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Serum Hepcidin Concentration is Elevated during Malaria Infection among Children in Sokoto Nigeria

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Abstract :*Malaria and iron deficiency are major public health problems for children living in sub-Saharan Africa. This study was aimed at evaluating serum hepcidin concentration dynamics during malaria infection among children in Sokoto, North-Western Nigeria. 402 children were enrolled in this study, comprising 200 males (49.8%) and 202 females (51.2%) aged between 1 – 17 years. The prevalence of malaria infection in this study was 31.3%, with subjects within the ages of 11 – 15 years having the highest prevalence (29.6%). Significant differences were observed for serum hepcidin concentrationand other iron indices between malaria infected children and non-malaria infected children, (control) ($p < .05$). Serum hepcidin concentration is increased during malaria infection among children in our study population ($p < .05$).We concluded that determination of serum hepcidin concentration in synergy with other iron parameters may be a useful marker in the prognosis and management of malaria infection in our setting.*

Keywords -*anaemia,ferritin, hepcidin, iron deficiency anaemia, malaria.*

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I. Introduction

In spite of the remarkable progress achieved in the fight against malaria, the disease continues to have debilitating impact on people's health and livelihoods. Global update from 2015 showed that 212 million cases of malaria led to 429 000 deaths of which most of the victims were children aged under 5 years in Africa [1]. Specifically, in the same year, 92% of death cases due to malaria occurred in the African Region, 6% in the South-East Asia Region, and 2% in the Eastern Mediterranean Region. Children under 5 are especially susceptible to malaria illness, infection and death. Reports from 2015 also showed that malaria killed an estimated 303 000 under-fives across the world, with 292 000 occurring in the African Region. The disease kills 1 child every 2 minutes [1].

The malaria parasite, acquires iron during various stages of its life cycle. There have been theories that the blood stage of the parasite acquires iron from serum transferrin, from iron produced during the breakdown of hemoglobin, or from a free pool of intracellular iron [2]. Malaria induces the destruction of infected and non-infected red blood cells. This hinders the ability of reticuloendothelial macrophages to recycle iron back to the bone marrow [3]. Iron deficiency anaemia (IDA) is the most prevalent anaemia globally, and it is more common in developing countries [4,5]; were malaria morbidity and mortality is predominant [3].

The primary iron storage protein that acts as a reservoir for iron is ferritin [6]. It plays a key role in the regulation of iron homeostasis and is also accepted as a biomarker in the evaluation of body iron stores [7]. Made up of an approximately spherical apoprotein shell (molecular weight of 480 000) that encloses a core of ferric hydroxyphosphate of about 4 000 iron atoms), human ferritin is composed of two immunologically distinct types: H and L [6]. The H-subunit functions as a ferroxidase and carries out oxidation of iron to the Fe^{2+} form, while the L-subunit is associated with iron nucleation, mineralization, and long term iron storage [8].

When iron is exported to serum, it is bound by the glycosylated iron-transport protein known as transferrin (Tf). Found in the blood plasma, lymph, and other body fluids, Tf is saddled with the responsibility of transporting iron to all cells. This keeps free iron at very low concentrations inimical to the survival of iron-dependent pathogens [9] like *Plasmodium* species. The enabling step in the uptake of iron from Tf requires that the protein is attached to specific receptors on the surface of the cell known as transferrin receptor (TfR1). Another receptor, TfR2 also binds Tf and may act as the homeostatic iron sensor, regulating hepcidin synthesis in response to diferricTf concentration [6].

Hepcidin is a peptide hormone with 25 amino acids [10]. Chromosome 19 carries the *hepcidin antimicrobial peptide* (HAMP) gene that encodes hepcidin, through the 84 amino acid precursor known as

preprohepcidin [6,10]. This precursor molecule, thereafter, undergoes two enzymatic cleavages in the hepatocyte cytoplasm and in the blood, to liberate the 25 amino acid-containing biologically active form known as hepcidin-25 [10]. The role of hepcidin is the inhibition and subsequent destruction of ferroportein (FPN1). FPN1 is the major iron export protein, located in the basolateral surface of gut enterocytes and the plasma membrane of reticuloendothelial (RE) cells like macrophages [11,12].

Malaria and iron deficiency (ID) are major public health problems for children living in sub-Saharan Africa. Many researchers have done work on malaria among children in these regions [13–17]. Serum hepcidin may be a good marker for the prediction of anaemia of inflammation (AI) [18]. This research work was aimed at evaluating serum hepcidin concentration dynamics during malaria infection among children in Sokoto, North-Western Nigeria.

