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Research Article

Effects of Malaria Infection on some Haematological and Biochemical Parameters in the General Population and Pregnant Malaria Patients Attending Two District Hospitals in Niger State, Nigeria

Abstract

The effects of malaria on some haematological and biochemical parameters among males, pregnant and non-pregnant female patients attending district hospitals in Bida and Kagara areas of Niger State, Nigeria was investigated. The subjects were volunteers confirmed to be positive for malaria and without other clinical symptoms. A total of 231 patients (103 males and 128 females) within the age range of 1-50 years were studied. Fifty (20 males and 30 females) apparently healthy individuals in the similar age bracket were used as controls. Haematological and biochemical parameters were analyzed using standard methods. Overall, the reduction in haemoglobin concentration and packed cell volume (PCV) in patients were significantly higher/lower from non-infected patients ($p>0.05$), while there was no significant differences observed in the male patients. The mean cell haemoglobin concentration (MCHC) (36.03 ± 1.29g/dl) in pregnant subjects was significantly higher ($p<0.05$) compared to the non-infected pregnant control group (32.65 ± 0.46g/dl). There was a significant reduction in neutrophils but increase in lymphocytes in all subjects irrespective of sex. Non-significant ($p>0.05$) increase existed in serum lipid levels among pregnant and non-pregnant women. When males and females were compared, serum lipids except triglycerides varied significantly. There were apparently no variations in glucose levels between pregnant and non-pregnant subjects, whereas males had significantly higher values. Some of these parameters can thus be useful tools in the monitoring and management of malaria.

Introduction

Malaria has been a major human health problem that threatens the lives of about 40% of the World's population [1], causing morbidity and mortality worldwide [2]. It is endemic in 100 countries making about half of the world's population to be at risk [3]. About 50% of Nigerian population is reported to suffer from at least one episode of malaria each year [4]. Nigeria has been reported to have the greatest burden of the disease among the endemic countries in the world, Kyu et al., (2013). The tropical climate of Nigeria accounts for the high prevalence of the disease, only south of Jos in Plateau State, Nigeria has lower incidence of malaria due to the low temperature of the area [5]. Environmental and daily fluctuation of temperature is said to be associated with the infection rate and malaria parasite development [6]. Malaria affects both sexes and all ages. It is reported to be a common cause of miscarriage in pregnant women, premature delivery, low birth weight, maternal anemia, intrauterine growth retardation and intrauterine death [7]. Diagnosis of malaria in pregnancy using blood film detection technique becomes difficult when the parasite sequestrates and replicates in the placenta and therefore may not be found in the film [8]. Placenta malaria parasites can cross placenta wall either during pregnancy or at birth resulting into vertical transmission to the baby [9]. Malaria has resulted to about 11% of maternal and 30% of childhood mortality in Nigeria [10,11].

Haematological and biochemical parameters are used as indices to monitor the severity of malaria [4,12,13]. The degree of changes in haematological and biochemical parameters depend on the level of parasitemia, nutritional status [14], malaria immunity [15] and the endemicity of the disease [16]. This study thus seeks to investigate the effects malaria

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infection will have on some haematological and biochemical parameters among males, pregnant and non-pregnant female patients attending district hospitals in Bida and Kagara areas of Niger State, Nigeria.

Materials and Methodology

Subjects

The subjects for this study were patients attending two district hospitals in Bida and Kagara, Niger State, Nigeria. The control groups comprised of apparently healthy individuals who tested negative to malaria.

Study population

A total of 231 Subjects consisting of 181 test samples and 50 control samples were used.

Ethical approval

Ethical approvals were obtained from each of the management of the two district hospitals before commencement of the work and the patients' consents were sought.

Sample collection and preparation

Four (4) milliliters of blood was collected and dispensed into serum separating tube (SST) while 2ml was dispensed into an Ethylene Diamine Tetra-Acetate Acid (EDTA) tube and mixed appropriately. Serum was separated from the clotted sample into serum tube and stored frozen until ready for analysis. Biochemical analysis was done within 72 hours of sample collection. Haematological parameters were done the same day as sample collection.

Haematological analysis

Haemoglobin was determined using cyanomethemoglobin reaction, haematocrit by centrifuging with microhaematocrit centrifuge in capillary tube; total white blood cell count was determined with turk's solution using Neubauer counting chamber, mean cell haemoglobin concentration determined by calculation and differential white blood cell count was done using peripheral blood smear. All methods were described by Ochei and Kolhatkar (2000).

Biochemical analysis

Serum Urea, Total Protein, Albumin, Total and Direct Bilirubin, Alkaline Phosphatase, Serum Cholesterol, Serum High Density Lipoprotein and Serum Triglycerides were determined using commercial diagnostic reagent kits from Randox Laboratories Limited, United Kingdom. Serum Sodium, Serum Chloride and Serum Creatinine were determined using commercial diagnostic reagent kits from TECO Diagnostics, U.S.A. Reflotron Strips used with Reflotron Plus chemistry analyzer from Roche Diagnostics GmbH, Germany were used for analysis of Serum Alanine Aminotransferase (ALT), Serum Aspartate Aminotransferase (AST), Serum Potassium and Serum Creatinine respectively.

