was used for the assay of MDA, and haptoglobin.

Determination of haptoglobin (Hp)

Haptoglobin (Hp) was analysed using the ELISA reagents (Melsin Medical Company Limited, China). This test uses enzyme linked immunosorbent assay-double antibody sandwich principle to assay Hp levels in the sample. The Micro strip plate previously coated by Purified Hp antibody to make the solid-phase antibody. The principle is based on the fact that when sample containing Hp is added to the wells, it combines with Hp antibody- labelled by HRP, to produce antibody - antigen - enzyme-antibody complex. The enzyme that has not combined with the antigen antibody complex is washed completely and Chromogen solution A and Chromogen Solution B were added. This addition facilitates a colour change to blue. The addition of the acidic solution changes the colour to yellow. The colour change was determined using a spectrophotometer at a wavelength of 450 nm. The concentration of Hp in the samples is determined by comparing the optical density of the samples to the standard curve.

Determination of serum malondialdehyde

Serum Malondialdehyde was determined using a chemical method [25]. Malondialdehyde in serum was separated and determined as conjugate with Thiobarbituric acid, (TBA). Serum proteins were precipitated by Trichloric acid, (TCA) and then removed by centrifugation. The MDA-TBA complex was measured at 534 nm.

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 20. We calculated the frequencies and percentages. Student t- test both independent t test and paired sample t-test as well as ANOVA were used to compare the data. The results were expressed as mean ± standard error of mean. A p- value of ≤ 0.05 was accepted as significant in all statistical comparisons.

Results

The sociodemographic variables of SCA subjects (steady and crises) and control group is shown in table 1. A significant number of SCD children were aged 5 years and above (80% in steady, 50% in crisis and 68.2% among the control group). We observed as equal distribution of males and females (50%) among subjects in the steady state but there is a minor increase of females than males in crisis and

control groups (56.7% and 68.2% respectively). Subjects of Hausa/Fulani ethnicity accounted for 90% SCD children and controls. Table 2 shows the comparison of MDA and Hp between SCD subjects and apparently healthy controls. The HP was significantly lower among the sickle cells subjects compared to controls (p = 0.00) while the MDA level was significantly higher among the SCD subjects compared to controls (p = 0.00). Table 3 shows the distribution of MDA and Hp among the SCD subjects (at steady state and crisis) and control individuals. The result shows statistically significant difference between A V C (p = 0.000 and 0.011) and B V C groups (p = 0.000). We did not observe a statistically significant difference between A V B groups (p > 0.05). Table 4 highlights the effect of age on the MDA and Hp among the sickle cell disease subjects. There was no age-related difference between the age groups (p = 0.191 and 0.520) respectively. Table 5 highlights the effect of gender on the MDA and Hp among sickle cell disease subjects. Our finding indicated that there was no statistically significance difference based on gender (p = 0.948 and 0.423) respectively. Table 6 shows comparison of MDA and Hp of sickle cell disease children among different ethnicities. No statistically significant difference was observed among all the groups (p > 0.05). Table 7 shows the MDA and Hp of SCD subjects based on the maternal level of education. Findings indicated that there was no significantly significance difference (p > 0.05) based on maternal level of education. Table 8 shows the MDA and Hp of the SCD subjects based on maternal occupation. There was no statistically significant difference (p < 0.05) based on the maternal occupation. Table 9 shows the MDA and Hp of SCD subjects based on maternal income. We observed no statistically significant difference (p > 0.05) among the parameters.

Group	Steady	%	Crisis	%	Control	%
N	30		30		22	
Age (Years)						
< 5Yrs	6	20	15	50	7	31.8
5 Yrs Above	24	80	15	50	15	68.2
Gender						
Male	15	50	13	43.3	7	31.8
Female	15	50	17	56.7	15	68.2
Ethnicity						
Hausa/Fulani	27	90.0	27	90.0	20	90.9
Yoruba	0	0.0	2	6.7	2	9.1
Igbo	3	10.0	1	3.3	0	0.0
Level of Education Mother						
Primary	3	10.0	6	20.0	0	0.0
Secondary	12	40.0	10	33.3	6	31.8
Tertiary	8	26.7	4	13.3	15	63.6
Non formal	7	23.3	10	33.3	1	4.5
Occupation of Mother						
Business	20	66.7	23	76.7	2	9.1
Civil Servant	1	3.3	2	6.7	13	59.1
House Wives	9	30.0	5	16.7	7	31.8
Mother's Income						
< 18,000	16	53.3	25	83.3	6	27.3
25,000 - 40,000	2	6.7	1	3.3	1	4.5
50,000 - 100,000	2	3.3	0	0.0	4	27.3
> 100,000	2	6.7			0	0.0
None	9	30.0	4	13.3	5	22.7

Table 1: *Socio-demographic variables among Sickle Cell Disease Children and control individuals.*

Group	N	MDA (nmol/l)	HP (ng/ml)
SCD	60	2.51 ± 0.09	22.78 ± 1.89
Control	22	0.92 ± 0.16	35.77 ± 2.31
p-value		0.000	0.000