II. Materials and Methods

2.1 Subjects and Setting

The subjects in this cross-sectional study comprised 402 children diagnosed with malaria, as well as malaria negative control subjects; all aged between 1 and 17 years. Informed consent was obtained from the guardian or parents of the subjects. The study was conducted in the paediatric department of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto and Specialist Hospital Sokoto which are Tertiary Hospital in Sokoto metropolis. Sokoto is located in North-West Geographical zone of Nigeria.

2.2 Laboratory

2.2.1 Blood Sample Collection

Blood sample was collected by venipuncture as described by Jurry*et al*[19]. 4mls of venous blood was obtained from each of the subjects, 2mls was dispensed into plain bottle. Sera were obtained after centrifuging the blood samples at 4500 × *g* for 15 minutes and were stored at -20^{o}C until analysis. The remaining two 2mls of blood was transferred in to EDTA bottle under aseptic condition. The samples collected in EDTA bottles were carefully mixed 5-6 times to prevent clotting. About 6µl and 2µl of EDTA blood samples were collected and placed on a clean-grease free glass slide; 6µl for thick film and the 2µl for thin film. The blood films were made immediately (within 30 minutes) after sample collection and thick films were handled with care avoiding it being washed away during staining [20,21].

2.2.2 Laboratory Analysis

2.2.2.1 Malaria Parasite Testing

The SD Bioline Malaria Ag P.f/Pan Rapid Test Kit was used to carry out the malaria parasite test (05CDA096A) [22,23]. This was then followed by microscopy using stained thick and thin blood film for effective determination of parasite density [20].

2.2.2.2 Serum Hepcidin Estimation

Serum hepcidin level was estimated using the enzyme-linked immunosorbent assay (ELISA) kit from Kamiya biomedical company, USA (KT-50560) [24].

2.2.2.3 Serum Iron Estimation

Serum iron was estimated using colorimetric assay kit procured from BiovisionInc.[25].

2.2.2.4 Serum Ferritin Estimation

Serum ferritin level was estimated using ELISA kit procured from Enzo-Life-Science, UK (ELS-20015).

2.2.2.5 Total Iron Binding Capacity (TIBC) Estimation

TIBC was estimated using colorimetric assay kit procured from Biovision Inc., USA (BID-10543).

2.3 Statistical Analysis

The data obtained were analyzed using SPSS Version 20. They were grouped into malaria positive and negative subjects, as well as various age groups, expressed empirically. The Kolmogorov-Smirnov test was used to determine the normality of the data. Analysis of variance (ANOVA) was used for comparison of 3 or more groups while the independent t-test was used for comparisons between 2 groups. Correlation test was also carried out using Pearson's linear correlation analysis. P value ≤ 0.05 was considered as statistically significant.

III. Results

A total of 402 children were enrolled for this study comprising 200 (49.8%) males and 202 (51.2%) females of age range 1-17 years. The results from Table 1 showed that 126 (31.3%) children were malaria infected while 276 (68.7%) were non malaria infected children. Table 2 compares the serum hepcidin concentration and some iron indices between the malaria infected and malaria negative subjects (control) showing statistically significant differences for all parameters. Results from Table 3 shows significant correlation between serum hepcidin concentration and malaria parasite density. Further details are found in the tables below.

Table 1: Demographic Details of enrolled Subjects

Features	Characteristic	Percentage (%)
Total Number of Subject	402	
Gender Distribution:		
Male	200	49.8
Female	202	50.2
Age Range (Years):		
1-5	109	27.1
6-10	108	26.9
11-15	119	29.6
16-17	66	16.4
Number of Malaria infected	126	31.3
No. of Non-Malaria infected	276	68.7
No. of Male infected	65	51.6
No. of Female infected	61	48.4
Age Range (Years) Infected with Malaria:		
1-5	38	30.1
6-10	35	27.8
11-15	33	26.2
16-17	20	15.9

Table 2: Effect of Malaria on Serum Hepcidin Concentration and other Iron Indices**.**

Parameters	Malaria infected Mean±SD	Non- malaria infected Mean±SD	*p*-value
SH (ng/ml)	273.88 ± 59.84	163.23 ± 70.81	.00*
SI(µg)	57.52 ± 17.07	106.96 ± 35.95	.00*
SF (ng/ml)	21.82 ± 38.82	99.70 ± 76.16	.01*
TIBC (mg/dl)	440.46 ± 54.01	353.20 ± 55.97	.00*

*Note: SH= serum hepcidin, SI= serum iron, SF= serum ferritin, TIBC= total iron binding capacity, ng/ml= nanogram per milliliter, µg= microgram, mg/dl= milligram/deciliter., *significant difference, P < .05*

Table 3: Pearson Correlation between Malaria Parasite Density, Serum iron, Serum Ferritin, TIBC and Serum Hepcidin Concentration.

