Oxidative stress in sickle cell disease – a tertiary hospital experience in western Odisha

Sumitra Bhoi1, Seema Shah2, Anil Kumar Goel3, Arti Dhingra3, Pramila Kumari Mishra1

1 Veer Surendra Sai Medical College, Burla, Orissa, India
2 Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India
3 Shaheed Hasan Khan Mewati Government Medical College, Nalhar (Mewat), Haryana, India

Correspondence to: Anil Kumar Goel (akgoel.paeds@gmail.com)

DOI: 10.5455/ijmsph.2014.300520141 Received Date: 29.04.2014 Accepted Date: 30.05.2014

ABSTRACT

Background: The prevalence of sickle cell disease (SCD) in western Odisha is 9.1% which is a quite high figure if one looks at the morbidity and mortality out of it. In sickle-cell disease, low-oxygen tension promotes RBC sickling and repeated episodes of sickling damages the cell membrane and makes it rigid. Free radical promotes increased and irreversible sickling of RBC membrane by damaging it. Several newly emerging biological markers have potentiality to be utilized in deciding the degree of oxidative stress and disease severity.

Aims & Objective: We studied the oxidative stress which was assessed in sickle cell disease patients of western Odisha by measuring serum MDA as a by product of lipid peroxidation, with simultaneous measurement of levels of antioxidant vitamins.

Materials and Methods: A prospective single center case control study was done over a period of 1 year ending in July 2012. A total of 50 sickle cell disease children and 40 healthy children aged between 5 to 15 years of either sex were evaluated clinically and were screened by sickling test and diagnosis confirmed by haemoglobin electrophoresis and were labelled as case (both AS and SS). The estimation of uric acid, serum malondialdehyde, serum vitamin E and plasma vitamin C were done.

Results: Out of 50 patients 30 cases were homozygous (SS) and 20 cases were heterozygous (AS) for sickle cell as per Hb electrophoresis. There was a significant decrease in the level of Hb in homozygous (SS) cases (8.82 ± 1.05) while a moderate decline was noted in heterozygous (AS) patients (11.79 ± 1.14).

Conclusion: The hyperbilirubinemia, hyperuricemia, decreased levels of antioxidants like vitamin C and vitamin E and simultaneous increased level of serum MDA, strongly points towards high oxidative stress in these patients. With increasing accuracy and utility of different methods for measurements of these biomarkers for sickle cell disease is shown to have a cause and effect relationship.

Key Words: Malondialdehyde (MDA); Oxidative Stress; Sickle Cell Disease; Vitamin C; Vitamin E

Introduction

The prevalence of sickle cell disease (SCD) in western Odisha is 9.1% which is a quite high figure if one looks at the morbidity and mortality out of it.1,2 Sickle cell disease is caused by point mutation in the β-Globin gene on chromosome 11, causing replacement of glutamic acid by valine at the sixth position of β-globin chain of Hb. It is inherited in an autosomal co dominant manner. Homozygous sickle cell disease (SS) is a result of inheritance of a sickle gene from each parent, whereas sickle cell trait (AS) results from inheritance of a sickle gene from one parent and normal Haemoglobin A (Hb A) gene from other.3,4 SCD is a hemoglobinopathy clinically characterized by chronic haemolysis, susceptibility to frequent infections, growth retardation, splenic sequestration and intermittent episodes of painful vaso-occlusive crisis affecting different organs.3,4 The altered amino acid content in β-globin chain promotes the non-covalent polymerization (aggregation) of haemoglobin causing red blood cells to become sickle shaped and this decreases the elasticity of RBCs which is more pronounced during conditions of decreased oxygenation or higher oxygen demand as at higher altitude, infections, exercises and during surgery. Decreased RBC’s elasticity is central to the pathophysiology of sickle-cell disease. In sickle-cell disease, low-oxygen tension promotes RBC sickling and repeated episodes of sickling damages the cell membrane and makes it rigid. Further, repeated sickling and unsickling generates free radicals, which can irreversibly damage cell wall increasing its rigidity and thus shortening its life span.5 These permanently sickled RBCs cells fail to pass through narrow capillaries, leading to vessel occlusion and ischemia. Several newly emerging biological markers have potentiality to be utilized in deciding the degree of oxidative stress and disease severity. Reactive oxygen species (ROS) cannot be measured directly because of their extreme reactive instability and short half-life hence comes the role of secondary markers of oxidative stress which are stable and measurable.6,7 The possible role of antioxidants and its level are best demonstrated by its correlation with...
oxidative stress biomarkers. As free radical promotes increased and irreversible sickling of RBC membrane by damaging it, oxidative stress was assessed in sickle cell disease patients of western Odisha by measuring serum MDA as a by-product of lipid peroxidation, with simultaneous measurement of levels of antioxidant vitamins (vitamin E and vitamin C) as a possible beneficial therapeutic supplements in prevention of sickle cell complications.

