Serum Iron, Serum Ferritin And Total Iron Binding Capacity In Patient With Severe Falciparum Malaria

1Onuoha, S.C., 1Uzuegbu, E.U. and 2Nwachoko, Ndidi

1Department of Biochemistry, University of Port Harcourt, Choba, Nigeria P.M.B 5323, Port Harcourt, Nigeria.
2Department of Medical Biochemistry, Delta State University, Abraka, Nigeria.

Abstract: Within the last 10 years, it has become evident that iron status and ferritin has a diagnostic and therapeutic potential, therefore, it is of great need to establish the reference interval for iron status and ferritin. The impact of age and sex on iron status and ferritin level in patient with severe falciparum malaria aged 1-60 years was studied in Benin City, where malaria is endemic. 81 volunteers were enrolled during the eleven weeks of study. 45 malaria subjects aged 1-60 years and 36 uninfected were screened for the study. Iron status was evaluated using three biochemical Parameters (serum iron “SI”, serum ferritin “SF” and Total Iron Binding Capacity “TIBC”). It was observed that plasmodium falciparum density in serum iron, serum ferritin and total iron binding capacity is higher in patient aged between 1-20 years (61.97µ/dl ± 3.70, 30.09µg/ml ± 3.81 and 316.45µg/dl ± 27.91), than those between 21-40 years (57.43µg/dl ± 1.18, 27.86µg/ml ± 1.12and 272.29µg/dl ± 9.04) and between 41-60 years of age (57.00µg/dl ± 1.07, 27.14µg/ml ± 0.83 and 271.71µg/dl ± 7.52) respectively. The rate of SI, SF and TIBC were significantly lower (60.44µg/dl ± 3.59, 29.33% ± 3.35 and 302.62µg/dl ± 6.74) in malaria patients than controls (89.67µg/dl ± 2.48, 47.94% ± 8.10 and 313.17µg/dl ± 15.67) (P < 0.01).

Key words: Plasmodium falciparum, Serum iron, Serum ferritin and Total iron binding capacity.

INTRODUCTION

Iron is an essential micronutrient necessary for the transportation of respiratory gases via haemoglobin in the red blood cells. Iron also intervenes in the constitution of enzymatic systems such as catalases, peroxidases and cytochromes that play an essential role in cellular respiratory mechanisms, in mitochondrial respiratory channel (Ghosh et al., 1995).

Anemia is defined as a haemoglobin concentration lower than the established limit defined by the World Health Organization (WHO). This limit ranges from 110g/dl for men. Anemia is one of the most widespread public health problems, especially in developing countries, and a major cause of morbidity and mortality in malaria-endemic areas of sub-Saharan Africa.

Malaria is a disease caused by protozoa of genus plasmodium; it is a serious health problem in tropical and subtropical areas. World wide, more than 400 million people are affected by malaria, with about 200 million in sub-Saharan Africa. In Cameroon, malaria is a public health priority (Breman et al., 2004).

Studies in Cote d’Ivoire and Benin estimated that iron-deficiency anemia account for approximately 50% of the anemia observed. In the Cote d’Ivoire study, the proportion of anemic individuals with iron deficiency varied by age and gender. Approximately 80% of the anemic pre-school – age children had iron – deficiency anemia, compared with 50% of the school age children and women and 20% of the men. Malaria and other infections or inflammatory disorders contributed significantly to a high prevalence of anemia, particularly in young children, but these infections and/or disorders and iron deficiency could not explain all of the anemia cases.

The prevalence of anemia and iron deficiency is commonly estimated from the blood haemoglobin level (Das et al., 1997). However, low iron is not easily quantified, for even with a significantly depleted body iron store, blood haemoglobin may still be acceptable. Serum ferritin concentration, therefore, is taken as a more specific indicator of the body iron status (Lipschitz, et al., 1974).

Malaria may cause severe anemia due to erythrocyte lyses and there is a consequent fall in blood haemoglobin, even though body iron stores may not be significantly depleted (Abdalla, 1990).

Plasma ferritin concentration are significantly higher in all the malaria groups than in a control despite associated anemia, ferritin is a positive acute phase protein (APP) and is known to increase in infection and injury (Harju, et al., 1984 and Fitzsimons et al., 1990).

In community-based studies, it is not uncommon for serum ferritin to be high, even in the presence of anemia. In recent survey of Indian pre-school children, high serum ferritin was positively associated with
erythrocyte protoporphyrin and negatively related to the mean corpuscular haemoglobin concentration (Raman et al., 1992).

