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Iron Status of Pregnant and Post-Partum Women with Malaria Parasitaemia in Aba Abia State, Nigeria

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Abstract

Malaria is the most uncontrollable public health problem worldwide. The study was aimed at determining the association between Iron and Malaria parasitaemia among pregnant and post-partum women. A total of 206 pregnant and 50 post-partum women who tested negative to HIV were recruited in the study. They were stratified into four groups; 144 malaria parasitaemia and 62 aparasitaemic pregnant women, 30 placental infected malaria and 20 malaria placental uninfected post-partum women. They constituted group 1, 2, 3 and 4 of the study subjects respectively. Also 20 malaria infected and 20 malaria uninfected non- pregnant women, classified as groups 5 and 6 respectively, represented the control subjects. The test groups were asymptomatic subjects and control groups were apparently healthy subjects. All were between 17 and 44years. Malaria and malaria parasite density were determined by the thick film technique, serum transferrin (STFR) and SF were measured by ELLSA. Full blood count and red cell indices were evaluated using sysmex Automated Hematology Analyzer model KX2IN Series. Serum transferrin (STFR-F) index was derived from values of SF and STFR. Student's t-test and ANOVA were used for comparison of groups. The mean parasite Density in the peripheral and placental blood was 685.56 ± 484.55 parasite /µ1 and 762.47 ± 459.62 parasite/µl of blood respectively but no significant difference was observed on comparison (P>0.05). Haemoglobin was lower in the infected but not significant on comparison with the uninfected pregnant subjects (P>0.05) while serum ferritin was higher in the infected, however, showed no significant difference in comparison with the uninfected (P>0.05). Serum transferin (STFR) was significantly higher in the infected than uninfected pregnant women (P>0.05). The infected pregnant subjects showed 40% anaemia of infected and 13% IDA. This study has shown that anaemia is the major cause of poor maternal and infant outcome in pregnant women in the area. Increase Parasitaemia leads to decrease iron level culminating to iron deficiency anaemia.

Keywords: Iron status; Pregnant women; Post-partum women; Palaria parasitaemia

Introduction

Malaria and Iron deficiency are the predominant causes of severe anaemia in sub-saharan Africa and have their respective deleterious impact on women of reproductive age, the neonates, infants and children [1,2]. The vulnerability of children and pregnant women to malaria is because children have naive immune system while the immune system of pregnant women is potentially compromised. Consequently, the two groups are considered to be the highest risk population for malaria- related death [3,4].

Iron is absorbed in the gastrointestinal tract and is essential for erythropoesis. In most cases, the amount of iron absorbed is not always enough for sustenance of pregnancy and may result to iron deficiency. The major reason for iron deficiency during pregnancy is that pregnancy places tremendous increase in the body's need for iron in order to match with the increase in plasma volume of the pregnant woman and the fetal need for proper development [5]. The need culminates in depleted iron stores. The progressive depletion of iron-stores

will eventually result to Iron Deficiency Anaemia (IDA) [6]. Body iron status is usually assessed by considering hemoglobin, red cell indices and serum Ferritin concentrations along with evidence of inflammation, infection and liver disease [7].

The assessment emphasizes on combination of parameters from the storage, transport and functional iron compartments. The best combination would be estimations of haemoglobin or haematocrit, serum transferrin receptor and serum ferritin. Such a combination would reflect functional impairment, tissue avidity for iron and iron storage [8]. Therefore, this study seeks to evaluate the alterations in iron components in pregnant women and post-partum subjects with malaria parasitaemia in Aba, Abia State.

Either malaria parasitaemia or iron deficiency has been emphasized as major etiological factors causing anaemia in pregnancy. Little observation has been made on the co-existence of such factors. Again, these studies paid attention to the assessment of the etiological factors of anaemia in pregnancy and pregnancy outcome(s) due to extreme severity of anaemia as the cause of maternal/infant morbidity and mortality. Anaemia is commonly observed in pregnancy and malaria infection in pregnant women may exacerbate the anaemia. Iron status in pregnant and post partum women should be determined.

Materials and Methods

Study area

The study took place at Aba, Abia State, Nigeria.