Table 2: *Comparison of MDA and Haptoglobin of SCD Subjects and apparently healthy Controls.*

Key: *MDA= Malondialdehyde (nmol/l), HP=Haptoglobin (ng/ml), N= number of subjects, SCD=Sickle Cell Disease, S=Significant, Correlation is significant at level of ≤0.05.*

Group	N	MDA (nmol/l)	Hp(ng/ml)
A	30	2.57 ± 0.09	25.19 ± 3.25
B	30	2.45 ± 0.18	20.36 ± 1.87
C	22	0.92 ± 0.16	35.77 ± 2.31
Post HOC			
AVB		0.531	0.200
AVC		0.000	0.011
BVC		0.000	0.000

Table 3: *Comparison of the Antioxidant enzymes, MDA and Haptoglobin between SCD Subjects (steady and crisis) and Controls.*

Key*: ANOVA=Analysis of Variance, MDA= Malondialdehyde, HP=Haptoglobin N= no. of subjects, ng/ml=nanogram per milliliter, pg/ml= Pico-gram per milliliter, nmol/l= nanomole per litre, A= Steady state, B= Crisis, C= Control, V= Versus.*

Parameter	< 5 Years	5 Years Above	p-value
N	21	39	
MDA (nmol/l)	2.31 ± 0.19	2.61 ± 0.11	0.191
Hp (ng/ml)	24.46 ± 3.21	21.88 ± 2.35	0.520

Table 4: *The comparison of MDA and Haptoglobin levels based on age groups of sickle cell disease Subjects.*

Key: *N= Number of subjects, MDA= Malondialdehyde (nmol/l), HP=Haptoglobin (ng/ml).*

Gender	Male N = 28	Female N = 32	p-value
Parameter			
MDA (nmol/l)	2.51 ± 0.15	2.51 ± 0.14	0.984
Hp (ng/ml)	21.18 ± 2.29	24.18 ± 2.93	0.423

Table 5: *Comparison of MDA and Haptoglobin between male and female Subjects with sickle cell disease.*

Key: *N= Number of subjects, MDA= Malondialdehyde (nmol/l), HP=Haptoglobin (ng/ml). Data were analyzed using student t-test and the results are presented as mean ±SEM.*

Ethnic Group						
Group	**Hausa/Fulani N=54**	**Yoruba N=2**	**Igbo N=4**	**Post Hoc**		
Parameters				H V Y	H V I	Y V I
MDA (nmol/l)	2.49 ± 0.11	3.18 ± 0.79	2.26 ± 0.37	0.545	0.576	0.436
Hp (ng/ml)	22.07 ± 1.99	36.41 ± 15.53	26.55 ± 7.66	0.524	0.606	0.642

Table 6: The comparison of MDA and Hp of sickle cell disease Subjects based on ethnicity.

Key*: N= Number of subjects, SEM= Standard Error of Mean, ANOVA=Analysis of Variance, MDA= Malondialdehyde (nmol/l), HP=Haptoglobin (ng/ml), H= Hausa/Fulani, Y= Yoruba, I= Igbo, V=VS. Data were analyzed using one-way ANOVA with turkey post-hoc test.*

Educational level					
	Primary	**Secondary**	**Tertiary**	**Non-formal**	**p-value**
Parameters					
MDA (nmol/l)	2.61 ± 0.25	2.05 ± 0.91	1.70 ± 0.24	2.43 ± 0.15	0.717
Hp (ng/ml)	17.57 ± 1.33	29.42 ± 3.31	30.93 ± 2.65	18.78 ± 2.33	0.152

Table 7: *MDA and Hp SCD Subjects based on maternal level of education.*

Key*: ANOVA=Analysis of Variance, Malondialdehyde (nmol/l), HP=Haptoglobin (ng/ml), Data were analyzed using one-way ANOVA.*

Occupation				
Occupation	**Business**	**Civil service**	**House wives**	
Parameters				
MDA (nmol/l)	2.47±0.11	1.15±0.22	1.97±0.26	0.339
Hp (ng/ml)	23.00±2.32	33.64±3.73	27.63±2.37	0.687

Table 8: *MDA and Hp levels among the SCD Subjects based on maternal occupation.*

Key: *ANOVA=Analysis of Variance, Malondialdehyde (nmol/l), HP=Haptoglobin (ng/ml). Data were analyzed using one-way ANOVA.*

Maternal Income				> 100,000	None	P-value
< 18000	**25 - 40,000**	**50 - 100000**				
Parameters						
MDA (nmol/l)	2.34 ± 0.13	2.41 ± 0.25	0.70 ± 0.34	1.77 ± 0.43	1.98 ± 0.28	0.907
Hp (ng/ml)	23.23 ± 2.03	41.75 ± 15.91	35.16 ± 4.78	27.22 ± 4.53	26.97 ± 2.73	0.766