MATERIALS AND METHODS

Equipment:
1) Incubator (Dup – 9022, surgifriend medicals, England)
2) Refrigerator (EHT – 17sk, whirt pool, USA)
3) Digital spectrophotometer (SNO 20403219, surgifriend medical, England)
4) Microtiter plate reader
5) Centrifuge (C56C clinascal, Vulcom Technologies, USA)
6) Calculator (TH-2000, Purpo®, China)

Reagents:
Iron buffer reagent
UIBC buffer reagent
Iron color reagent
Iron standard (500µg/dl)

Specimen:
Blood sample

Biochemical Method:
Blood samples were drawn from antecubital vein between eight and twelve A.M. from subjects after the detection of a falciparum – positive blood smear. In all cases, blood was collected before anti-malaria therapy. The sample was transferred to a heparinised tube for biochemical investigations into an EDTA – containing tube for hematological investigation – samples from the field were transported immediately to the laboratory in screw-capped tubes. Malaria parasite density was determined by counting the number of parasitized erythrocytes per 1000 erythrocytes from a thin smear and expressed as percentage parasitaemia. All tests were done immediately except for ferritin, transferring and iron.

The later were done every 2 weeks on sample stored at 20°C.
Plasma transferring was estimated by immunoturbidimetry, ferritin by two-step sandwich assay, Fe by reducing Fe³⁺ to Fe²⁺ with sodium dithionite and developing the colour complex with bathophenanthroline disulfonate. All this tests were done using kits (Boehringer Mannheim) (Henry, 1984).

Determination of Iron and Total Iron Binding Capacity (TIBC):
The iron in serum is dissociated from its Fe³⁺ - transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduces the Fe³⁺ to Fe²⁺ the chromogenic agent, ferrozine, forms a lightly coloured Fe²⁺ - complex that is measured spectrophotometrically at 560nm.

The unsaturated iron binding capacity (UIBC) is determined by adding Fe²⁺ ion to serum so that they bind to the unsaturated iron binding sites on transferrin – the excess Fe²⁺ iron are reacted with ferrozine to form the coloured complex; which is measured spectrophotometrically, the difference between the amount of Fe²⁺ added and the amount of Fe²⁺ measured represents the unsaturated iron binding, the total iron binding capacity (TIBC) is determined by adding the serum iron value to the UIBC value.

RESULT AND DISCUSSION

The serum iron, serum ferritin and total iron binding capacity (TIBC) of subjects (malaria infected) and control (non-malaria infected) were determined using the procedures described and the result obtained are presented below. The results were obtained by ANOVA method of statistic analysis as mean ± standard deviation (SD).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>N o: 45</th>
<th>Serum iron (µg/dl)</th>
<th>TIBC(µg/dl)</th>
<th>Serum ferritin (µg/ml)</th>
<th>Transferrin saturation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 20</td>
<td>31</td>
<td>61.97±3.70</td>
<td>316.45±27.91</td>
<td>30.09±3.81</td>
<td>19.57±1.23</td>
</tr>
<tr>
<td>21 - 40</td>
<td>7</td>
<td>57.43±1.18</td>
<td>272.29±9.04</td>
<td>27.86±1.12</td>
<td>20.80±0.58</td>
</tr>
<tr>
<td>41 - 60</td>
<td>7</td>
<td>57.00±1.07</td>
<td>271.71±7.52</td>
<td>27.14±0.83</td>
<td>20.93±0.57</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation: SD among subject reference value: serum iron: 60 – 150(µg/dl), serum ferritin: 12 – 300(µg/ml); total iron binding capacity: 250 – 400(µg/dl) and transferring saturation: 0 – 55%.
Table 1 shows a decrease in serum iron, serum ferritin, and total iron binding capacity with an increase in age while transferring saturation increasing with an increasing age among subject (malaria).

Table 2: Age factor in serum iron, serum ferritin, transferring saturation and total iron binding capacity “TIBC” among healthy individual “control”.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No: 45</th>
<th>Serum iron (µg/dl)</th>
<th>TIBC(µg/dl)</th>
<th>Serum ferritin (µg/ml)</th>
<th>Transferrin saturation( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 20</td>
<td>31</td>
<td>89.27±2.58</td>
<td>315.68±12.70</td>
<td>49.41±8.38</td>
<td>28.48±1.49</td>
</tr>
<tr>
<td>21 - 40</td>
<td>7</td>
<td>90.14±2.17</td>
<td>311.71±17.09</td>
<td>46.4±7.83</td>
<td>28.99±1.63</td>
</tr>
<tr>
<td>41 - 60</td>
<td>7</td>
<td>90.43±2.19</td>
<td>307.57±19.91</td>
<td>45.14±6.17</td>
<td>29.67±2.28</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation: SD among healthy individuals "control" reference value; serum iron: 60 – 150(µg/dl), serum ferritin: 12 – 300(µg/dl); total iron binding capacity: 250 – 400(µg/dl) and transferring saturation: 0 – 55%.

The result in table 2 above shows an increase in serum iron and transferring saturation with increase in age while total iron binding capacity and serum ferritin decrease with increase in age.

Table 3: Gender sensitivity among subject “malaria”.

