Effect of Sickle Cell and Malaria Induced Anaemia on Some Biochemical Parameters

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Abstract: The effects of sickle cell and malaria on anaemia were observed in humans using some biochemical parameters as indicators. The study was performed with 150 anaemic patients comprising cases of 46 known malaria induced anaemia and 15 established cases of sickle cell induced anaemia. A further 150 apparently healthy individuals were used as controls. The biochemical parameters measured include some antioxidant agents such as vitamins C and E, albumin and uric acid. The results showed that the mean concentrations of albumin and vitamin E respectively were significantly lower (p< 0.05) in anaemic individuals when compared with the controls while the mean levels of uric acid was significantly higher in sickle cell and malaria induced anaemic cases. The study showed that the biochemical parameters studied were affected both in sickle cell and malaria induced anaemia. Hence the biochemical parameters assayed can be used as markers in the assessment and management of anaemia.

Key words: Sickle cell • Malaria • Anaemia • Antioxidants

INTRODUCTION

Anaemia is a public health problem world wide. Although the primary cause is iron deficiency, it is seldom present in isolation. More frequently it coexists with a number of other causes, such as malaria, parasitic infection, nutritional deficiencies and haemoglobinopathies [1]. The factors other than iron deficiency in the development of anaemia has been underestimated by public health officials, because for a long time anaemia has been confused with iron deficiency anaemia and this has influenced the development of strategies and programmes designed to control anaemia [2].

Sickle cell anaemia results from a point mutation in the genetic code such that a single amino acid (glutamic acid) is replaced by another amino acid (valine) in the globin chain of haemoglobin [3]. Sickling of red cells leads to two major consequences: a chronic haemolytic anaemia and occlusion of small blood vessels resulting in ischemic tissue damage. Sickle cell anemia is a condition in which there is observed oxidative stress in the body [3].

Malaria is a global health problem, causing disease on a vast scale. Malaria infection in humans is caused by Plasmodium falciparum species and is associated with reduction in haemoglobin levels and this frequently leading to anaemia [4].

Antioxidant is defined as any substance that when present at low concentration compared with those of an oxidazable substrate, significantly delays or prevents oxidation [5]. Antioxidants interact with oxidant and terminate the chain reaction before vital organs are damaged. They donate an electron to stabilize an oxidant. Vitamins are potent organic compounds, which are found in small concentration in food. They perform specific and vital functions in the body chemistry. Except for a few exceptions, they cannot be manufactured or synthesized by the organism and their absence or improper absorption results in specific deficiency disease. Vitamins as antioxidants donate an electron to stabilize a free radical, hence terminating the danger in the reaction of free radicals with important cellular components such as DNA. Therefore vitamins as
antioxidants act as defence system. All vitamins do act as antioxidants. It has been reported that ascorbic acid may act synergistically with vitamin E, the antioxidant effect may be due to quenching of singlet oxygen in the aqueous medium [6].

Serum albumin may act as an indirect and sacrificial antioxidant [7, 8] and inhibits peroxidases, free radical generation and haemolysis. Albumin also helps in the transport of certain substances through the blood and is important for tissue growth and healing [9].

Uric acid is an endogenous antioxidant and represents part of the tissues’ natural defense against oxidative stress. It is an important antioxidant in the serum representing a final product in the metabolism of the purines and act as a potent free radical scavenger [10]. Waring [11] also proposed that uric acid is the most abundant aqueous antioxidant in humans and contributes as much as two thirds of all free radical scavenging capacity in plasma. The aim of this study is to determine the effect of malaria and sickle cell induced anaemia in humans, using vitamin c and E, albumin and uric acid as markers.

**MATERIALS AND METHODS**

**Subject Selection:** Three hundred (300) subjects divided into two groups were used in this study. The first group was the apparently normal healthy individuals. A total number of 150 individuals were used as controls for this study, 78 of the 150 were females while 72 were males. All controls for the study gave their consent in writing to participate in the study by filling questionnaires given to them and allowing blood samples to be taken from them. Subjects were picked randomly from all social classes. The control subjects were free from acute illnesses like malaria, typhoid, pneumonia, hepatitis, sinusitis; upper respiratory tract infection and none had history of concomitant illness like rheumatic heart diseases, diabetes mellitus, hypertension, leukaemia and others. Smokers and patients with history of blood transfusion were excluded from the study.

