Impact of Malaria Severity on Serum Levels of Hepcidin and Iron Status in Children

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Citation

Submitted: Jan 31, 2019; Accepted: Apr 14, 2019; Published: Apr 30, 2019

Abstract: Malaria is a life threatening disease and one of the leading causes of death in Africa especially in children. This study aimed to assess the impact of malaria on levels of hepcidin and iron status (haemoglobin, serum iron and total iron binding capacity) based on the severity of malaria infection. A total of ninety three subjects between the ages of 6 months-15 years were investigated for malaria infection using microscopic method. Thirty three subjects had severe malaria; thirty subjects had non-severe malaria and thirty apparently healthy subjects served as control. Hepcidin was estimated using enzyme linked immunosorbent assay (ELISA), serum iron and haemoglobin were estimated spectrophotometrically. The results obtained showed that there were increased levels of hepcidin and decreased levels of haemoglobin, serum iron and total iron binding capacity in severe and non-severe malaria compared with apparently healthy subjects (P<0.05). Hepcidin also showed inverse correlation with serum iron, TIBC and haemoglobin in both severe and non-severe malaria. The study concluded that severe malaria may cause the upregulation of hepcidin and the increased hepcidin could put the subject at risk of iron deficiency anemia. The study suggests that hepcidin could help in the evaluation of severe and non-severe malaria and also be used to monitor the progression of malaria infection.

Key Words: Hepcidin, Malaria, Children

Introduction:
Malaria is currently one of the most endemic diseases especially in Africa. According to World Health Organization (WHO) report [1], there were 212 million new cases of malaria worldwide in 2015 (range 148–304 million). The WHO African Region accounted for most global cases of malaria (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%). It was also stated that in 2015 [1], there were an estimated 429 000 malaria deaths (range 235 000–639 000) worldwide. Most of these deaths occurred in the African Region (92%), followed by the South-East Asia Region (6%) and the Eastern Mediterranean Region (2%). Children under 5 years are particularly susceptible to malaria illness, infection and death. In 2015, malaria killed an estimated 303 000 under-fives globally, including 292 000 in the African Region. Between 2010 and 2015, the malaria mortality rate among children under 5 fell by an estimated 35%. Nevertheless, malaria remains a major killer of under-fives, claiming the life of 1 child every 2 minutes [1]. Malaria causes a lot of disturbances in physiological iron distribution and utilization, through mechanisms such as: hemolysis, release of heme, anemia, deposition of iron in macrophages, and inhibition of dietary iron absorption [2]. Malaria-induced destruction of infected and non-infected red blood cells both stresses and impedes the capacity of macrophages to recycle iron back to the bone marrow [3]. Acute malarial hemolysis represents a major stress on the homeostatic mechanisms of iron flux regulation that have evolved primarily to maintain erythroblast supply while minimizing the oxidant stress-associated effects of iron [4]. The production of hepcidin is regulated by iron, so that more hepcidin is produced by hepatocytes when iron is abundant, limiting further iron absorption and release from stores. When iron is deficient, hepatocytes produce less or no hepcidin, allowing more iron to enter plasma [5]. Hepcidin plays a complex but important role in both iron restriction that occurs during malaria infection and determination of iron status, thereby, influencing disease susceptibility [6]. It has been shown that elevated level of hepcidin causes an increase in impaired iron absorption and metabolism which in turn could cause iron deficiency anemia. The upregulation of hepcidin leads to iron accumulation in macrophages and a decrease in serum iron, possibly contributing to the dyserythropoiesis and anemia that could accompany malaria infections [7]. Additionally, hepcidin upregulation directly blocks dietary iron absorption: children with post-malarial anemia have high hepcidin levels and poorly incorporate orally administered...
iron into their red blood cells [8]. It has also been shown that in humans, iron deficiency appears to protect against severe malaria, while iron supplementation increases risks of infection and disease [9]. Haemoglobin was measured because most of the iron in the body is in haemoglobin of red cells which contain about 1 mg of iron per millilitre of erythrocytes, or about 2–3 g of total iron. Both iron deficiency and iron excess cause cellular and organ dysfunction. Low plasma iron concentrations restrict iron uptake by erythrocyte precursors, limiting haemoglobin synthesis and causing anaemia [10]. Therefore as a result of this interplay, the study was designed to assess the impact of malaria on levels of hepcidin and iron status in malaria infected children (during severe and non-severe infection) since they are highly susceptible to malaria infection.