Study population

Two groups of subject were recruited for the study. Asymptomatic pregnant and immediate post- partum women and were within 17- 44 years. Each of the groups was further sub-divided into parasitemic and aparasitemic group based on the detection of malaria parasite in the blood of the pregnant subjects and blood from the placenta of the immediate post-partum. The pregnant subjects were on enrolment for antenatal care while the immediate post-partum had their delivery shortly before the collection of specimen. Both groups were drawn at antenatal care and delivery units of Abia State University Teaching Hospital (ABSUTH) and Living Word Hospital WMH). The Malaria parasitemic pregnant women were 144 in number and considered as the first group of the study subjects while the malaria aparasitaemic pregnant subjects were 62 in number and considered as the 2nd batch of the study group. The malaria parasitaemic immediate post-partum were 30 in number and taken as the third group of the study subjects while the malaria aparasitaemic immediate post-partum were 20 in number and taken as the 4th batch of the study group. Similarly, 20 malaria parasitaemic and 20 malaria aparasitaemic apparently healthy non- pregnant women of the same age with the study groups were recruited as controls and served as the 5th and 6th groups respectively. The control subjects were drawn from the staff and students of school of Nursing, ABSUTH. Both haematological and biochemical parameters were determined at the Haemtology research and the Chemical Pathology Specialist Unit of New Covenant Medical Laboratories and Blood Bank Services LTD, Aba.

Specimen collection

Peripheral blood was obtained from pregnant and non-pregnant women between 9am- 11am while blood from the placenta was obtained within 30 minutes after delivery. Collection of peripheral blood was by venipunture technique. Without the use of tourniquette, about 8ml of blood was collected each from the anti-cubital vein by the phlebotomist. About 8ml of blood from placenta was obtained by biopsy pool method; this was carried out by the matrons.

Laboratory procedures parasitological and biochemical analysis

Screening for Malaria parasite was carried out using Rapid Diagnostic Test by (SD BIOLINE INC, 2013) BATCH NO.05 FK30-02-8. A pink line at the positive and control band indicated a positive reaction whereas only one pink line at the control band indicated negative reaction. No pink line at both positive and negative control bands indicates an invalid result.

Microscopic detection of malaria parasite was carried out using Giemsa stained thick film reported by Cheesbrough [9]. Quantitative parasite count using thick film was carried out as described by WHO (1991), the numbers of asexual parasitic forms (trophozoites,Schizonts) present were counted over 200 Leukocytes. The qualitative test for HIV antibodies in blood was carried out with Determine as described by Alere [10] while the confirmatory test for HIV antigens in blood was carried out using HIV1 and HIV 2 Comb firm kit as described by Orgenics in 2013. A serum sample yielding a minimum of two circular, coloured antigen spotsjncluding gp41 or gp36 is defined as HIV positive.

The quantitative determination of STFR was by Elisa technique as described by Monobind Inc. while serum ferritin was estimated by Elisa technique as described by Monobind Inc.

Haematological estimation

Estimation of full blood count with Differential and Red cell indices was carried out using sysmex automated 3part haematology analyzer model KX-2IN manufactured by Sysmex, Kobe Japan.

Statistical analysis

The computational analyses were performed using statistical package for social science (SPSS) Version 21.0. The results were expressed in mean and Standard deviation. Student's t-test and analysis of Variance (ANOVA) were used for comparison of different in various groups.

Results and Discussion

According to WHO standard, anaemia in pregnancy is present when hemoglobin concentration in the peripheral blood is <11.0 g/L.

In this study, anaemia was found to be prevalent in 64% of the malaria infected pregnant subjects. The prevalence of anaemia at first antenatal visit in this study was lower than 94.07% reported by Nduka et al. [11]. It did not differ from 60.8% reported by Okafor et al. [12]; 59.6% reported by Agan et al. [13], 62.4% reported by Ogbodo et al. [14] and 66% reported by Erhabor et al. [15] **(Tables 1 and 2).**

Table 1 Severity of anaemia among pregnant women using Hb (Haemoglobin).

Functional Iron Compartment	Malaria Infected Pregnant Women		Malaria Uninfected Pregnant Women	
	Frequency	Percentage	Frequency	Percentage
Hb (g/L)	(No)	(%)	(No)	(%)
>110 (Normal)	51	35.42	23	37.1
90-109.0 (Mild)	82	56.94	35	56.45
70-89.9 (Moderate)	11	7.64	2	3.23
<70 (Severe)	0	0	2	3.23

Table 2 Evaluation of iron in pregnancy using sTfR assay.

