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Full Length Research Paper

Biochemical and haematological changes in pregnant malaria patients and pregnant non-malaria women

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Malaria has been known to alter hematological and biochemical parameters during pregnancy and is one of the causes of anemia in pregnancy. In a cross sectional study, changes in hematological and selected biochemical parameters were investigated in pregnant malarial patients (17) compared with 20 healthy pregnant non-malarial women (controls). Assay for transferrin (TF), total iron binding capacity (TIBC), serum iron, protein, cholesterol and triglyceride were evaluated on sera while hemoglobin concentration, total white blood cell count were carried out on whole blood using standard laboratory methods and enzyme colorimetric kits. The mean values of serum transferrin (4.31 ± 0.54 g/l), TIBC 112.45 ± 14.22 µmol/L serum iron 29.64 ± 2.78 µmol/L in pregnant malaria parasitaemic group were significantly ($p < 0.01$) higher than the respective values of 3.13 ± 0.45 g/l, 81.70 ± 11.70 µmol/L and 21.36 ± 2.41 µmol/L in the control. Total serum cholesterol (4.28 ± 1.1 mmol/L) and triglycerides (1.56 ± 0.14 mmol/L) were significantly ($p < 0.01$) elevated in the pregnant malarial parasitaemic group compared with the control values of 2.9 5 ± 0.71 and 1.17 ± 0.28 mmol/L, respectively. The white blood cell (WBC) count did not show any significant change and there were no changes in hemoglobin, transferrin saturation as well as serum protein in pregnant-malaria group compared with the control. The results suggest that malaria may aggravate the hypertransferinaemia and hyperlipidemia syndrome of human pregnancy.

Key words: Pregnancy, malaria, hematological indices, transferrin, cholesterol.

INTRODUCTION

The pregnancy state in women is highly susceptible to infection which in most instances may prove not only life threatening to the mothers but may also have profound impact on foetal outcome (Brabin, 1983). The risk of adverse pregnancy outcome including prematurity, intrauterine death, spontaneous abortion and stillbirth may increase during malaria attack (World Health Organization, 1993). Concern has been expressed as a result of most studies from sub-Saharan Africa, where approximately 25 million pregnant women are at risk of *Plasmodium falciparum* infection annually and one in four women have placental infection at birth (Desai et al., 2007). In South-east Nigeria, the rate of malaria and anemia were 215 and 327 per 1000 pregnant women while the incidence of anaemia due to malaria was 571 per 1000 pregnant women (Ekejindu et al., 2006). The management of malaria infection has become a major challenge to public health particularly in pregnancy and

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Abbreviations: TF, Transferrin total; **TIBC,** iron binding capacity; **WBC,** white blood cell; **LDL,** lowers low density lipoprotein; **HDL,** high density lipoprotein; **EDTA,** ethylene diamine tetra-acetic acid; **HB,** blood hemoglobin.

with the emergence of resistance to many antimalarial drugs. This is so because of the danger involved in the treatment of pregnant women with most anti-malarial drugs. Repeated infection, for people in malaria endemic regions impacts partial immunity to the disease. Unfortunately, pregnant women who lose some of this immunity during pregnancy are at risk of malaria (Gamain et al., 2007). At present, diagnosis of malaria is by microscopic counting of the number of parasites in the red blood cells of suspected patients. Quite often, however, a patient may show symptoms of malaria (chills, pyrexia, and splenomegally) but the results of laboratory test and investigation to confirm the diagnosis of clinical malaria may indicate absence of parasites in blood smear due to absence of current tools to detect malaria parasites especially in low parasitaemia (Nosten et al., 2007). This is true in instances of complicated malaria where the parasites become sequestered in deep tissues. There is need to employ some biochemical and hematological indices in laboratory diagnosis of malaria along side with the standard microscopy approach.

Several reports have indicated changes in hematological indices, serum transferrin and iron concentration in humans with subsequent hemolytic anemia as a common feature of malaria (Eteng, 2002; Aremu, 1989). Similarly, Mendez et al. (2001) have described increased serum transferrin levels and also of transferrin receptors associated with malaria parasitaemia. Transferrin not only plays a central role in iron metabolism as the principal carrier of iron in blood of all vertebrate species but also in defense mechanism of the body against infections (Underwood, 1977).

Malaria affects iron ferrokinetics, hemoglobin levels and serum proteins. This is true as iron released from haemoglobin of lysed red blood cells in the course of malaria attack is transported bound to transferrin, while the globin part of the hemoglobin degraded into amino acids for synthesis of tissue proteins. Alterations in several other parameters in malaria with parasitaemia have also been reported. Increase in cholesterol and triglycerides have been reported as indicators of the severity of malaria in clinical retrospective study in returned travelers Parola et al. (2004).

