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Iron status of malaria and control subjects attending selected hospitals in Sokoto Metropolis, Nigeria

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ABSTRACT
The association of nutritional status and malarial disease is complex with multiple dimensions. Deficiencies in some of these nutritional parameters rethought to lead to malnutrition with subsequent susceptibility to malaria infection. The objective of this study is to determine iron status of malaria and control subjects attending selected hospitals in Sokoto metropolis, Nigeria. Serum iron was measured by the method of Nitro-PAPS. TBC was assessed calorimetrically while Transferrin and UIBC were determining using specific sharing formula. Result of the study observed significant($p<0.005$) increased in TIBC level of most male infected individuals compared to female infected whereas a decreased in Transferrin level in about all male infected individuals than in female infected and vice-visa. The serum iron levels are comparatively low due to redistribution of iron from the serum into the tissue storage forms (transferrin). Also, the percentage transferrin is low, perhaps due to the body absorbing more transferrin than needed, inadequate production of transferrin by the liver which is the major site of malaria infection or excess excretion of transferrin in the urine by the kidneys. After a general analysis, the study observed that serum iron, TIBC and transferrin are highly affected by the malaria.

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INTRODUCTION

Iron serves as a trace nutrient essential and vital for the transportation of respiratory gases through hemoglobin in the red cells. Iron plays important role in the functioning of a variety of enzyme systems like catalases, peroxidases, and cytochromes, which play essential importance in cellular respiration in the mitochondria (Gouadu *et al.,* 2008; Banjoku *et al.,* 2013). Iron in the human body can exists basically in three forms, namely: functional iron in hemoglobin, tissues and harmonic enzymes; stored iron in form of ferritin in the plasma; hemosiderin and circulating iron which is attached to transferrin in the plasma (Gouadu *et al.,* 2008; Akodu *et al.,* 2013). Ferritin is a large molecular weight protein composing of approximately 20% of iron which occurs nearly in most tissues of the body; let alone hepatocytes and reticuloendothelial cells, where it serves as iron reserve (Akodu *et al.,* 2013). Likewise, in serum minute amounts of ferritin exists to reflect the iron stores in healthy individuals. Ferritin functions in the absorption, storage and release of iron. It remains in the body tissues until it is required for erythropoiesis, where it releases iron to bind transferrin for onward transport to the erythropoietic cells. Consequently, serum ferritin, transferrin, serum iron, total iron binding capacity are regarded as indicators revealing levels/ concentrations of iron in human subjects (Akodu *et al.,* 2013; Banjoku *et al.,* 2013). However, pathophysiologies such as those of malaria are key factors causing variation or distortion of iron status in the body essentially due to derangement in iron metabolism (Banjoku *et al.,* 2013).

Malaria is a serious and sometime death-causing disease caused by a parasite that is transmitted through mosquito bite on humans (Centers for Disease Control and Prevention, 2022). Malaria is a common disease in many parts of the world, especially in Africa and Nigeria. This infection is caused by a protozoan parasites belonging to Plasmodium species. Symptoms of malaria includes fever, chills, headache, nausea, muscle aches, tiredness, vomiting. Other possible symptoms are anemia, jaundice; and in severe cases seizures, mental confusion, kidney failure, coma, and even death (CDC, 2022). Therewith, in Africa, the most prevalent and dominant species believed to be causing malaria is the *Plasmodium falciparum*; while other species causing malaria are of the extractions of *P. vivax*, *P. ovale*, *P. malariae*. All these microbes causing malaria are due to vector nature of Anopheles mosquitoes (*Umar et al*., 2010; Nkumama *et al*., 2016; Saleh etal., 2017; Sarkingobir *et al*., 2020). Malaria is transmitted to humans by being bitten by an infective female anopheles mosquito, being transfused with an infected blood, received an organ transplant from an infected donor, being pierced by an infected sharp object like needle/ syringes, being involved in congenital malaria (CDC, 2022). The people who are at risk of malaria infection live mostly in poor countries like Nigeria, because the transmission is endemic. However, *P*. falciparum a typical form of malaria in Africa and relations causes severe and life-threatening infection especially in people with little or no immunity, such as youngsters, pregnant women, travellers from non-malaria regions are more affected with morbidity and mortality to malaria. The situation is mostly aggravated due to poverty to seek for healthcare, inadequate healthcare services, and the likes (CDC, 2022).