Table 9: *MDA and Hp of SCD Subjects based on maternal income.*

Key: *ANOVA = Analysis of Variance, Data is presented as mean ± SEM, Malondialdehyde (nmol/l), HP = Haptoglobin (ng/ml). Data were analyzed using one-way ANOVA.*

Discussion

SCD is genetic disease of global significance. Nigeria has a large burden of the disease particularly with an estimated 150,000 Nigerian children born each year with SCD [1-2]. The disease is responsible for up to 20% of neonatal mortality [5-6]. As at 2006, the WHO declared disease a problem of main public health significance and a burden that must be addressed if recent improvements in overall child survival are to be consolidated [7]. There is scarcity of data on oxidative stress markers and haptoglobin level among SCD children in Sokoto. The present study highlights MDA and Hp of homozygous sickle cell children compared with normal controls with haemoglobin AA.

Our finding in this study indicates that there is a significant increase in MDA level of SCD subjects when compared with HbAA controls ($p = 0.00$). However, when we compared the MDA level among the SCD subjects in the steady and VOC with control group, there was a statistically significant difference ($p = 0.00$ and 0.00) respectively. But when SCD subjects in the steady state were compared with those with vaso-occlusive crisis, there was no significant difference ($p = 0.531$). Our finding is in agreement with previous reports [26-28]. Accumulation of MDA disturbs the organization of phospholipids in the human erythrocyte membrane bilayer. The oxidation of phospholipids in the plasma and internal organelle membranes (mitochondria) damage their function [29]. The increase of MDA in SCD patients may also be associated to the auto-oxidation of iron commonly seen in these patients [30]. Also, the excess creation of MDA has added toxic effects that often leads to alterations of the proteins, modifications of amino-acid side chain, and lipids structure. These changes can potentially cause a partially or completely affect the function of proteins including antioxidant enzymes and relevant protein receptors [31]. These challenges can potentially increase complement activation and associated lysis of the erythrocyte [32]. Our observation in human model is consistent with previous reports [33-35] in animal and human model which indicated that scavenger plasma proteins haptoglobin (Hp) are depleted and malondialdehyde formation, an end product of lipid peroxidation was increased in BERK-SS mice, SCD and patients and in patients with haemolytic-related diseases. Several mechanisms are thought to contribute to the high oxidative burden commonly seen in sickle cell disease patients; excessive levels of cell-free haemoglobin released from lysed red cells [36-37], pro-inflammatory challenge associated with free haemoglobin in the circulation [38], recurrent free-radical associated ischemia-reperfusion injury [39-40] and increased autoxidation of erythrocytes containing sickle haemoglobin (HbS) [41].

In this study, we also observed that the mean serum Haptoglobin (Hp) level was significantly lower among the SCA subjects compared with the HbAA controls. However, there was no significant difference between the serum Hp level of SCD subjects in the steady state and those in crisis. When the Hp levels of SCD subjects in the steady and VOC were compared with control group, there was a significant difference ($p = 0.011$ and 0.000) respectively. However, when SCD subjects in the steady state were compared with subjects with vaso-occlusive crisis, we observed that there was no statistically significant difference ($p = 0.20$). This finding agrees with previous reports [42-43]. The reduced Hp is as a result of consequences of haemolysis due to breakage of sickled RBCs. One third of the erythrocytes are damaged in the intravascularly often leads to increased cell-free plasma Hb and heme levels [44]. The pathophysiological challenge associated with free Hb/heme includes; acute haemodynamic instability and acute or chronic vascular injury. The toxicity and inflammatory nature of free Hb is responsible for the greater nitric oxide consumption seen in SCD patients which promotes the consequent accumulation of hydroxyl radicals and ROS in the blood vessels. The body's first defense mechanism against the harmful effects of free Hb involves haptoglobin (Hp), whose principal role is to bind to free Hb in the plasma, thus averting the excretion of iron by the kidneys and protecting blood vessels from its oxidative effects. In addition, Hp also has immunomodulatory properties [45-46]. Our observation in human model in this study is consistent with previous reports [33-35] in animal model which indicated that scavenger plasma proteins haptoglobin (Hp) are depleted in BERK-SS mice. Finding from this study may be a viable clinical indication for interventions that can potentially increase plasma haptoglobin levels in SCD [47]. This may be beneficial by preventing oxidative reactions with haemoglobin and the release of free heme into the vasculature [48].

We observed that there were no significant differences in the MDA and Haptoglobin levels among the sickle cell disease children based on age, gender, ethnicity, maternal level of educational attainment, level of education and income ($p > 0.05$). The reason for this observation is unknown.

Conclusion and Recommendation

Conclusion

This study confirms that SCA children have lower values of Hp but higher values of MDA and compared to controls. Antioxidant enzymes, MDA (index of lipid peroxidation) and Hp could potentially be used as effective therapeutic targets in the management of patients with SCA. We recommend that antioxidant supplementation be implemented as an affordable and accessible intervention for sickle cell disease patients (in the steady or crisis states) to prevent further oxidative damage to red cells.

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