Original Research Article

Antioxidative Potential of Foetal Haemoglobin in Sickle Cell Disease.

Comment [KF1]: You should also give a little background on SOD, GPx and MDA, so people will understand what you are talking about in your introduction.

Abstract

Background: Oxidative stress is a clinical condition in sickle cell disease (SCD) that results from increased production of reactive oxygen species (ROS). High Foetal haemoglobin (HbF) is beneficial in sickle cell disease due to its ability to resist polymerization with sickle haemoglobin. The aim of this study is to determine the anti-oxidative potential of HbF in subjects with SCD.

Methods: Whole blood was used for the determination of HbF concentration while serum was used for the assay of Glutathione peroxidase (GPx), Super-oxide dismutase (SOD), and malondialdehyde (MDA). Alkali denaturation method was used for the determination of HbF while spectrophotometric method was used to assay for the various oxidative stress markers.

Results: The concentrations of HbF and MDA were significantly higher in the case subjects compared to the normal subjects. A direct relationship was observed between GPx (r = 0.47) and SOD (r = 0.46) with HbF. However, an inverse relationship was observed between MDA with HbF (r= -0.33), GPx (r= -0.18) and SOD (r= -0.26).

Conclusion: We conclude that HbF potentially associates with the antioxidant enzymes (GPx and SOD) to counteract the oxidative effect of ROS in SCD.

Keywords: Sickle cell disease, Foetal haemoglobin, Oxidative stress, lipid peroxidation, Glutathione peroxidase, Super oxide dismutase.

INTRODUCTION

Despite years of intensive research, Sickle cell disease still remains a disease of public interest, accounting for significant number of deaths globally. [1] This genetic blood disorder is characterized by an abnormal haemoglobin (HbS) that results from a point mutation in the beta (β)-globin chain of the haemoglobin [2]. The sickle haemoglobin (HbS) is responsible for all the clinical outcomes of the disorder including polymerization, vaso-occlusion and elevated rate of oxidative stress [3].

Oxidative stress (OS) is a state where the production of reactive oxygen species overwhelms the body's anti-oxidant defence system [4]. Several factors including HbS polymerization, decreased antioxidants activity, chronic haemolysis and inflammation have been reported to be responsible for increasing the rate of OS in SCD. The effect of these processes is an increase in the rate of tissue destruction [5].

Foetal haemoglobin, plays a beneficial role in SCD due to its ability to neutralize HbS polymerization [6 & 7]. Neonates and some adults with SCD experience a mild form of disease due to production of high concentration of HbF in their blood [8 & 9]. However, Sickle cell subjects from African countries including Nigeria have been reported to have a low concentration of this haemoglobin [10]. This study therefore is aimed at assessing the effect of foetal haemoglobin concentration from this population on oxidative stress in sickle cell subjects.

METHODS

METHODOLOGY

Subjects

The study was made up of twenty-five (25) steady state sickle cell subjects and seventeen (17) normal subjects. Subjects were randomly recruited after giving consent to be part of the study.

Inclusion Criteria

The criteria for inclusion of the sickle cell subjects included adults from 18 years and above, subjects not on hydroxy urea treatment and have not received blood transmission three months from the date of study. For the control subjects only adults with no history of ill health were recruited for the study.

Sample collection and analysis

About 4.0ml of venous blood obtained from the participants was used for analysis of all the biochemical parameters. Whole blood was used for foetal haemoglobin analysis while serum was used for the analysis of oxidative stress markers; Glutathione peroxidase (GPx), super-oxide-dismutase (SOD) and malondialdehyde (MDA). Alkali denaturation method was used for the determination of foetal haemoglobin concentration while spectrophotometric method was used for determination of the oxidative stress parameters.

Statistical analysis

Statistical analysis was performed with SPSS (version 20). Descriptive data was reported as means and standard error while students T-test was used to compare the means between groups. Pearson's correlation test was used to perform correlation analysis. All statistical values within $P<0.05$ were regarded as significant.

RESULTS

The concentration and activities of oxidative stress markers in sickle cell subjects and normal subjects were estimated (Table 1). The level of lipid peroxidation (MDA) in sickle cell subjects was significantly higher than in normal subjects (P=0.001). Similarly, the Foetal haemoglobin concentration (HbF) of the sickle cell subjects was also significantly higher than the control subjects (P=0.001). However, the activity of the anti-oxidant enzymes in subjects with sickle cell anaemia were lower than in the normal subjects.

Biochemical markers	Sickle cell Subjects	Normal subjects	P-value
HbF (%)	5.36±0.58	2.49±0.39	0.001*
MDA (µmol/mL)	14.09±1.20	4.95±1.35	0.001*
GPx (mg/mL)	34.77±3.09	42.29±4.95	0.246
SOD (µ/mL)	35.18±3.53	41.37±6.75	0.425

Table 1: Oxidative stress markers in Sickle and Normal Subjects.
* significant at P < 0.05
HbF: Foetal haemoglobin, MDA: Malondialdehyde, GPx: Glutathione peroxidase SOD: Super-oxide-dismutase,

The composition of the oxidative stress markers within the mean foetal haemoglobin concentration (HbF≤ 5) and those outside the range (HbF≥ 5.) were estimated (Table 2). The level of lipid peroxidation (MDA) in subjects with HbF≤ 5 was significantly higher than those with HbF≥ 5. However subjects with HbF≥ 5 showed an insignificant increase in activity of GPx, and SOD compared to those with HbF≤ 5.