Materials and Method
Ethical clearance for sample collection was obtained from Federal Medical Centre, Ido-Ekiti, Ekiti state and Afe Babalola University, Ekiti State before the commencement of the investigation. The nature and purpose of research was explained to each subject for those who could understand and the mothers of the younger children for the purpose of obtaining their consent. A total of ninety three children between the ages of 6months-15 years were investigated. The subject stratification was based on severity of malaria infection since the World Health Organization (WHO) had categorized malaria density of greater than 250,000 parasites/µl of blood as severe malaria and those less than 250,000 parasites/µl as non-severe malaria [11].

A total of 33 subjects had severe malaria, 30 subjects had non-severe malaria and 30 apparently healthy subjects without malaria served as control.

Malaria detection: Malaria parasites were detected microscopically following Giemsa staining of thick and thin blood films [12].

Estimation of hepcidin: This was done using enzyme linked immunosorbent assay for determination of hepcidin level in serum [13].

Estimation of serum iron: This was measured spectrophotometrically at 560nm. The absorbance measured at this wavelength is proportional to serum iron concentration [14]

Estimation of total iron binding capacity: The total iron binding capacity was estimated using the sum of serum iron concentration and unsaturated iron binding capacity [14].

Estimation of hemoglobin concentration: This was measured spectrophotometrically at 540nm wavelength [15].

Statistical analysis
Results obtained were subjected to statistical analysis using software package for social sciences (SPSS); version 21.0 (SPSS Inc. Chicago, Illinois, USA). All parameters were expressed as mean ± SD. The student r-test was the tool of choice in comparing means. Correlations of the parameters were also done. Values were statistically significant at p=0.05 or 0.01.

Results
Table 1 shows the result of mean ± standard deviation of hepcidin, Hb, iron and total iron binding capacity for malaria positive subjects based on age group. There was significant decrease in hepcidin as the age increased while Hb, total iron and TIBC were significantly higher in older age groups.

Table 2 shows the result of mean ± standard deviation of hepcidin, Hb, iron and total iron binding capacity for malaria positive subjects based on age group. There was significant decrease in hepcidin as the age increased while Hb, total iron and TIBC were significantly higher in older age groups.

Table 3 shows the correlation of hepcidin and other parameters in subjects with severe and non-severe malaria. There was negative correlation (inverse relationship) at p<0.01 when hepcidin was correlated with other parameters.

Discussion
Malaria and its complications have been described to be one of the leading infections and causes of death in children [16]. This research was set to assess the impact of malaria severity on hepcidin and iron status relative to apparently healthy children without malaria infection.

In this research, there was significant increase in hepcidin levels in severe and non-severe malaria compared to control group. This study agrees with other works which stated that

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<th>Severe Malaria</th>
<th>Non-severe Malaria</th>
<th>Control</th>
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<tr>
<td>Hepcidin (ng/ml)</td>
<td>127.79 ± 28.55</td>
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<td>Hb (g/dl)</td>
<td>9.38 ± 1.28</td>
<td>11.46 ± 1.39</td>
<td>12.86 ± 1.13</td>
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<td>Iron (µg/dl)</td>
<td>54.06 ± 8.37</td>
<td>96.96 ± 48.15</td>
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<td>TIBC (µg/dl)</td>
<td>260.16 ± 16.73</td>
<td>271.68 ± 34.04</td>
<td>284.33 ± 32.08</td>
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<td>Hepcidin (ng/ml)</td>
<td>115.04 ± 49.40</td>
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<tr>
<td>Hb (g/dl)</td>
<td>9.14 ± 1.13</td>
<td>10.72 ± 1.05</td>
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<tr>
<td>Iron (µg/dl)</td>
<td>60.24 ± 25.58</td>
<td>66.09 ± 20.45</td>
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<tr>
<td>TIBC (µg/dl)</td>
<td>257.78 ± 18.70</td>
<td>259.86 ± 17.86</td>
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<td>1.000</td>
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<td>Hb (g/dl)</td>
<td>-0.494**</td>
<td>-0.441* 0.15</td>
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<tr>
<td>Total Iron</td>
<td>-0.784**</td>
<td>0.000</td>
</tr>
<tr>
<td>TIBC (µg/dl)</td>
<td>-0.589**</td>
<td>0.000</td>
</tr>
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</table>