Functional Iron Compartment	Malaria Infected Pregnant Women		Malaria Uninfected Pregnant Women	
	Frequency	Percentage	Frequency	Percentage
sTfR (nmol/L)	(No)	(%)	(No)	(%)
>64 (Normal)	70	48.61	42	67.74
67.1-114 (increasderythropoisis)	73	50.69	18	29.03
>114 (Marked erythropoiesis)	1	0.69	2	3.23

Furthermore, it was higher than 30% reported by Douamba et al. [16] and 20.3% reported by Buseri et al. [17]. The difference in the prevalence rates could be related to various levels of transmission of malaria in different locations and the interactions of other aetioloical factors of anaemia in pregnancy. The prevalence rate of anaemia in pregnant women in this study is high and worrisome. The reasons were not far-fetched. It may be related to several factors such a dirty environment, government's approach to prevention and treatment of malaria and the attitude of most pregnant women to combating malaria. On the environment, the stagnant water in drainage provides enabling habitat for the breeding of mosquitoes. On the part of the government, the inability to curb the incessant and protracted industrial action (strike) in the health sector which shuts down government medical centers could contribute adversely to the pregnant women not having access to antenatal cares. Also the situation where Insecticide Treated Nets (ITNs) are not adequate and evenly distributed, contributes to the persistently increased rate of anaemia. It is reported that some pregnant women sleep outside the insecticide Treated Nets thereby exposing themselves to Mosquito bites. Greater numbers of pregnant women report to antenatal late mostly in the second and third trimester paving way for late administrations of necessary interventions. Furthermore, some pregnant women resort in intake of anti-malaria drugs from drug vendors without considering the efficacy of such drugs. This may contribute to the high rate of malarial anaemia as the efficacy of these drugs cannot be guaranteed even in the wave of prevailing fake and counterfeit drugs. Considering the severity of anaemia among the malaria infected pregnant women, 56.9% and 7.6% were recorded for mild and moderate anaemias respectively. That no severe anaemia observed in this study was expected because only apparently healthy pregnant women who were on first antenatal booking were enrolled in the study. Since pregnant women with clinical malaria were excluded, the rate of anaemia and its severity could be an underestimation of the overall prevalent rate. The low prevalence of moderate anaemia and lack of severe anaemia could be attributed to wide use of haematinics and prophylactics anti-malarial prior to antenatal booking. It is a common practice in Nigeria for women in particular to place themselves on anti-malarial and haematinics.

This study also revealed 62% anaemia among the uninfected pregnant subjects and by severity 56.45% mild anaemia, 3.23% moderate while 3.23% severe anaemia was similar to the result obtained by Erhabor et al. [15] who reported 48% anaemia. The incidence and severity of anaemia may be used as indicators of the several health conditions of the pregnant women or women of the reproductive age. In addition, some subjects that might be anaemic were undetected and

unattended to. Also, other factors apart from malaria, may contribute to the anaemia, such as deficiencies of iron, folic acid, zinc and copper.

On the distribution of iron in the store compartment [shown by concentration of serum ferritin (SF)] of the malaria infected subjects, 25.6% had decreased iron store while 2.0% had elevated iron store. But with the malaria uninfected pregnant women, 24% had decreased iron store while there was no elevated iron store. A remarkable observation in this study is the excessive increase in concentration of serum ferritin that was present in 2.08% of the infected pregnant subjects. The higher value of the SF might be attributed to the fact that ferritin is an acute phase reactant and may have been indicated in some latent infection or sub-clinical disorders. This is as a result of changes in the release and storage of iron in the monocyte-macrophage systems specifically in inflammatory disorders. In inflammatory disorders, there is a reduction in the rate of release of iron by the phagocytes and an increase in the storage of iron by the phagocyte/macrophage systems.