Table 2: Classification of oxidative stress biomarkers based on Foetalhaemoglobin concentration in sickle cell subjects

Oxidative stress markers	HbF≤ 5 (13)	HbF≥ 5 (12)	P-value
MDA (µmol/mL)	16305.71±1272.02	11684.63±1914.01	0.05*
SOD (µ/mL)	30.24±4.07	40.54±5.67	0.15
GPx (mg/mL)	31.46±3.22	38.35±5.39	0.28

HbF: Foetal haemoglobin, MDA: Malondialdehyde, GPx: Glutathione peroxidase SOD: Super oxide dismutase, CAT: Catalase

The correlation between the antioxidant enzymes with foetal haemoglobin and malondialdehyde was assayed (Table 3). Foetal haemoglobin significantly correlated positively with GPx and SOD and inversely with MDA. GPx and SOD also correlated inversely with MDA.

Table 3: Correlation between anti-oxidant enzymes with Foetalhaemoglobin and Malondialdehyde in sickle cell subjects

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Biochemical markers	r	P-value
HbF-MDA	-0.333	0.104
HbF-SOD	0.464	0.020*
HbF-GPx	0.473	0.017*
MDA-Gpx	-0.184	0.378
MDA-SOD	-0.257	0.215

DISCUSSION

This study was carried out to determine the anti-oxidative potential of foetal haemoglobin in subjects with sickle celldisease. Foetal haemoglobin has been defined as the best modulator of clinical severity in sickle cell disease because of its ability to resist HbS polymerization [(11)]. Twenty-five (25) steady state sickle cell subjects with an average age of 23.0 years participated in the study. Seventeen (17) normal age matched subjects were used as control for the study. The foetal haemoglobin concentration, concentration of oxidative stress markers of the study population and their relationship was assayed.

Sickle cell subjects had significantly higher foetal haemoglobin concentration compared to the normal subjects. This difference has been associated to genetic factors in sickle cell subjects capable of maintaining the expression of the haemoglobin into adulthood [(12)]. The level of expression of foetal haemoglobin has also been reported to vary between sickle cell subjects; with some having a higher expression while others having a relatively lower expression of the haemoglobin. This finding is in agreement with previous studies by Olaniyi*et al.*[(13)], who reported a similar foetal haemoglobin concentration of 5.16% using the same method of assay.

The rate of lipid peroxidation (MDA) in Sickle cell subjects was also significantly higher than in the normal subjects. The elevation in lipid peroxidation in sickle cell subjects has been associated with high concentration of reactive oxygen species (ROS) produced from processes including HbS-polymerization, haemolysis and ischemic reperfusion [(14)]. This observation is in agreement with the studies of Nader *et al.* [(15)], Okorie et al, [(16)] and Afolayan*et al.*[(5)], who also observed a significantly higher levels of MDA in sickle cell subjects compared to normal subjects.

The activities of glutathione peroxidase and super oxide dismutase (anti-oxidative enzymes) were lower in sickle cell subjects compared to normal subjects. The reduced activity of these enzyme is associated to the high amount of ROS generated in sickle cell subjects. At this rate, the expression of anti-oxidative enzymes can barely keep up with the elevated amount of ROS resulting in reduced expression of the enzymes [(17)]. Previous studies by Biswal *et al.* [(18)], reported a similar finding. However, unlike the current study, they reported a significant difference in the antioxidant activities of the enzymes in the study population.

A comparison of the concentration of GPx, SOD and MDA in different HbF concentrations revealed a significant elevation of MDA in subjects with HbF≤5% compared to those with HbF>5%. This observation suggests the beneficial role of higher HbF concentration against cellular damages caused by lipid peroxidation [(19)]. Conversely, the activities of GPx and SOD was slightly higher in subjects with HbF>5% than those with HbF≤5%. This difference suggest that the foetal haemoglobin concentration plays a role in determining the concentration of anti-oxidative enzymes in sickle cell disease. however, more studies are needed to verify this finding.

The analysis of the association between HbF and the oxidative stress markers reveals that HbF significantly correlates with GPx and SOD while MDA inversely correlates with HbF. This observation suggests that the anti-oxidative properties of GPx and SOD is increased when the HbF is increased while the lipid peroxidation seems to prevail when the HbF concentration is low. Furthermore, the correlation between MDA with GPx and SOD revealed an inverse relationship. This finding further stress the role of GPx and SOD as antagonists of lipid peroxidation (MDA). Previous studies by Lia *et al.* [(20)] and Zhu*et al*[(21)]have linked erythroid-derived necrosis factor-2 (NRF2) as a stimulant for the production of both antioxidant proteins

and HbF in sickle cell disease. Hence, there is a possibility for individuals with higher expression of NRF2 to have higher expression of HbF, GPx and SOD.

Conclusion

The findings of this study suggest that foetal haemoglobin potentially associates with anti-oxidant enzymes to counter the effect of oxidative stress produced by reactive oxygen species in subjects with sickle cell disease.

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