Materials and Methods

This prospective single centered case control study was conducted at the department of Pediatrics and Biochemistry and sickle cell research centre at a tertiary care centre (VSS Medical College, Burla, Sambalpur) over a period of 1year ending in July 2012. The study protocol was approved by the institutional ethical committee and informed consent was obtained from the parents of the children before inclusion into the study group. A total of 50 sickle cell disease children aged between 5 to 15 years of either sex were evaluated clinically and were screened by sickling test and diagnoses confirmed by hemoglobin electrophoresis and were leveled as case (Both AS and SS). Same tests were carried out in 40 healthy children of matched age and sex and leveled as control. Children with history of blood transfusion in last 90 days of admission, any associated comorbidity from rheumatologic diseases or those presenting with other medical or surgical complications were excluded from the study. Initial evaluation included detailed history including previous episodes of painful crisis and thorough clinical examination. Venous bloods of all patients were collected in EDTA tube for measurements of hematological parameters. Venous blood was also collected in plain tube; sample centrifuged and serum stored till further analysis of biochemical parameters. The estimation of uric acid was done in semi auto analyzer by uricase method, Serum Malondialdehyde by methods of Satoh et al, 1978[9], Serum Vitamin E by Baker and Frank, 1968[9] and Plasma Vitamin C by Roe, 1961[10]. The results obtained were statistically analyzed and compared with using Students’ t test & the correlation evaluated using Pearson’s correlation coefficient.

Results

Out of 50 patients 30 cases were homozygous (SS) and 20 cases were heterozygous (AS) for sickle cell as per Hb electrophoresis. There was a significant decrease in the level of Hb in homozygous (SS) cases (8.82 ± 1.05) while a moderate decline was noted in heterozygous (AS) patients (11.79 ± 1.14). The serum bilirubin level was found to be high in both homozygous (2.82 ± 0.69) and heterozygous (1.42 ± 0.45) sickle cell disease patients in comparison to control group. The level of MDA was significantly high in SS group (3.96 ± 0.45) as compared to AS group (2.01 ± 0.45). The mean Vitamin E level in SS patients and AS patients was 18.35 ± 1.89, 24.78 ± 3.90 respectively with p value of < 0.001. The level of Vitamin C was low in both study groups which was also statistically significant (p < 0.001).

Table 1: Biochemical Parameters in sickle cell disease and control cases

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HbSS (Homozygous)</th>
<th>HbAS (Heterozygous)</th>
<th>Control (HbAA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (gm%)</td>
<td>8.82 ± 1.05 ***</td>
<td>11.79 ± 1.14</td>
<td>12.98 ± 0.36</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>2.82 ± 0.69***</td>
<td>1.42 ± 0.45</td>
<td>1.0 ± 0.96</td>
</tr>
<tr>
<td>Direct Bilirubin (mg/dl)</td>
<td>1.38 ± 0.42*</td>
<td>0.89 ± 0.25*</td>
<td>0.7 ± 0.34</td>
</tr>
<tr>
<td>MDA (μmol/L)</td>
<td>3.96 ± 0.45 **</td>
<td>2.01 ± 0.45</td>
<td>1.43 ± 0.59</td>
</tr>
<tr>
<td>Uric acid (μmol/L)</td>
<td>6.9 ± 0.56***</td>
<td>6.01 ± 0.52</td>
<td>5.71 ± 0.82</td>
</tr>
<tr>
<td>Vitamin E (μmol/L)</td>
<td>18.35 ± 1.89***</td>
<td>24.78 ± 3.90**</td>
<td>27.46 ± 5.68</td>
</tr>
<tr>
<td>Vitamin C (μmol/L)</td>
<td>21.61 ± 2.25***</td>
<td>27.29 ± 4.22**</td>
<td>34.04 ± 0.36</td>
</tr>
</tbody>
</table>

*p < 0.05; ** p < 0.01; *** p < 0.001 when SS compared with AS. *p < 0.05; **p < 0.01 when AS compared with control.

Table 2: Correlation of serum level of MDA with antioxidant levels (Uric acid, Vitamin E and Vitamin C) in SCD patients

<table>
<thead>
<tr>
<th>Sickle cell anemia (SS)</th>
<th>Uric acid (μmol/L)</th>
<th>Vitamin -E (μmol/L)</th>
<th>Vitamin -C (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'p'</td>
<td>0.640</td>
<td>-0.79</td>
<td>-0.519</td>
</tr>
<tr>
<td>'p'</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Discussion