Pregnancy is a time of considerable maternal adaptation during which iron status is affected. Malaria is thought to produce anaemia by lowering haemoglobin levels through several mechanisms including direct haemolysis, accelerated splenic removal of red blood cells and reduced erthropoiesis. These account for the reduced haemoglobin concentration. In some areas of malaria endemicity however, many other causes of low haemoglobin concentration have been identify including nutritional and non-nutritional factors [17]. The soluble transferrin receptor (sTfR) was significantly higher in the malaria infected pregnant women than the uninfected. It suggests that asymptomatic malaria may also be associated with increased haemolysis of red blood cells. Elevated sTfR concentration is possible in subjects who had asymptomatic or mild malaria and this occurs because of haemolysis of red blood cells [18-20] **(Tables 3 and 4).**

Table 3 Evaluation of store iron in pregnancy using serum ferritin (SF).

Store Iron Compartment	Malaria Infected Pregnant Women		Malaria Uninfected Pregnant Women	
	Frequency	Percentage	Frequency	Percentage
SF (ug/L)	(No)	(%)	(No)	(%)
<15 (Decreased)	37	25.69	15	24.19
15 – 200 (Normal)	104	72.22	47	75.81
>200 (Increased)	3	2.08	0	0

Table 4 Comparison of the mean ± SD of iron status of malaria infected and uninfected pregnant subjects.

Parameters	Infected Pregnant (n = 144)	Uninfected Pregnant (n = 62)	P-values
Hb (g/L)	102.42±10.98[a]	105.79±14.16[a]	0.067
MCV (fl)	88.20±11.03[a]	89.70 ±8.34[a]	0.34
sTfR (nmol/L)	62.78±21.97[a]	54.07±23.73[b]	0.012
SF (ug/L)	43.61±84.99[a]	31.31±28.18[a]	0.267
C-RP (mg/dl)	6.09±4.88[a]	4.49±2.74[b]	0.017

Malaria and other conditions that may be associated with increased haemolysis of red blood cells such as Glucose-6-phosphate dehydrogenase deficiency, sickle cells anaemia and thalassemias, may lead to increased erythropoiesis. Increased erythropiesis in turn, results to increase sTfR [21].

In this study, the concentration of SF was increased in the infected pregnant women than the uninfected pregnant women although it did not show any statistical relevance. The presence of the parasite can induce chronic or mild acute phase response even in asymptomatic subjects resulting to elevated SF concentration. Besides, the inclusion of subjects without any correction or adjustment made on values of SF and those with marked level of SF (>200 u.g) gave rise to the increased values of SF beyond that of uninfected non-pregnant women. Ordinarily, one may suggest exclusion of subjects with marked SF (>200 µg) but it may lead to loss of valuable diagnostic data.

With regards to age, gestational age and gravidity, this study failed to find any consistency on the effect of age and gravidity on iron status parameters. However, iron status was affected by gestational age. The data followed the normal physiological pattern in which Iron parameters decreases with increasing gestational age. This is expected as normal pregnancy progresses. Hemoglobin concentration was high in the first trimester but reduces as pregnancy progresses to the second and third trimesters although it tends to increase again in the third trimester above the second trimester. The data clearly shows that the increased haemoglobin level in the first trimester could be as a result of reduced iron requirement since there is usually a temporary ceasation of menstruation in the first trimester. Moreover, the amount of iron transferred to the fetus is still minimal. From the beginning of second trimester, there is a major expansion in the maternal red cell mass which continues until the third trimester [22]. During this period, iron is transported through the placenta to the developing fetus therefore maternal functional and store iron are expected to be reduced.

The third trimester increase was expected because of less demand for iron by the maternal-fetal placental unit and the fetus. This also is necessary to accommodate delivery that would be expected soonest. Another possibility to the increase

level of iron in the third trimester could be that the pregnant women may have dabbled into intake of haematinics on their personal volition.