*correlation is significant at the 0.05 level (2-tailed) **correlation is significant at the 0.01 level (2-tailed)
high levels of hepcidin were observed in non-severe malaria subjects compared to control subjects [17,18]. However, this finding is not in line with the work which stated that there is suppression of hepcidin in severe malaria cases [19]. The results did not also support the works which stated that children with non-severe malaria had higher hepcidin levels than those classified as severe malaria subjects [20]. This could be as a result of the presence of inflammatory diseases or other activities of hepcidin that were not investigated because hepcidin has been shown to also increase in inflammatory diseases. There was significant increase in the level of serum iron in control subjects compared with severe malaria subjects and non-severe malaria subjects. This study agrees with a previous finding that malaria is the leading cause of iron deficiency with other micronutrients [21]. It was also observed that there was significant increase in total iron binding capacity in healthy control subjects compared with severe and non-severe malaria subjects. Total iron-binding capacity (TIBC) measures the blood’s capacity to bind iron with transferrin (its transport protein) in blood and it is therefore a measure of the maximum amount of iron that it can carry which indirectly measures transferrin [22]. There was significant increase in total iron binding capacity when the age group 11-15 years was compared with other age groups (6months-5 years and 6-10 years). This is justified by the fact that in the course of infection, nutrients move from circulation to tissues causing a reduction from circulation. Also in this study, it was observed that there was decreased hepcidin level with increase in age. This is to say that there was increased hepcidin level in the <5 years age group compared with other age groups. Therefore from the findings of this study, it can be said that children is inversely proportional to age. This study agrees with another report which stated that hepcidin level was highest in youngest children and decreases as age increases [23]. It was also observed that there was increased level of serum iron with increase in age. The hemoglobin concentration of control subjects was higher compared with severe malaria subjects and non-severe malaria subjects, although the level of hemoglobin concentration in subjects with non-severe malaria was higher than those with severe malaria. These findings agree with another finding which showed that hemoglobin concentration decreases based on the severity of malaria and this could be as a result of excessive breakdown of hemoglobin during malaria infection [24]. Moreover, there was negative correlation observed between hepcidin and hemoglobin concentration in both severe and non-severe malaria. Also, there was significance negative correlation when hepcidin was correlated with total iron binding capacity in severe malaria subjects and non-severe malaria subjects. This work also supports the work which stated that high levels of hepcidin reduce the binding capacity of iron thereby preventing iron absorption [25]. There was negative correlation found between the levels of hepcidin and serum iron in both malaria cases. This study is also in agreement with another research which reported that hepcidin blocks the uptake of dietary iron from the intestine, and increase the accumulation of iron in macrophages [26]. The result is a decrease in serum iron levels which routes iron away from pathogens that could potentially exploit circulating iron, but may also render the host anemic by restricting iron availability to the erythrocytes.

Conclusion
This study showed that subjects with severe malaria had higher levels of hepcidin, lower levels of serum iron and lower hemoglobin concentration compared with non-severe malaria subjects and control subjects who had lower hepcidin levels and higher serum iron levels and hemoglobin concentration. This study concluded that severe malaria may cause the upregulation of hepcidin. The study suggests that hepcidin could help in the diagnosis of severe and non-severe malaria and also be used to monitor the progression of malaria infection.

Acknowledgement
The authors acknowledge the staff members in the Phlebotomy unit as well as the staff members of Department of Chemical pathology and Haematology of Federal Teaching Hospital, Ido-Ekiti, Ekiti State, Nigeria for their assistance during sample collection.

Competing interests
The authors declare that there is no competing interest. There was no financial assistance whatsoever.

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