	Malaria Parasite Density [MPD(/µl)] r	*p*-value
SI(µg)	-0.06	.52
SF (ng/ml)	-0.04	.62
TIBC(mg/dl)	0.09	.32
SH (ng/ml)	0.03	.05*

*Note: r= correlation coefficient, SI= serum iron, SF= serum ferritin, TIBC= total iron binding capacity, MPD= malaria parasite density, *significant correlation p = .05, µl = microliter, µg= microgram, ng/ml= nanogram per milliliter, mg/dl= milligram per deciliter.*

IV. Discussion

Malaria, ID, anaemia, and high infection rate are intertwined factors common among young African children [26]. The epidemiological data of malaria varies with geographical location. Thus, specific data obtained for a given location can be of help when designing strategic malaria control programs [16]. In Nigeria, the disease is endemic and shows seasonality in the various geographic zones of the country [27]. In 2009, the Nigerian Federal Ministry of Health showed that malaria contributed directly to 25% of infant mortality and 30% of under-five mortality [28].

Out of a total of 402 subjects, aged between 1 – 17 years, who were enrolled for this study, results showed that 126 (31.3%) children were infected with malaria (Table 1). This result is higher than that of Abdullahi*et al.*[16] who reported 27.29% prevalence rate and lower compared to the results of the study of Umaru and Uyaiabasi[29] who reported 35.7% prevalence rate. This result is also lower when compared to the result of the study of Jiya*et al.*[30] in Sokoto, who reported the prevalence rate of 45.4% among children. The prevalence of *Plasmodium falciparum* infection in this study was also lower than the 60.6% obtained by Dawaki*et al*. [31] among Hausa communities in Kano state, Nigeria. This relatively low prevalence could be due to absence of breeding sites for the Anopheles vector in some months of the year.

The proliferation of *Plasmodium* during malaria infection requires iron during the clinically silent liver stage of growth and in the pathological phase of erythrocyte invasion [2]; and the small molecule hepcidin, has been identified as a vital controller of iron bioavailability [32,33]. In this study, there was a statistically significant increase (p = .00) in the serum hepcidin concentration in malaria infected children (273.88 ± 59.84 ng/ml) in comparison to non-malaria infected children (163.23 ± 70.81 ng/ml) (Table 2). This result indicate

upregulation of hepcidin response during malaria infection and agrees with that of several studies [26,33–35]. Moreover, malaria parasite density showed a strong positive correlation (r = .03, p = .05) with serum hepcidin concentration (Table 3).Humans and mice infected with Plasmodium have been shown to have elevated plasma hepcidin levels with positive correlation to parasitemia and plasma IL-6 [36]. The upregulation of hepcidin during malaria infection may contribute to anaemia[2,32], through its binding to FPN1 and subsequent internalization in lysosomes [6]. This may have protective effect as shown in mice during experimental malaria. Immunoneutralization of hepcidin was associated with parasitemia and death in *Plasmodium berghei* infection. This was not the case when the animals were pretreated with a hepcidin-expressing lentivirus that conferred some form of protection to the infection [36].

Results from this study, on the contrary, showed a significant decrease (p = .00) in the serum iron concentration between malaria infected children (57.52 ± 17.07 µg) and non-malaria infected control (106.96 ± 35.95 µg). This was similar to the serum ferritin concentration for the 2 groups (Table 2). These results were similar to those obtained by Jeremiah *et al.*[37], and Onuoha[38] who showed in their study that serum iron, serum ferritin, and total iron binding capacity were affected by malaria at p< .01. This may be the consequence of intravascular hemolysis of parasitized red cells, phagocytosis of parasitized and unparasitized red cells, and hypersplenism associated with the anaemia seen in acute malaria [3].

V. Conclusion

Our study had shown that serum hepcidin concentration is increased during malaria infection among children in our study population. We further infer that determination of serum hepcidin concentration in synergy with other iron parameters may be a useful marker in the prognosis and management of malaria infection in our setting.

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