At world level, about 228 million cases of malaria were recorded in 2018. Specifically, African continent have the highest burden of malaria morbidity, 213 million cases (93%) in the year 2018; then South-East Asia region have 3.4%, and East Mediterranean region have the lowest score of 2.1% (WHO, 2019). Despite, the control strategies embarked upon by African countries, leading to some sort of progress; the problem of drug resistance, poor environmental sanitation, and other related impediments are still making it difficult to disgorge malaria out of the affected areas (Diallo *et al*., 2017). Likewise, in 2020, WHO reports that 241 million clinical cases occur due to malaria, and 627, 000 deaths occurred due to malaria, therewith most of them are from African countries (CDC, 2022).

Malaria is transmitted all over Nigeria; 76 % of the population lives in high transmission areas while 24 % of the population lives in low transmission areas. More than 90% of the inhabitants in the country are of the risk of contracting Malaria, and 50% of them have been affected with one or more episode of malaria every season of the year (Abdullahi *et al*., 2009). Moreover, malaria transmission season is unhalted all year round in the south and is also almost transmitted throughout the entire months of the years in the northern part of the country. The primary

vector across most of the country is Anopheles (Cheesebrough, 2006). According to the 2020 World Malaria Report, Nigeria had the highest number of global malaria cases (25 % of global malaria cases) in 2019 and accounted for the highest number of deaths (23 % of global malaria deaths) (WHO, 2019).

In Sokoto state, a pocket of studies was conducted on malaria (Abdullahi *et al*., 2009; Sarkingobir *et al*., 2020) but there is haply published epidemiological data in the area is scarce. Therefore, p comprehensive study of malaria situation of the locality is expected to provide base-line information, which will be useful in the effective making of control measures, which could thus help move the locality towards achieving the Millennium Development Goals (MDGs) (Abdullahi *et al*., 2009). The aim and objectives of this study were therefore to determine the nutritional status such as serum iron, TIBC, UIBC and serum Transferrin of malaria infected individuals and control subjects in Sokoto, north western Nigeria (Umar *et al*., 2010; Sarkingobir *et al*., 2020). There is paucity of data on the level of transferrin, serum iron, UIBC and serum total iron binding capacity among population of different sex and age in Sokoto Nigeria. However, the study is timely and relevant that can be used to fill the gap for the awareness on the nutritional benefits among the communities (Abdullahi *et al*., 2009). The objective of this study is to determine iron status of malaria and control subjects attending selected hospitals in Sokoto metropolis, Nigeria.

OBJECTIVES OF THE STUDY

The objective is mainly to determine iron status of malaria and control subjects attending some hospitals in Sokoto metropolis, Nigeria.

MATERIALS AND METHODS

Study area

Sokoto state is one of the 36 states of Nigeria, specifically it is located in the Northwest geopolitical zone. It has a population of about 4, 427 as related by 2006 census (Sarkingobir *et al*., 2020). The Sokoto is the capital of Sokoto state. In term of demography, the state is mostly populated with Hausa/ Fulani tribes, then *Gobirawa*, *Zabarma*, *Adarawa*, *Arawa*, and the likes. The average rainfall in the year is about 550mm with peak in august. The city of Sokoto is commercial and educational hub of its own (Abdullahi *et al*., 2009; Saleh *et al*., 2017).

Study population;
20 patients from some hospitals in Sokoto, who were enrolled to participate in this study.

