Original Research Article

Status of Oxidative Stress and Lipid Profile in Patients of Sickle Cell Anemia

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ABSTRACT

Background: Sickle cell anaemia (SCA) is a hereditary disorder with higher potential for oxidative damage due to chronic redox imbalance in red cells. Several biomarkers have been associated with SCA clinical prognosis. This study was designed to determine the oxidative stress & lipid profile that can be used in monitoring the prognosis & management of SCA patients.

Aim & Objectives:
1. Study of Glutathione as oxidative stress marker in patients of SCA.
2. Evaluation of lipid profile in patients of SCA.

Method: This was an observational study. We analysed the oxidative stress marker i.e. reduced glutathione (GSH) along with lipid profile in 50 patients of SCA & compared the values with 50 control subjects.

Results: Compared with values of controls, SCA subjects had significantly lower erythrocyte GSH ($P \leq 0.05$).The plasma cholesterol (138.2 ± 2.4 mg/dl) in sickle cell anaemia were significantly decreased ($p \leq 0.05$) when compared with the control (162.3 ± 7.6mg/dl). Also the plasma high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol in sickle cell anaemia (33.46 ± 0.96 mg/dl and 89.3 ± 2.5 mg/dl) were significantly decreased ($p \leq 0.05$) when compared with the control (37 ± 1.8 mg/dl and 105.4 ± 8.3 mg/dl). There was no significant variation in the mean level of triglyceride in sickle cell disease when compared with the control.

Conclusion: The results are indicative of enhanced oxidative stress along with dyslipidaemia in the form of lower HDL-C, LDL-C & Total Cholesterol. Additional studies are warranted to test this hypothesis & the probable mechanism involved in this complex network of markers & their role in SCA pathogenesis.

Key Words: Sickle Cell Anaemia, Oxidative stress, Lipid profile, GSH, Total cholesterol, LDL-C, HDL-C

INTRODUCTION

Sickle cell disease (SCD) results from a single base-pair mutation in the gene for the β-globin subunit of adult haemoglobin (HbA). An adenine-to-thymine substitution in the sixth codon replaces glutamic acid with valine in the resulting β-globin chain. Polymerization of sickle haemoglobin (HbS) is the origin of the molecular pathophysiology in SCD. It is actually through erythrocyte membrane damage that HbS produces most of the

clinical features of disease, from haemolytic anaemia to micro vascular obstruction. [1] The protean manifestation is the vaso-occlusive or painful crisis, in which episodic micro vessel occlusion at one or many sites induces pain and disability, accompanied by local inflammation. [2] In Maharashtra the prevalence of disease ranges from 1.9% to 33.5% . We are residing in an endemic zone and the prevalence of sickle cell anaemia here in Vidarbha region of Maharashtra is from 9.4% to 22.2%. [3] However, the disease is yet to be viewed as a major public health problem; as many babies are born each year with sickle cell disease are more prone to death in the presence of opportunistic infections, diarrhoea, measles and malnutrition. Atherosclerosis is characterized by increased accumulation of cholesterol in arterial wall macrophages and is exacerbated by oxidant stress. [4] Patients with SCA are subjected to increased oxidative stress, particularly during vaso-occlusive crises and acute chest pain. Oxidative stress represents the imbalance between enhanced generation of reactive oxygen species and low cellular content of antioxidants. The major defence systems include those that scavenge free radicals such as glutathione, vitamin C, vitamin E and superoxide dismutase and that reduce hydroperoxides formed by glutathione peroxidase and catalase. [5] In the cell, glutathione (GSH) is the most abundant low-molecular-weight thiol containing molecule GSH inside the cell is considered to be the most sensitive indicator of the cell's overall health, and of its ability to resist toxic challenge. GSH depletion in cell can trigger suicide of the cell by a process known as apoptosis. [6]

On other hand, lipid profile is a group of tests that are often ordered together to determine risk of coronary heart disease. It is a good indicator of whether someone will have a heart attack or stroke caused by blockage of blood vessels or hardening of arteries. [7] We attempted to study a possible involvement of dyslipidemia along with oxidative stress marker, reduced glutathione (GSH) in adult patients of sickle cell anaemia in steady state.

MATERIALS AND METHODS

This study is an observational study conducted in the year 2012 - 2013 at Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha, Maharashtra, India. 50 sickle cell patients of age group 16-50 years were selected from the sickle cell clinic run by the Hospital. All subjects studied were diagnosed as SCA based on positive sickling tests and haemoglobin electrophoresis at a pH of 8.6 on cellulose acetate paper. Equal number of age and sex matched sickle cell negative healthy subjects were selected as a control group. All the procedures were conducted after Institutional Ethical Committee approval and informed consent was obtained from the subjects.

5ml of venous blood was collected by clean venipuncture from each patient via the antecubital vein using a plastic syringe. Hemolysates were used for estimation of reduced glutathione (GSH). The assay of GSH with DTNB was performed by a standard Beutler method (1963). The method described is based on the development of a yellow colour when 5,5'-dithiobis (2-nitrobenzoic acid) (Ellmans Reagent, DTNB) is added to sulphydryl compounds due to redox reaction between GSH and DTNB. The colour which develops is fairly stable for about 10 min. The reaction is read at 412nm. [6,8] The serum was separated by centrifuging the coagulated blood for 5 min & lipid profile was estimated. The plasma lipid profile (cholesterol, triglyceride and HDL-C) was measured by the colorimetric method using Biosystem kits; while LDL-

cholesterol was determined by using the Freidwald formula. Data were analysed using SPSS version 16.0.The results were expressed as mean ± standard deviation and student t-test was used to compare the values of cases to controls and p value of ≤ 0.05 was considered to be significant.

