ABSTRACT

Sickle cell anaemia is associated with elevated oxidative stress via increased generation of reactive oxygen species (ROS), and decline in antioxidant defences. Increased oxidative stress is thought to play a role in the development of sickle cell anaemia complications. In the current study, vitamins A, C, and E levels were estimated in 35 sickle cell anaemics attending the Paediatric Medical Clinic of the Usman Danfodiyo University Teaching Hospital, Sokoto, Nigeria and the results compared with those of apparently healthy non-sickle cell anaemic volunteers of comparable age and social status. Serum levels of vitamin A, C, and E were 31.06 ± 2.97 µg/dl, 0.27 ± 0.05 mg/dl, 0.20 ± 0.01 mg/dl and 69.51 ± 4.54 µg/dl, 0.77 ± 0.10 mg/dl and 0.49 ± 0.02 mg/dl in sickle cell anaemics and non-sickle cell anaemic subjects respectively. There was significantly (P<0.05) decreased levels of antioxidant vitamins in sickle cell anaemic subjects. Age and gender did not have significant (P>0.05) difference. The results suggest that sickle cell anaemics in the study area have low serum levels of antioxidant vitamins, an indication that the sickle cell anaemics are predisposed to increased oxidative onslaught.

Keywords: Sickle cell anaemia, serum vitamins A, C, and E.

INTRODUCTION

Patients with sickle cell anaemia (SCA) suffer from many complications, including growth retardation, chronic haemolytic anaemia, recurrent and painful vaso-occlusive episodes, acute chest syndrome and impaired immune function (El-Hazmin, 1979). Although the exact reasons are not well established. Literature indicates that low levels of antioxidant vitamins, zinc and folic acid could be contributing factors (El-Hazmin, 1979; Powars, 1975). Sickle cell anaemia is a hereditary disorder caused by the substitution of valine for glutamic acid in the sixth position of the ß-Chain of the haemoglobin (HbSS) with high potential for oxidative damage due to a chronic redox imbalance in red cells that often results in continuous generation of oxygen species and clinical manifestations of mild to severe haemolysis (Rank et al., 1985). The production of reactive oxygen species (ROS) can be grossly amplified in response to a variety of pathophysiological conditions such as hypoxia, inflammation, infection, dehydration and deficiency in antioxidant vitamins (Bunn, 1997).

The deleterious effects of the excessive production of free radicals or reactive oxygen species can be prevented by the body’s antioxidant defence mechanism which may include antioxidant vitamins (A, C and E) and enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Essien, 1995). Antioxidant vitamins help in neutralizing the effect of free radicals by mopping them off which further limit the sickle cell disease complications (Sies, 1997).

Sickle cell anaemics are under chronic oxidative stress induced by excessive production of free radicals from the sickle cell haemoglobin (Adam et al., 2001). This may overwhelm the normal antioxidant defences and deplete antioxidants including vitamins A, C and E, and increases the frequency of sickling crises accompanied by tissue or organ damage (Adam et al., 2001). Sickle cell anaemia crises (haemolytic anaemia, jaundice, painful swelling of hands and feet and skeletal changes due to erythroid hyperplasia) is becoming rampant and is highly life threatening most especially in paediatric patients. It is expected that this study will stimulate interests, discussion and further studies on the role of antioxidant vitamins vis-à-vis complications of sickle cell anaemia. In this study serum vitamins A, C and E were estimated in sickle cell anaemics and the results compared with those of apparently healthy non-sickle cell anaemics of comparable socio-economic status. The correlation co-efficient between the serum levels of antioxidant vitamins A, C and E and the frequency of SCA crises were also determined.

MATERIALS AND METHODS

Participants: The subjects employed for this study were 35 sickle cell anaemic patients of both sexes who were attending the Paediatric medical clinic of the Usman Danfodiyo University Teaching Hospital, Sokoto, Nigeria. Also 35 apparently healthy participants of both sexes were recruited to serve as control. The consents of all the subjects were sought for and obtained. Ethical committee approval was also obtained for the research.

Blood samples: - Blood samples were collected by venipuncture and delivered into clean dry tubes and allowed to clot at room temperature. The samples were centrifuged at 3000 rpm for 5 minutes using benchtop centrifuge and the serum separated and kept in labeled sample bottles at (-20°C) until required.
Reagents: All chemicals and reagents were of analytical grade and purchased from Sigma Chemical Company, USA.

Analytical methods: Serum vitamin A level was determined by method of Bassey et al. (1946), vitamin C level was determined by method of Roe and Kuether (1943), and vitamin E level was determined by method of Neild and Pearson, (1967).

Statistical Analysis: Results are presented as mean ± standard error of mean and separated on the basis of gender. Significant differences in mean at 5% level were determined using ANOVA. Serum levels of antioxidant vitamins were correlated with the sickle cell anemia crises and correlation coefficients determined. SPSS Version 18 was used for the analysis.