Determination of serum Iron;
Methodology;
Nitro-PAPS Method
Iron ions are dissociated from its carrier protein, transferring in an acid medium and simultaneously reduced to the ferrous form. The ferrous ions react with the chromate Nitro-PAPS to a color-complex highly specific. The resulting absorbance is directly proportional to the iron contents (MEDICHEM Middle East Chemistry Reagents, 2010).

Principle;
Transferrin-Fe-complex ————> Apotransferrin + Fe3+
Fe3+ ————————> Fe2+
Nitro PAPS + Fe2+ ————————> colored complex

Table 1: Procedure for determination of iron

	Test (µl)	Standard (µl)	Blank (µl)
Distilled water	-	-	50µl
Reagent	1000	1000	1000
Serum	50µl	-	-
Standard	-	50µl	-

The contents were mixed and incubate at 37ºC for 3 minutes and absorbance was read at 578nm. And the absorbance of the sample and standard were measured against blank.
Calculation
Iron concentration = ODA (sample). × 30 (µmol/L)

ODA (STD.)

Determination of total iron binding capacity (TIBC).
Methodology;
Colorimetric method
Principle;
Surplus iron was put into the sample in a manner that transferrin is saturated. The rest of iron would be taken in by Magnesium- carbonate. The bound iron in the supernatant as collected the TIBC is determined similar to that of iron.
Concentration of working reagents;
Reagent 1.
$FeCl_3$ 89.5mmol/L
Reagent 2.
$MgCO_3$
Procedure 1 (iron saturation);
Temperature used 25^0C
The reagent and sample was pipette into test tubes:
Reagent 1. 500 µl
Sample. 250 µl

Mixed and allowed to stay for 30 minutes at 25^0C. Then, one spatula of reagent 2 was put in the mixture, allowed to relax for additional 60 minutes. Within the period occasionally they were shakes 5 times. Then the mixture was centrifuged for 10 minutes at 4000rpm and clear supernatant was taken for iron measurement with centronic iron ferene.

Procedure 2 (Iron determination);
Formation of Sample blank reagents;
'Dissolved reagent 3 (according the amount of 2 spatula included in the test kit) in 50 ml of solution 1.'

Table 2: Reagent mixture for determination of total iron binding capacity
Pipette 1 ml of solution 2 into 5 ml of sample blank reagent.

	Sample(µl)	sample blank (µl)
Clear supernatant	100	100
Sample blank reagent	—	1000
Reaction mixture	1000	—

The contents were mixed and incubated at 37ºC for 5 minutes and absorbance was read at 578nm. Absorbance of the sample measured against sample blank.

Calculation;
TIBC was deduced using the formula below;
TIBC Concern = A (sample) - A (sample blank) × 570 µmol/L

Determination of Unsaturated iron binding capacity (UIBC);
UIBC was calculated using the formula below;

UIBC Concern = TIBC - serum iron in µmol/L
Determination of Transferrin (ST);
Transferrin was calculated using the formula below;

$$\text{Serum transferrin} = \frac{\text{serum Iron}}{\text{TIBC}} \times 100 \text{ in percentage \%}$$

RESULTS AND DISCUSSION

Table 3. 1: Serum iron, serum total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), and serum transferrin among malaria and control subject based on age.

Age (years)	No: 20	Serum iron (µmol/L)	TIBC (µmol/L)	UIBC (µmol/L)	Transferrin %
14 – 25 (Control)	4	8.3 ± 2.13[a]	76.67 ± 10.36[a]	68.54 ± 8.81[a]	10.17 ± 2.20[a]
14 – 25 (Infected)	2	5.97 ± 1.39[a]	71.54 ± 3.14[a]	65.57 ± 1.75[a]	8.28 ± 1.59[a]
26 – 35 (Control)	3	10.01 ± 3.88[a]	5.15 ± 2.48[a]	44.14 ± 5.34[a]	18.80 ± 7.22[a]
26 – 35 (Infected)	3	3.42 ± 0.09[b]	77.52 ±10.14[a]	74.10± 10.22[a]	4.60 ± 0.79[b]
36 – 45 (Control)	2	16.18 ± 2.71[a]	71.63 ± 14.63[a]	55.45± 17.34[a]	24.38 ± 8.76[a]
36 – 45 (Infected)	4	5.38 ± 1.48[a]	90.06± 18.31[a]	84.68± 18.10[a]	6.51 ± 2.30[b]
46 – 55 (Control)	1	6.53 ± 0.30[a]	59.28 ± 0.10[a]	52.75 ± 0.11[a]	11.02 ± 0.12[a]
46 – 55 (Infected)	1	9.17 ± 010[b]	74.67 ± 0.11[a]	65.50 ± 0.12[a]	12.28 ± 0.11[a]