There are however no reports in literature in which changes in biochemical and hematological parameters have been compared in pregnant women with parasitaemic malaria and pregnant non-malaria women. This study therefore, compares the changes in hematological and selected biochemical parameters in pregnant individuals with parasitaemic malaria and pregnant subjects without malaria and also evaluated the impact of malaria on the biochemical and hematological indices of the pregnancy state.

PATIENTS AND METHODS

Subjects

The study took place in Calabar, a malaria endemic coastal town in the Southeastern part of Nigeria. A total of 37 pregnant women of different ethnic origins and socio-economic classes resident in Calabar and aged between 20 - 43 years were enlisted for this study. Those in this age bracket being adults possess a certain degree of immunity to malaria, hence; do not develop severe anaemia in the face of mild malaria peripheral parasitaemia which would have influenced the results (Gamain, 2007).

Selection of patients

The patients (17 pregnant women with malaria parasitaemia) were drawn from pregnant women attending antenatal clinic at the General Hospital, Calabar. Each patient specifically presented with clinical symptoms indicative of malaria namely; fever, headache, body weakness, and nausea, followed by the actual detection of *Plasmodium falciparum* parasites by microscopic examination of the Giemsa (pH 7.2) stained blood smear from finger pricks.

Furthermore, a Widal test was done to exclude those who had typhoid infection, since typhoid infection has similar symptoms with malaria. They had not also been on iron therapy or treatment. These patients were assigned into the pregnant malaria parasitaemic group. Patients who presented with clinical symptoms such as fever, headache, body weakness and nausea but without parasites obtained in blood smears were excluded. Also, pregnant women who presented with clinical malaria parasitaemia but who had been on iron therapy or haematinic drugs for the past of one month were omitted from this group.

Subject of control group

Twenty (20) healthy pregnant non-malaria women from across section of the university community who came to enroll for the first time in the antenatal clinic of the General Hospital Mary Slessor Way, Calabar comprised the control group. A pre-tested questionnaire was randomly administered and information on ages, residence and health status (absence or presence of parasitaemic malaria or other infections, presence or absence of iron or haematinic drug therapy) were obtained.

Subjects who were healthy as defined by absence of malaria or other infections, those not on haematinic drugs or iron therapy and those within the age range of study were recruited into the control group, and actual microscopic screening done on them revealed absence of *P. falciparum*. For ethical reasons, consents of the subjects were obtained after the purpose of the research had been communicated to each of them before samples were collected. Sampling lasted for seven (7) weeks during the wet season between the hours of 7.00 and 9.00h GMT to minimize diurnal variability of blood iron Laurell (1953).

Collection of blood samples and assays

Approximately 8 ml each of venous blood samples were collected by vein puncture from the antecubital vein of the forearm of each patient and subject with a sterile needle and syringe and immediately divided into two approximately equal portions. One half was placed in a screw cap plastic bottle which has been acid- washed and rinsed with double distilled deionized water to render it iron free, and allowed to clot at ambient temperature for between one to two hours. Serum was obtained after centrifuging at 10,000 g for 5 min using an M.S.E. tabletop centrifuge by decanting from the clot into iron free screw cap centrifuge tubes. The remaining half was placed in an ethylene diamine tetra-acetic acid (EDTA) bottle and gently mixed to prevent clotting. This was used for the determination of blood hemoglobin (HB) and white blood cell count (WBC). All glass ware used for assays were similarly acid-washed and

Table 1. Hematological indices in healthy pregnant non-malaria subjects and pregnant women with *Plasmodium falciparum* malaria parasitaemia.

Parameter	Pregnant non-malaria subject (20)	Pregnant malaria patient (17)
Age (years, mean ± SD)	30.50 ± 3.42	29.80 ± 6.80
Range (years)	19 - 42	20 - 43
Gestational age (weeks mean ± SD)	13.5 ± 0.85	13.2 ± 0.74
Range (weeks)	12 -15	12 - 14
Transferrin (g/L)	3.13 ± 0.45	4.31 ± 0.54**
TIBC (µmol/L)	81.70 ± 11.70	112.45 ± 14.23**
Serum Iron (µmol/L)	21.36 ± 2.41	29.64 ± 2.78**
Transferrin saturation (%)	26.30 ± 6.30	25.80 ± 3.40
Hemoglobin (g/100 ml)	11.60 ± 1.40	11.90 ± 1.30
WBC-Tc ($\times 10^9$/L)	6.10 ± 0.60	6.80 ± 1.90

TIBC= Total iron binding transferrin capacity, WBC= White blood cell (total count)
Values are mean ± SD, ** P<0.01 compared significantly with the control group (Pregnant non-malaria subjects).

rinsed with double deionized water to minimized contamination with iron.