Decreased level of haemoglobin and increased level of bilirubin in sickle cell anemia (SS) (Table1) patients indicates increased haemolysis. There was a significant decrease in the level of Hb in homozygous (SS) 8.82 ± 1.05 than heterozygous (AS) patients (11.79 ± 1.14) indicating greater degree of haemolysis in SS patients as compared to AS patients (p < 0.001). The hyperbiliruinemia, a constant finding in both the groups, indicates accelerated haemolysis. Study done by West et al proposes the similar findings. Several studies have proposed that reactive oxygen species causes externalization of phosphatidyl serine on RBC surface, signalling it for phagocytosis and subsequent removal from the circulation thus leading to intravascular haemolysis and hyperbilirubinemia. Premature destruction of RBC and oxidative stress lead to chronic haemolysis. HbS oxidizes at 1.7 times the rate of HbA resulting in accelerated production of superoxides and formation of hydrogen peroxide (H2O2). H2O2 in turn produces hydroxyl radicals by ‘Haber-weiss’ reaction and damages the red blood cell membrane, proteins and
lipids by thiol oxidation and lipid peroxidation. Oxidative degradation of arachidonic acid and polyunsaturated fatty acids of different cell membranes leads to the production of F2-isoprostanates, Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) which can be measured as oxidative stress biomarkers in urine or blood, to indicate the degree of oxidative stress and they have been demonstrated to be increased in SCD. In our study MDA was found to be increased in both SS (3.96 ± 0.45) and AS (2.01 ± 0.45) which was statistically significant (p <0.001). Patrick B. Walter, 2008 reported the mean level of MDA as 2.61 ± 0.97 in SCD patients while Emokpae Mathias Abiodun et al in 2014 reported the level of MDA in 87 sickle cell disease patients as 5.54 ± 0.02. Jyoti Titus reported increased levels of MDA (4.15 ± 0.30) in SS patients while the values in AS and control groups were 2.52 ±0.33 and 1.83 ±0.22 respectively. Similar statistically significant results were reported by Talufer Tamer et al (2000) and Prakash S et al in 2011.

Total (Peroxyl) Radical-trapping Antioxidant Parameter (TRAP) of antioxidants was measured by Warner et al in human Plasma and their contributions were reported to be as 35 - 65 % for urate, 0-24 % for ascorbate, 5-10 % from Vitamin E and 10-50 % from plasma protein. In our study, we found a rise in serum uric acid (Table1) in both SS (6.9 ± 0.56) and AS (6.01 ± 0.82) groups. The increased level of serum uric acid was statistically significant as compared to that of the control group (5.7±0.82). K De Ceulaer reported hyperuricemia in 44% of SS patients and Herbert S. found hyperuricemia in 26 of 67 adults (39%) of Sickle cell disease. Increased uric acid might be a consequence of increased cell turnover because of exaggerated haemolysis or an attempt to scavenge increased free oxygen radicals as uric acid also behaves as a powerful antioxidant and scavenger of singlet oxygen radicals. The increased uric acid level in our study was found to be positively correlated with MDA.

The mean Vitamin E level in our study in SS patients and AS patients is (18.35 ± 1.89) and (24.78 ± 3.90) respectively. This indicates more pronounced oxidative stress in homozygous sickle cell patients as compared to heterozygous patients resulting in a greater fall in vitamin E levels in former group. Vitamin E is a lipid soluble chain breaking antioxidant, which has protective role in almost all cells of the body. It scavenges free radical by its ability to transfer phenolic hydrogen to a peroxyl free radical of peroxidized polyunsaturated fatty acids. The antioxidant action of Vitamin E is effective even at high oxygen concentration and thus it is concentrated in those lipid structures which are exposed to higher partial pressure of oxygen for example membranes of erythrocyte, respiratory tract & retina. A study published by Hoewitt MK reports significantly increased hydrogen peroxide induced haemolysis following a drop in the level of vitamin E below 0.6 mg/dL. The lowered level of vitamin E in our study was found to be negatively correlated with increased level of serum MDA. It can be explained on the basis of their increased utilization in an attempt to scavenge the increased production of free oxygen radicals. Decreased vitamin E level in sickle cell anaemia patients might behave as a cause or an effect to increased haemolysis.

Vitamin C another anti-oxidant was also decreased significantly in both SS and AS group when compared to control group. Jyoti Titus in 2004 observed a similar trend of decrease level of Vitamin C in SS patients when compared with AS and control group (p<0.001). Vitamin C, a free radical scavenger, directly accepts electron from superoxide hydroxyl anion as well as from various lipid hydroxyl peroxides. The lowered vitamin C level in sickle cell anaemia patients indicates their exhausted status in an attempt to quench increased free radicals. Ascorbic acid (Vitamin C), an aqueous phase antioxidant, has excellent protective role in regeneration of the reduced form of other powerful antioxidants namely glutathione peroxide and Vitamin E and thus stops free radical chain reaction. As sickle cell anaemia (SS) patients are more prone to haemolysis and have a greater propensity for the formation of superoxide and hydrogen peroxide, the vitamin C levels decreases and show a negative correlation with serum MDA level.

Antioxidant vitamins supplementation might delay the progression and complications of disease.

**Conclusion**

The high prevalence of sickle cell disease in western Odisha contributes a major role in morbidity and mortality out of it. The hyperbilirubinemia, hyperuricemia, decreased levels of antioxidants like vitamin C and vitamin E and simultaneous increased level of serum MDA, strongly points towards high oxidative stress in these patients. With increasing accuracy and utility of different methods for measurements of these biomarkers for sickle cell disease
is shown to have a cause and effect relationship. Multicentric large scale studies are needed to prove their uses as a potential marker of the severity of the disease. Further, potential use of these antioxidants in delaying the onset of organ complications in SCD patients deserves thorough research.

References


Source of Support: Nil
Conflict of interest: None declared