RESULTS

Serum lipid profile values of SCD cases and controls have been shown in table-1. Total cholesterol; HDL and LDL cholesterol have shown significant decrease in patients of SCD while triglyceride level was not significantly different in cases and control. The mean reduced glutathione level was significantly lower among patients with SCA compared with controls (p≤0.05). The mean value of GSH in the SCA group was about four times less than half that of control group.

Table 1. Lipid profile and Glutathione (GSH) level in cases and controls

Parameter	Cases (SCD) (n=50) (Mean ± SD)	Controls (HbAA) (n=50) (Mean ±SD)	P value
Mean Age	23.8 ± 2.4	24.1 ± 3.2	≥0.05
Total Cholesterol(mg/dl)	138.2 ± 2.4	162.3 ± 7.6	≤ 0.05*
TG (mg/dl)	100 ± 4.9	106 ± 13.3	≥ 0.05
HDL-C (mg/dl)	33.46 ± 0.96	37 ± 1.8	≤ 0.05*
LDL-C (mg/dl)	89.3 ± 2.5	105.4 ± 8.3	≤ 0.05*
GSH (µmol/l	15.2 ±1.4	59.8±2.7	≤ 0.05*

* - Significant 'p' value

DISCUSSION

When a red blood cell containing sickle haemoglobin gives up its oxygen to the tissues it changes from its usual doughnut shape to sickle shape and becomes stiff rather than soft and flexible like normal red blood cells. The abnormal viscoelastic properties of oxygenated sickle cell erythrocytes and formation of irreversibly sickled erythrocytes are related to abnormal properties of their membrane which affect the plasma lipid profile. [7]

Reduced glutathione is one of the components of membranes and cytoplasmic compartments of RBC that maintains their integrity. In our study we found that there were significant changes in all the parameters of lipid profile along with oxidative stress marker of RBC i.e. reduced glutathione (GSH). [7,9-11]

GSH maintains redox homeostasis by two mechanisms, namely by reacting with hydroperoxides and by conjugation reactions. GSH is converted to its oxidized form GSSG when it reduces hydroperoxides, a reaction catalyzed by glutathione: hydrogen-peroxide oxidoreductase (also known as glutathione peroxidase, EC 1.11.1.9). Reduced GSH in turn is regenerated by glutathione: NADP oxidoreductase with NADPH as cofactors. [12]

In this study, subjects with SCA had markedly lower erythrocyte concentrations of GSH compared with controls (≤0.05) this was similar to the study reported by Morris C R (2008). [13] Another study by Marvin Reid (2006) [14] has proposed that the lower erythrocyte GSH of SCD patients is not due to suppressed synthesis or impaired regeneration but rather to increased consumption. Previous research shows that sickle red blood cells are more susceptible to oxidative lipid damage.

In this study, it was observed that plasma cholesterol in sickle cell disease was significantly decreased when compared with the control (≤0.05). This is similar to findings of previous studies by Zorca S, 2011, Nnodim J K, 2012, & Seixas M O,

2010. The decrease in plasma cholesterol could be that the "sickled cell" gets stuck in the tiny blood vessels blocking the flow of blood and causing pain. This condition probably could reduce the circulating cholesterol and hence low cholesterol level. [4,7,10]

It was also observed that there was no significant difference in triglyceride of sickle cell disease subjects when compared with the control. This is in line with the work of J.K. Nnodim [7] But it is in contrast to other studies by Zorca S [4] & Seixas M O [10] where triglycerides levels were significantly high in patients with sickle cell disease as compared to control groups. Triglyceride is mainly used in diagnosis and treatment of patients with diabetes, nephrosis, liver obstruction and other disease involving lipid metabolism. In addition, of all dietary lipids, plasma triglyceride levels are the most dependent on fasting vs. non fasting status of the subject at the time blood is drawn, and in non-fasting subjects, the amount and type of fat or fatty acids in the diet and time elapsed since the fats were consumed can strongly affect the blood triglyceride levels which possibly can be a reason for such insignificant finding in our study. [4,7,15]

In the present study we have found significantly lower levels of LDL-C which documented in virtually every previous study. [4,7,10] Total cholesterol, in particular LDL-C, has a well-established role in atherosclerosis. The low levels of LDL-C in SCD are consistent with the low levels of total cholesterol and the virtual absence of atherosclerosis among SCD patients. [4,10]

Similarly, the plasma high density lipoprotein cholesterol was depleted in sickle cell disease when compared with the control (≤0.05). The low HDL-C level seen in sickle cell patients would be expected to predispose them to increase risk of coronary heart disease HDL cholesterol is inversely related to the risk of developing coronary artery disease. The higher the HDL-C the less chance of developing coronary heart disease. [4,7,10]

Additional markers of oxidative damage should be identified to further provide evidence that oxidative stress is the major aggravator in sickle cell disorder. Sickle red blood cells will also be investigated in situations where ROS is scavenged to prove that oxidative stress is fundamental to problems associated with sickle cell disease. [12,13]

CONCLUSIONS

We have found low HDL-C, LDL-C & total cholesterol including the oxidative stress marker reduced Glutathione (GSH) in SCD patients. Decreased glutathione levels occur in SCD and may contribute to alterations in the erythrocyte redox environment, leading to compromised erythrocyte integrity. There can be a possibility that the fatty acids carried by triglycerides may become oxidized in SCD patients with haemolytic oxidative stress and serve as signalling molecules, or alternatively, haemolytic oxidative stress may co-ordinately regulate these pathways. By gaining further insight into the pathogenic mechanisms, additional studies are warranted to test this hypothesis and the probable mechanisms involved in this complex network of markers and their role in SCD pathogenesis.

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