In characterizing anaemias using iron status parameters. The malaria infected pregnant woman showed 16% iron deficiency, 13% iron deficiency anaemia, 40% anaemia of infection, 2% anaemia of inflammation in association with iron deficiency anemia and 4% other anemias. The prevalence of iron deficiency in this study was lower than 18% reported by Isah et al. [23], 47% and 32% in primigravidae and multigravidae respectively reported by Brabin [24] and 55% by Van den broek and Letsky [25]. In the same perspective, the prevalence of IDA found in this study was higher than 6% recorded by Duffy et al. [26]. On the other hand, it was lower than 22.6% recorded by Usanga et al. [27]. Several factors may have contributed to different prevalent rates. First and foremost are the criteria adopted in defining iron status; as no universal criteria have been adopted and various scholars use different criteria. Also it is reported that the use of multiple criteria could falsely reduce the prevalence findings for iron deficiency. Assessment of iron status in this study adopted the combination of haemoglobin, soluble transferrin receptor and serum ferritin. This is in conformity with the recommendation of Pippard and Hofbrand [28] who emphasized the choice of parameters to embrace different iron compartments as well as evidence of infection and inflammation. Another factor that may contribute to different prevalent results could be the nature of the study subjects and the inclusion criteria. Whereas this study concentrated on asymptomatic malaria parasitized pregnant subjects and without prescription of iron supplements, other studies recruited the entire pregnant subjects or anaemic pregnant women even some subjects on iron prophylactics. However, it shows that the prevalence of iron and iron deficiency anaemia varies with geographical location. Moreover, it reflects the standard of living of the subjects which may be associated with their income, medical care and education. Variation may still occur as a result of the composition of different food or dietary intake and some cultural practices such as geophagy. Most grain based diets may contain high phytate and the soil with respect to geophagy, may contain some salts or lead (Pb) that inhibit iron absorption [29]. Excess intake of diet saturated with copper may result to increased levels of copper in the serum. Eventually, iron absorption is negatively affected by elevated levels of copper [30]. Moreover, iron deficiency or iron deficiency anaemia may results from inadequate intake of bioavailable iron promoters. Iron promoters enhance iron absorption and such iron promoters include vitamin A and ascorbic acid.

The high rate of anaemia of infection in the study was expected. Granted that the recruited subjects were malaria subjects but the tendency for susceptibility to other infections was possible. Infections from the gastrointestinal tract such as hookworm are known to contribute to the anaemia [31]. In view of the result of this study, screening of iron status of pregnant women could be reviewed to include investigation on gastrointestinal tract and genito-urinary tract infections.

In this study, anaemia of inflammation was characteristically defined by the use of soluble transferrin receptor assay. Soluble transferrin receptor is a good indicator of-tissue iron deficiency and is independent of iron stores. It is not elevated in case of inflammation; therefore it was adopted to differentiate between iron deficiency anaemia and anaemia of chronic inflammation or both [20]. The presence of inflammation/infection underscores the benefit of prophylactic iron administration [32]. Thus, iron administration with the intention of providing available iron to the haemopoetic sites may be reduced or inhibited.

On the overall, the prevalence of iron deficiency was reduced in the uninfected than the infected group. This could be associated with the effect of immune activation on iron status parameters. The other anaemias showed at 4% may not have been unconnected with heredity factors and deficiencies in vitamin B12, folate and some other micronutrients such as zinc.

Conclusion

This study demonstrated that pregnancy is a period of increased need for iron however; malaria induces the redistribution of iron. This study observed that IDA is more prevalent in the malaria uninfected pregnant subjects while anaemia of infection followed by IDA is more prevalent in malaria infected pregnant women. So, it would be vital to re-evaluate pregnancy screening procedure to include assessment of malaria parasitaemia and detailed iron parameters. Other micronutrient status such as folate, vitamin A, pyridoxine and cobalamine could be evaluated. Subjects identified with IDA may require iron supplementation and iron absorption enhancing molecules such as ascorbic acid. In addition, those with anaemia of infection in which iron supplementation might not be of immense benefit could be treated for infection before iron administration. One of the remarkable findings of this study is the identification of subjects with anaemia of inflammation and those with IDA in association with anaemia of inflammation. In such situations, there will be an underestimate of iron deficiency. This unimaginable condition should be borne in mind in administration of iron interventions.

Furthermore, increased serum ferritin (SF) was indicated. This confirmed that SF could be considerably influenced by malaria or inflammatory processes. Therefore SF could not reflect the exact iron content in the store in such conditions. For the actual iron concentration in the store, it might be ideal to adjust the value of SF using a correction factor. The result obtained may serve as background information for further studies especially on the role-of hepcidin on iron metabolism in pregnant women and post-partum with malaria parasitaemia.

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