Values are expressed as mean ± standard error of mean using GRAPHPAD PRISM version 9.
Values with the same superscript latter with controls are not significantly different (P > 0.05).
Values with the different superscript latter with controls are significantly different (P> 0.05)

Table 3.2: Serum iron, serum total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), and serum transferrin among malaria and control subject based on gender.

Subject malaria patient and controls	No: 10	Serum iron (µmol/L)	TIBC (µmol/L)	UIBC (µmol/L)	Transferrin **%**
Male (control)	6	6.86 ± 0.90[a]	70.11 ± 4.25[a]	62.45 ± 3.76[a]	9.72 ± 1.11[a]
Male (infected)	4	3.83 ± 1.14[b]	86.07 ±19.47[a]	83.09± 18.71[a]	4.33 ± 1.21[b]
Female (control)	4	3.34 ± 0.05[a]	86.93 ± 6.56[a]	83.60 ± 6.56[a]	3.84 ± 0.31[a]
Female (infected)	6	10.90 ± 1.72[b]	68.39 ± 5.99[a]	57.27 ± 6.18[a]	16.79 ± 3.20[b]

Values are expressed as mean ± standard error of mean using GRAPHPAD PRISM version 9.
Values with the same superscript latter with controls are not significantly different (P > 0.05).
Values with the different superscript latter with controls are significantly different (P> 0.05)

Table 3.3: Comparison of serum iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC) and serum transferrin between subject "malaria" and "control".

Subject Controls	No: 20	Serum iron (µmol/L)	TIBC (µmol/L)	UIBC (µmol/L)	Transferrin %
Control	10	5.29 ± 0.79^{a}	81.05 ± 7.63^{a}	75.76 ± 7.60^{a}	6.87 ± 1.16^{a}
Malaria	10	10.18 ± 1.71^{b}	67.16 ± 5.50^{a}	57.03 ± 5.53^{a}	15.69 ± 3.07^{b}

Values are expressed as mean ± standard error of mean using GRAPHPAD PRISM version 9.
Values with the same superscript latter with controls are not significantly different (P > 0.05).
Values with the different superscript latter with controls are significantly different (P> 0.05)

Table 4: Reference range of the parameters

parameters;	Serum iron (µmol/L)	TIBC (µmol/L)	UIBC (µmol/L)	Transferrin %
Ref. Range;	Men 11– 28 Women 6.6– 26	42.96 — 80.55	Men 12–43. Women 13– 56	10 — 40%

DISCUSSION

The Total Iron Binding Capacity (TIBC) in 65% of the subjects in the study area (as depicted in 3.1 table 1; age factor) fall within the normal range (42 - 80µmol/l). Although, the TBIC level of malaria infected subjects in the present study are found to be higher compared to the control subjects. The outcome of the present study resemble that of Gouado *et al.,* (2008) who reported increase level of TIBC in malaria infected in different ages among the study subjects. This can be related to the fact that most of the study subjects show a correlation between TIBC and transferrin is generally considered as good. However, low TBIC in 15% of the subjects in the present work is similar to the report of Gouado *et al.,* (2008). This may probably due to anemic factor, in which red blood cells RBCs are being destroyed quickly (hemolytic anemia) and that may imply a severe case of malaria. Whereas, an increase in TBIC in 20% of the subjects of this study resemble that of the report of Gouado *et al*., (2008) was observed, hence the high TIBC implies low iron and may be from the women of child bearing either pregnant or those that have used oral contraceptives prior, as all these have been reported to increase TIBC, however not covered in this study Gouado *et al*., (2008).