The second group of the human subjects consisted of 150 anaemic patients, attending the Braithwaite Memorial Hospital (BMH) Port Harcourt within a period of 2 months. Patient’s data, diagnosis and causes of illness were obtained from patients’ folders. Out of the 150 patients, 77 were females while 73 were males, age ranging between 15-67 years in both cases. Data obtained from patients’ diagnosis indicated that about 46 out of the 150 cases were admitted for malaria fever, 15 were sickle cell cases, 23 were diabetic cases and 39 of the cases were reported for diseases such as rheumatoid arthritis, leukaemia, retroviral, malnutrition, septicaemia, liver cirrhosis and glomerula nephritis, while source of anaemia for the remaining 27 was yet to be established.

**Biochemical Studies:** Blood samples were collected into EDTA and plain bottles for analysis and all cases of anaemia were confirmed by carrying out haemoglobin assay on patients’ samples.

Determination of vitamin C in blood plasma was measured according to the method described by Omaye et al., [12]. To 0.5ml of plasma in tube labeled test, 1.5ml of 6% TCA was added, centrifuged for 20mins at 1000g. 0.5ml of the supernatant was pipette into separate clean tube and 0.5ml of DNPH reagent was added, incubated for 3hrs at room temperature .thereafter, 2.5ml of 85% H$_2$SO$_4$ was added to test tube, allowed to stand for 30mins and absorbance read at 530nm. Concentration of vitamin C was obtained from the calibration curve of vitamin C (stock 2mg/ml).

Determination of vitamin E was performed according to the method previously described by Quaife et al., [13]. Test tubes were labelled test, standard and blank. Plasma(1.5ml) was pipetted in tube labelled test, while 1.5ml of vitamin E standard ( D-α tocopherol, 10mg in ethanol), was pipetted into tube labelled standard and 1.5ml of water was pipetted in tube labelled blank. Absolute ethanol and xylene, 1.5ml each was pipetted into all tubes, mixed and centrifuged at 1000g for 10mins. Thereafter, 1ml of xylene layer was transferred into separate test tubes labelled test, standard and blank. Then 1ml α’ α’ dipyridyl reagent was added to all tubes, mixed and absorbance read at 460nm. Finally, 0.33ml of ferric chloride was finally added to all tubes, mixed and absorbance read at 520nm after 10mins. Concentration of vitamin E  was obtained using the above formula.

The measurement of albumin was based on its quantitative binding to the indicator 3, 3’, 5, 5’, tetrabromo-m-cresol sulphonephthalein (bromocresol Green, BCG). The test tubes were labeled blank, standard, sample and control. Bromocresol green reagent (3mls) was pipetted into each tube, while 0.01ml of distilled water, standard, sample and control was pipetted into their respective tube mixed and incubated at 25°C for 5minutes. The absorbance was measured at 578nm against the reagent blank. The concentration of albumin was determined by multiplying the absorbance of sample with concentration of standard divided by absorbance of standard.
Table 1: Effects of sickle cell on anaemia using Vitamin C and E, albumin and uric acid as parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>App. Normal healthy individuals (n=150)</th>
<th>Sickle cell induced anaemic patients (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>0.61±0.29</td>
<td>0.31±0.23</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Vitamin E (mg/l)</td>
<td>7.82±2.38</td>
<td>4.42±1.60</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>42.76±8.10</td>
<td>28.20±4.87</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Uric Acid (µmol/l)</td>
<td>163.69±45.08</td>
<td>336.60±99.38</td>
<td>P&gt;0.05</td>
</tr>
</tbody>
</table>

Table 2: Effect of malaria on anaemia using Vitamin C and E, albumin and uric acid as parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>App. Normal healthy individuals (n=150)</th>
<th>Malaria induced anaemic patients (n=46)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>0.61±0.29</td>
<td>0.29±0.18</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Vitamin E (mg/l)</td>
<td>7.82±2.38</td>
<td>5.56±1.92</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>42.76±8.10</td>
<td>32.34±8.67</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Uric Acid (µmol/l)</td>
<td>163.69±45.08</td>
<td>277.17±120.21</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Uric acid was measured according to the method previously reported by Fossati et al., [14]. The test tubes were labeled blank, standard, sample and control. One millilitre (1 ml) of uric acid reagent was pipetted into each tube, while 0.01 ml of distilled water, standard, sample and control was pipetted into their respective tube mixed and incubated at 37°C for 5 minutes. The absorbances were measured at 520 nm against the reagent blank. The concentration of uric acid was determined by multiplying the absorbance of sample with concentration of standard divided by absorbance of standard.