<table>
<thead>
<tr>
<th>Subject malaria patient</th>
<th>No: 45</th>
<th>Serum iron (µg/dl)</th>
<th>TIBC(µg/dl)</th>
<th>Serum ferritin (µg/ml)</th>
<th>Transferrin saturation( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>25</td>
<td>60.04±3.64</td>
<td>296.48±30.20</td>
<td>27.16±4.92</td>
<td>20.30±1.37</td>
</tr>
<tr>
<td>female</td>
<td>20</td>
<td>60.90±3.48</td>
<td>310.30±31.03</td>
<td>20.00±3.31</td>
<td>19.54±0.90</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation: SD among subject “malarial” reference value; serum iron: 60-150(µg/dl), serum ferritin: 12 – 300(µg/dl); total iron binding capacity: 250 – 400(µg/dl) and transferring saturation: 0 – 55%.

The result in table 3 show no significant difference in serum iron transferring, but with increase in serum ferritin in male than in female which is inversely proportional to TIBC.

Table 4: Gender sensitivity among subject “control”.

<table>
<thead>
<tr>
<th>Subject control</th>
<th>No: 36</th>
<th>Serum iron (µg/dl)</th>
<th>TIBC(µg/dl)</th>
<th>Serum ferritin (µg/ml)</th>
<th>Transferrin saturation( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>23</td>
<td>89.65±2.68</td>
<td>302.96±9.43</td>
<td>53.30±4.64</td>
<td>29.67±1.66</td>
</tr>
<tr>
<td>female</td>
<td>13</td>
<td>89.69±2.09</td>
<td>331.23±3.47</td>
<td>35.69±9.30</td>
<td>27.37±0.28</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation: SD among subject “control” reference value; serum iron: 60 – 150(µg/dl), serum ferritin: 12 – 300(µg/dl); total iron binding capacity: 250 – 400(µg/dl) and transferring saturation: 0 – 55%.

Table 4 shows no significant difference in serum iron but serum ferritin concentration increase in male with an increase transferring saturation and decrease TIBC in male than in female.

Table 4: Comparision of serum iron, total iron binding capacity (TIBC), serum ferritin and transferring saturation between subject “malaria” and control.

<table>
<thead>
<tr>
<th>Subject</th>
<th>N 36</th>
<th>Serum iron (µg/dl)</th>
<th>TIBC(µg/dl)</th>
<th>Serum ferritin (µg/ml)</th>
<th>Transferrin saturation%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>45</td>
<td>60.44±3.59</td>
<td>302.62±67.74</td>
<td>29.33±3.35</td>
<td>19.97±1.23</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>89.67±2.48</td>
<td>313.17±15.67</td>
<td>47.94±8.10</td>
<td>28.84±1.73</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation: SD among subject ( healthy individual “contro” ) and malaria patient reference value; serum iron: 60 – 150(µg/dl), serum ferritin: 12 – 300(µg/dl); total iron binding capacity: 250 – 400(µg/dl) and transferring saturation: 0 – 55%

Table 5 show a significant decrease in serum iron, serum ferritin, total iron binding capacity and transferrin.

Summary:

Iron and ferritin measurement might turn to be useful in different diagnosis of iron deficiency or iron overload disorders in the near future. It is required to establish a reference interval for this hormone hepcidin and to investigate important aspects of diurnal variations, the half-life, age, sex and race related differences. The present study examines the changes in serum iron (SI), total iron binding capacity (TIBC), serum ferritin (SF) as a function of gender and age in patient with severe falciparum malaria and also with healthy individual (control) between the ages of 1 – 60 years. The result of table one above show age factor in serum iron, serum ferritin, transferring saturation and total iron binding capacity (TIBC) among ill/infected subject (malaria); the biochemical analysis using t-test shows that TIBC,SF, SF is significantly higher in patient’s age between 1 – 20 years. But TS increase with an increasing age. In table two, the result by age factor in SI, SF, TS and TIBC among healthy subjects (control), showed that TIBC, ST and TS has a significant increase of 1% with an increasing age, but there was 7.09% increase in age 1 – 20 years of serum ferritin than those of 21 – 40 and 41 – 60 years respectively. In table three, the result by gender sensitivity among ill subjects (malaria) shows no significant difference in SI and TS, but with increase in SF in male than in female which is inversely proportional to TIBC. In table four, result by age sensitivity among healthy subjects (control) show no significant difference in SI, but DF concentration increase in male with an increase TS and decrease TIBC in male that in female. In table five. Comparison of serum iron (SI) total iron binding capacity (TIBC), serum
ferritin (SF) and transferrin saturation (TS) show a significant decrease in SI, TS, TIBC and SF with 32.10%, 30.79%, 3.39% and 38.81% respectively.

A general analysis of the result showed that serum iron, serum ferritin and total iron binding capacity are highly affected by malaria parasite (P<0.01).

REFERENCES