Determination of biochemical and hematological parameters

Serum transferrin, serum iron, serum protein, triacyglycerol and total cholesterol assays were done on each sample within 24 h of sample collection. Serum transferrin was determined indirectly as total iron binding capacity (TIBC) using the magnesium trioxocarbonate IV ($MgCO_3$) method in combination with the international committee for standardization in hematology (ICSH, 1978). The value of TIBC in µg/100 ml serum was divided by a factor 1.45 to obtain the level of transferrin in mg/100 ml of serum Aremu (1989) which was then expressed in international units (g/L) serum iron was determined by ICSH (1978) method. In brief, iron was separated from transferrin using hydrochloric acid (HCl).

The protein was then precipitated with trichloroacetic acid. Ferric ions were reduced to the ferrous state with mecaptoethanol and a bathophenanthroline colour reagent was then added. The purple or pink colour proportional to iron present was measured in a spectrophotometer against reagent blank at 450 nm. Serum protein was estimated by the method of Dogmas et al. (1975). Serum total cholesterol and tricylglycerol were estimated by use of enzyme colorimetric kit (Randox Laboratories, Ltd., Admore Antrim, United Kingdom). White blood cell count was determined by Dacie and Lewis (1975). Blood hemoglobin by cyanomethaemoglobin method of Crosby et al. (1954) in which the ferricyanide in drabkins reagent oxidizes hemoglobin to a non-toxic methemoglobin (cyanomethaemoglobin), whose transmittance is read at 540 nm and the concentration in gramme hemoglobin per 100 ml of blood read from the table developed by Pla and Fritz (1971) for conversion of percentage transmittance (T) to gramme hemoglobin per 100 ml of blood

Statistical analysis

Values were expressed as mean ± standard deviation and differences between controls and test group were analyzed using unpaired students t- test with SPSS statistics software (version 10). Probability value of $p < 0.05$ was accepted as the level of significance.

RESULTS

A total of 37 pregnant human sera were assayed for hematological indices, transferrin, TIBC, serum iron, hemoglobin, total white blood cell count and biochemical parameters (serum protein, total serum cholesterol and triglycerides). Microscopic screening revealed the presence of *P. falciparum* only in the sera of the 17 individuals who constituted the pregnant malarial group while the remaining 20 were the healthy pregnant non-malarial control group.

Hematological and biochemical parameters

Table 1, shows the mean value of hematological indices in pregnant malarial patients and healthy pregnant non-malarial (control) group. From the data obtained, serum transferrin (g/L), TIBC (µmol/L) and serum iron (µmol/L), values in the pregnant malarial group were 4.13 ± 0.54, 112.45 ± 14.22 and 29.64 ± 2.78, respectively. The corresponding control (pregnant non-malarial group) values were 3.13 ± 0.45, 81.70 ± 11.70 and 21.36 ± 2.41, respectively.

There were statistically significant ($p < 0.01$) increases in serum transferrin, TIBC and serum iron concentration in the pregnant malaria group compared with control (pregnant non-malaria group). There was no significant change in transferrin saturation and hemoglobin but white blood cell count increased slightly in pregnant malaria patients (6.80 ± 1.90) compared with the control (6.10 ± 0.60).

The values of serum protein, total cholesterol and triglycerides are shown in Table 2. There was a significant elevation ($p < 0.01$) in total serum cholesterol and triglycerides in the pregnant malarial group compared with control. No changes in serum protein were observed in the experimental group compared with the control.

Table 2. Mean ± SD values of serum protein, total serum cholesterol and triglycerides in pregnant malaria patients and pregnant patients without malaria.

Parameter	Pregnant non-malaria subject (20)	Pregnant malaria patient (17)
Gestational age (weeks)	12 - 15	12 - 14
Age weeks (range; mean ± SD)	(13.50 ± 0.85)	(13.20 ± 0.74)
Serum protein (g/100ml)	6.50 ± 1.00	6.20 ± 0.60
Total serum cholesterol (mmol/L)	2.95 ± 0.71	4.28 ±1.16**
Triglycerides (mmol/L)	1.17 ± 0.28	1.56 ± 0.41**

Values are mean ± SD; ** $p < 0.01$ compared with the control group.