Gender sensitivity in terms of TIBC (as depicted in tables 3.2, and 3.3 respectively) indicated that male (40% of the infected subjects) have higher TIBC above the normal reference range (42 - 80µmol/l), whereas females (60% of the infected subjects) have normal TIBC values. Contrarily, the reverse is the case among the control subjects where females (40% of the control subjects) have higher than normal TIBC values and the males (60% of the control subjects) TIBC values fall within the normal range. This could be due to inherited genetics change which is commonly course hemochromatosis and it could be also due to timely menstruations by females, especially in pregnant women where there is relative increase in TIBC value.

The serum iron levels in 50% of the study subjects were below the reference range (6.6 - 28 µmol/l). This may be due to the redistribution of iron from circulation to tissues (stored in form of transferrin). This ensures a decreased level of serum iron and increased tissue transferrin levels. The unsaturated iron binding capacity, UIBC in 70% in the study cohort falls above the normal reference range (12 – 56 µmol/l). This is undoubtedly because unsaturated iron binding capacity is the summation of serum iron and that TIBC is higher than normal in 20% of the study subjects (Gouado *et al.,* 2008).

The serum iron levels in malaria infected (40%) as well as control males (60%) are below the normal range (11-28µmol/l), whereas it is normal for malaria infected females (60%). However, in control female subjects (40%), it is lower than normal reference range, perhaps due to the redistribution of minerals. Thus, it may be considered that serum iron in male is more likely to be affected. Although, further study is necessary to find out the exact mechanism of how malaria interact with iron (Gouado *et al.*, 2008).

The percentage transferrin levels in 45% of the study subjects were below the normal range (10 – 40%) This may be due to the body absorbing more transferrin than needed. And this has contradicted the findings of Keusch (1998) as reported by (Gouado *et al.*, 2008) in which he attributed the increased percentage transferrin to the increased synthesis and increased transferrin activity. However, 55% of the subjects in the study subject show % transferrin levels within the normal range. This can be attributed to the normal distribution of iron and minerals in plasma and tissues in normal state. The percentage transferrin among the infected males (40%) is lower than normal, whereas in infected female subjects (60%) is normal. This might be as results of insufficient transferrin made by the liver. Improper production by the liver is a consequence of being the major site for the malaria infection or over-excretion of transferrin by the kidney.

After a general analysis, the data depicted that, serum iron, TIBC and transferrin TF are deeply touched by malaria infection. Clinical symptoms upon malaria infection, such as fever, pulse acceleration, sweating and shivering are implicated in the display of dissimilarities between the malaria patients and controls. This is because malaria biochemically distorts normal iron metabolism as stated by Gouado *et al*., (2008). Likewise, in the course of an infection, parable, malaria, minerals are redistributed from circulation to tissues and cause a reduction of minerals in the circulation (Gouado *et al*., 2008). However, the low values of TIBC observed in this study may be attributed to the state of nutrition and the diet consumed among people of the region. Moreover, malaria, being a hemolytic anemia results in increased hemolysis of RBCs there by releasing their iron content of the hemoglobin, making the TIBC comparatively low Gouado *et al*., (2008). All these explain why TIBC in malaria infected subjects are remarkably below than in controls and vice versa for transferrin. TIBC increment noted here is portend that stored iron has been utilized. Moreover, it is an outline iron liberation in the plasma, and an increment in transferrin synthesis coupled with transferrin activity (Cheesebrough, 2006).

CONCLUSION AND RECOMMENDATION

This study has concluded that both serum iron and percentage transferrin are lower in malaria infected subjects, but normal in the control subjects. In the liver, a major avenue of malaria metabolism, poor churning out of transferrin or increment in transferrin clearance occur as a result of malaria status.

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