RESULTS

The results of the current work showed significant difference (P<0.05) between serum antioxidant vitamins of the sickle cell anemic and non sickle cell anemic participants (Table 1). Gender appears not to have significant (P>0.05) effect on serum antioxidant vitamins.

Table 1: Serum Antioxidant vitamins of Sickle cell Anaemics (SCA) in Sokoto, Nigeria.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sickle Cell Anaemic</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Vit. A (µg/dl)</td>
<td>44.46±8.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.18±5.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vit. C (mg/dl)</td>
<td>0.33±0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.19±0.02&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vit. E (mg/dl)</td>
<td>0.19±0.01&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.19±0.01&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values bearing same superscript differ significantly (P<0.05) using ANOVA.

Table 2: Age Distribution of Serum Antioxidant vitamins in Sickle cell Anaemic Children in Sokoto, Nigeria.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sickle Cell Anaemics*</th>
<th>1 – 5 Yrs</th>
<th>6 – 10 Yrs</th>
<th>11 – 15 Yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td>Male</td>
</tr>
<tr>
<td>Vit. A (µg/dl)</td>
<td>40.44±7.99</td>
<td>39.30±9.71</td>
<td>31.06±6.93</td>
<td></td>
</tr>
<tr>
<td>Vit. C (mg/dl)</td>
<td>0.30±0.08</td>
<td>0.23±0.06</td>
<td>0.21±0.07</td>
<td></td>
</tr>
<tr>
<td>Vit. E (mg/dl)</td>
<td>0.20±0.01</td>
<td>0.18±0.01</td>
<td>0.20±0.02</td>
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</tr>
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</table>

Values are means ± SEM. Values show no significant different (P>0.05) using ANOVA.

Table 3 indicate significant (P<0.05) negative correlation coefficients between sickle cell anemia crises and serum antioxidant vitamins.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sickle Cell Anaemics (r)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-0.17</td>
<td>-0.23</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.10</td>
<td>-0.07</td>
<td>-0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.08</td>
<td>-0.30</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

All the serum antioxidant vitamins are significantly (P<0.05) negatively correlated with sickle cell anemia crises of the sickle cell anemias.

DISCUSSION

Sickle cell anemia is characterized by elevated oxidative stress via increased generation of reactive oxygen species (ROS), and decline in antioxidant defences (Ayyub et al., 2003). Increased oxidative stress is thought to play a role in the development of sickle cell anemia complications (Ayyub et al., 2003). Antioxidant vitamins (A, C and E) are thought to be effective in increasing the activities of antioxidant defence enzymes, scavenging free radicals, preventing oxidative damage and thereby sparing lipid components of the cells against lipid peroxidation. Oxidative stress and hereditary disorder are suggested to be a potential contributor to the development of sickle cell anemia and the associated complications (Zingg et al., 2000). This may be connected to the fact that the antioxidant status may be inadequate in sickle cell anemia subjects. The metabolic significance of the evaluation of antioxidants in sickle cell anemias is therefore of paramount importance. The results of the current study indicate that serum vitamins A, C and E of the sickle cell anemic subjects, in the study area were significantly (P<0.05) lower than the values obtained for the non-sickle cell anemic subjects. The results further reveal no significant difference in levels of antioxidant vitamins between male and female sickle cell anemic subjects (P>0.05).

The decreased levels of antioxidant vitamins are connected to increased oxidative stress in sickle cell anemia patients, resulting in higher utilization of these vitamins and consequently leading to their deficiencies. Thus, increased intake of synthetic or natural antioxidant vitamins could help to avert sickle cell anemia complications (Bunn, 1997).
Since patients with sickle cell anaemia are under continuous oxidative stress due to sickle cell redox imbalance (Aslan et al., 2000), a deficiency in antioxidant vitamins may contribute to the severity of sickle cell manifestations, which could be aggravated further by elevated copper, which is a well known pro-oxidant (Chan et al., 1999). The deficiency of vitamins also could account for some of the observed manifestations of sickle cell disease complications such as increased susceptibility to infection and haemolysis (Adelekan et al., 1989).

The negative correlation established between the serum levels of antioxidant vitamins and sickling crisis indicates that, the increases in the number of sickle cell crises are accompanied by a decrease in serum antioxidant vitamins. Sickle cell anaemic crises is associated with an over production of free radicals which are generated by the sickle cell haemoglobin (Habbel et al., 1997). This phenomenon may result into an increased utilization of the antioxidant vitamins A, C and E which have been used in scavenging the excess amount of free radicals produced which therefore explain the depletion of the vitamins at period of sickling crises.

CONCLUSION
In conclusion, there is significant decrease in serum antioxidant vitamins in sickle cell anaemic subjects. Gender has no significant effect on antioxidant vitamins. Turkey-Kramer Multiple Comparison test (In Stat 3 Software, SanDiego,USA) was used. Further studies are needed to find out whether supplementation of antioxidant vitamins may ameliorate some sickle cell disease complications.

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REFERENCES