DISCUSSION

The mean serum iron values (21.36 ± 2.41 µmol/L) in the control non-malarial group (Table 1), falls within the range of 14 - 36 µmol/L reported by Eastham (1972). Also, mean serum transferrin (3.13 ± 0.45g/L) is within the transferrin concentrations of 2.50 - 4.00 g/L reported in the plasma of normal subject by Tavil and Monton (1978). The value of serum transferrin for the malarial group is somewhat above the upper limit of 4.00 g/L given by Tavil and Monton, (1978) but that of serum iron falls within the normal range of 14 - 36 µmol/L reported by Eastham (1972). The data taken together suggest that the levels of serum transferrin and TIBC are on the average raised in pregnant malaria subject compared with pregnant non-malarial subjects. The rise in these iron indices is attributed to the fact that 85% heme iron liberated during parasitaemia induced haemolysis of red blood cell enters plasma causing elevation in serum iron Sussman (1974). Hemolytic anemia stimulates transferrin synthesis hence, the rise in serum transferrin and TIBC, which is needed to mobilize extra iron.

Pregnancy and malaria have opposing effect on serum iron. Pregnancy particularly towards the third trimester lowers the serum iron (Sussman, 19740) while in malaria serum iron is raised (Aremu, 1989 and Mendez et al. 2001). Thus, the low serum levels caused by pregnancy are raised by malaria infection. In spite of the raised serum iron, transferrin saturation did not show any significant changes, so also, was the blood hemoglobin. In normal pregnancy without infection, serum transferrin and TIBC are raised while serum iron is decreased. It therefore, appears that malaria infection amplifies the elevation in transferrin and TIBC occasioned by pregnancy but opposes the impact of pregnancy on serum iron as the latter is elevated.

Anemia in pregnancy is a major health problem in many developing countries where malaria and other infections contribute to increased maternal and prenatal mortality and morbidity Mahomed (2006). This iron deficiency is due to increased demand for iron to meet up with needs of the developing foetus. Upon check up, clinicians always correct this condition with haematinic drugs. In our research design, those who had been on iron therapy for the past one month were excluded. It would have been expected that anemia associated with pregnancy state would have been worsened by malaria in pregnancy since malaria is known to induce anemia (Ekejindu 2006). This was not the case from the data of the present study. It is probable that the haemolysis due to the parasites raised the hemoglobin concentration and therefore masked the absence of anemia.

Hyperlipidemia is a well known condition in human pregnancy though there are various underlying mechanisms (Suga et al., 1998; Butte, 2000). The pregnancy hormone, estrogen lowers low density lipoprotein (LDL), total serum cholesterol, but elevates high density lipoprotein (HDL) cholesterol. However, serum cholesterol and triacylglycerol are raised. This is in agreement with a previous report (Parola et al., 2004). Cholesterol is not a metabolic fuel and therefore, it is not stored, hence, its level in plasma is raised but for triglycerides (TG), it is under control by lipoprotein lipase enzymes and hormone sensitive TG lipase which are under endocrine control. Thus, it has good cholesterol effect. The picture appears to be different in the presence of malaria infection as serum cholesterol was raised. It is however, well known that serum cholesterol and triglyceride levels are increased during the second and third trimester as a result of changes in sex hormones, genetic and environ-mental factors. The present study has found serum cholesterol to be increased in the first trimester of pregnancy, whereas, other authors have identified this increase in the second and third trimester. The probable reason may be that since majority of our patients studied were in the last week of the first trimester, endocrine changes may not be different with those of the early phase of the second trimester. The severity of the malaria infection may also be another contributory factor. Thirdly, patients may have been on cholesterol rich diets and some patients may be obesed or over weight. These factors taken together may explain the rise in cholesterol levels in the first trimester observed in the present study. Malaria may worsen the lipid status in pregnancy.

The present study is limited to the assay of hemato-logical and biochemical parameters in the first trimester of pregnancy. We do not know what the changes in these

parameters may be in the second or third trimester with the subjects and patients of the present study. There were no changes in percentage transferrin saturation, hemoglobin concentration and serum proteins but the increase in some iron indices of hematological status (serum transferrin, TIBC, serum iron) and selected bio-chemical parameters (total cholesterol and triglyceride) is significant, as it could provide a fundamental framework for further research on the possible use of these indices in differential diagnosis of malaria thereby, justifying the need to employ biochemical and hematological indices in laboratory diagnosis of malaria along side standard microscopy approach.

Conclusion

The results of the study suggest that malaria may aggra-vate the hyper transferrinnaemia and hyperlipi-demia associated with pregnancy but not the anemia of human pregnancy. Further investigation in this regard and in cases of resurgent malaria in the 2nd and 3rd trimesters of patients and subjects is needed.

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