Statistical Analysis: All statistical analysis of the results was performed using Statistical Package for Social Sciences (SPSS) version 16.0 software produced by SPSS Inc., Chicago, IL. Results are expressed as Mean ± SD and they were compared using students’t test. Values of P<0.05 indicate statistical significant difference in the mean values while P>0.05 means no significant difference.

RESULT

Vitamin C concentration 0.31 ±0.23 (mg/dl) was decreased in the sickle cell induced anaemia when compared with 0.61 ±0.29 (mg/dl) in the control. There was also decrease in Vitamin E concentration 4.42 ±1.60 (mg/l) in the sickle cell induced anaemic patients when compared with 7.82 ±2.38 (mg/l) in the control. Albumin concentration of 28.20 ±4.87 (g/l) was significantly decreased in sickle cell induced anaemia when compared with the control concentration of 42.76 ±8.10 (g/l). Whereas, significant increase was recorded in uric acid concentration (µmol/l) 336.60±99.38 in the sickle cell induced anaemia when compared with uric acid concentration of 163.69±45.08 (µmol/l) obtained in the control as shown in table 1 below.

Vitamin C concentration 0.29 ±0.18 (mg/dl) was decreased in the malaria induced anaemia when compared with 0.61 ±0.29 (mg/dl) in the control. There was also decrease in Vitamin E concentration 5.56 ±1.92 (mg/l) in the malaria induced anaemic patients when compared with 7.82 ±2.38 (mg/l) in the control. Albumin concentration of 32.34 ±8.67 (g/l) was significantly decreased in the malaria induced anaemia when compared with the control concentration of 42.76 ±8.10 (g/l). While, there was a significant increase in uric acid concentration (µmol/l) 277.17±120.21 obtained in the malaria induced anaemia when compared with uric acid concentration of 163.69±45.08(µmol/l) obtained in the control as shown in table 2 below.

DISCUSSION

In the studied anaemic group, malaria induced anaemia appeared to be more frequent than the sickle cell induced anaemia. Overall, there was decrease in the antioxidants( Vitamin C, vitamin E and albumin) obtained in both the malaria and sickle cell induced anaemic patients, but significantly decrease of these antioxidants were more in the sickle cell induced anaemic patients. Also, significant increases in uric acid concentrations were observed in both sickle cell and malaria induced anaemic cases.

The observed change in the parameters assayed in this study is suggestive that sickle cell and malaria induced anaemia have high potentials for pathophysiologic changes and oxidative stress. It is generally known that vitamin E is a lipid-soluble antioxidant in cell membrane, functioning as a scavenger of peroxyl radicals, it is probably the most important inhibitor of the free- radical chain reaction of lipid peroxidation [15]. This finding is also suggestive of the existence of low antioxidant defense in anaemic patients.
This is similar to reports by other authors Titus, et al., [3] and Nwajo, [16]. Decrease albumin concentration is most likely due to more utilization of albumin to remove excess free radicals produced by anaemic patients even though malnutrition could also be attributed to the decrease in albumin levels. This is similar to previous studies by Agarwal et al.,[17]. However, this finding is not in agreement with previous reports by Titus et al., [3].

The high levels of uric acid observed could be attributed to increase oxidative stress activities in sickle cell and malaria induced anaemia. Previous reports by Rothschild et al.,[18] and Waringi, [11] had also associated sickle cell disease with hyperuricaemia. Uric acid can act as a pro-oxidant, particularly at increased concentrations and may thus be a marker of oxidative stress Becker, [19]; Strazzullo and Puig, [20].

The key to successful management of anaemia requires confirmation of diagnosis through investigation of the underlying cause, drug therapy, proper nutritional and dietary modifications.

CONCLUSION

This study has shown that sickle cell and malaria can induce anaemia and vitamins C and E, albumin and uric acids are some of the antioxidants that are affected. The investigations of these parameters are therefore necessary in the management of anaemia.

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