Statistical analysis

Results were analyzed with statistical package for social sciences version 19.0 using one way analysis of variance (ANOVA). Duncan's test was used to compare the means at 5% probability level.

Results and Discussion

Results

Haematocrit, neutrophils and eosinophils were significantly ($p<0.05$) reduced in the general population of malaria patients as shown in table 1 while reduction in haemoglobin and monocytes were not significant ($p>0.05$). Mean Cell Haemoglobin Concentration (MCHC) and the Total White Blood Cell Count (TWBC) were however elevated non-significantly ($p>0.05$). Whereas increase in lymphocytes and basophils were significant ($p<0.05$) as compared to the controls. Renal function parameters (urea, creatinine, sodium, potassium and chloride) were reduced non-significantly in malaria patients except chloride reduction that was significant ($p<0.05$) as indicated in table 2. The liver function parameters in patients as shown in table 3 were non-significantly ($p>0.05$) elevated in the activity of Aspartate Aminotransferase (AST), but significant ($p<0.05$) increase in Alkaline Phosphatase (ALP) activity and

Table 1: Haematological Parameters in the General Population of Malaria Patients.

Parameters	Control	Patient
Haematocrit (PCV) (%)	38.76 ± 0.52^{b}	34.47 ± 0.42^{c}
Haemoglobin (g/dl)	12.61 ± 0.27^{b}	11.70 ± 0.16^{b}
MCHC (g/dl)	32.63 ± 0.63^{b}	34.06 ± 0.31^{b}
Total WBC ($X10^{9}$/l)	6.28 ± 0.19^{d}	6.47 ± 0.12^{d}
Neutrophil (%)	63.40 ± 0.55^{b}	59.89 ± 0.54^{c}
Lymphocytes (%)	32.00 ± 0.54^{b}	36.25 ± 0.54^{c}
Monocytes (%)	3.96 ± 0.19^{b}	3.42 ± 0.09^{b}
Eosinophils (%)	0.62 ± 0.10^{b}	0.34 ± 0.04^{c}
Basophils (%)	0.02 ± 0.02^{b}	0.06 ± 0.02^{c}

Values are mean ± standard error of mean (SEM).
Test values with the same superscript as control are not significantly different ($p>0.05$).

Table 2: Renal Function Parameters in the General Population of Malaria Patients.

Parameters	Control	Patient
Urea (mmol/l)	3.43 ± 0.22^{b}	3.23 ± 0.22^{b}
Creatinine (mg/dl)	0.73 ± 0.02^{b}	0.68 ± 0.01^{b}
Sodium (mmol/l)	139.97 ± 4.80^{c}	124.39 ± 2.18^{c}
Potassium (mmol/l)	4.29 ± 0.14^{b}	3.98 ± 0.05^{b}
Chloride (mmol/l)	95.16 ± 2.05^{b}	84.66 ± 1.95^{c}

Values are mean ± standard error of mean (SEM).
Test values with the same superscript as control are not significantly different ($p>0.05$).

Table 3: Liver Function Parameters in the General Population of Malaria Patients.

Parameters	Control	Patient
AST (U/l)	18.72 ± 1.12^{b}	19.77 ± 0.54^{b}
ALT (U/l)	18.01 ± 1.34^{b}	16.60 ± 0.62^{b}
Alkaline Phosphatase (U/l)	187.24 ± 11.03^{c}	196.40 ± 8.46^{d}
Total Protein (g/dl)	4.31 ± 0.36^{b}	5.04 ± 0.20^{b}
Albumin (g/dl)	4.29 ± 0.17^{b}	3.69 ± 0.11^{b}
Total Bilirubin (mg/dl)	0.60 ± 0.10^{b}	0.42 ± 0.01^{c}
Direct Bilirubin (mg/dl)	0.13 ± 0.01^{b}	0.13 ± 0.01^{b}

Values are mean ± standard error of mean (SEM).
Test values with the same superscript as control are not significantly different ($p>0.05$).

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non-significant reduction in Alanine Aminotransferase (ALT) activity were obtained. Non-significant increase in total protein was obtained while albumin indicated non-significant decrease. There was significant ($p<0.05$) reduction in total bilirubin, but not in direct bilirubin levels. Table 4 shows glucose and lipid profile in which cholesterol and low density lipoprotein (LDL) were significantly ($p<0.05$) increased whereas increase in high density lipoprotein (HDL) was non-significant and triglycerides showed a non-significant ($p>0.05$) decrease. Reduction in blood glucose level in patients was not significant.

Haematological parameters of pregnant and non-pregnant malaria patients are shown in table 5 while biochemical parameters are shown in tables 6,7 and figure 1. Haematocrit values were significantly ($p<0.05$) decreased in both pregnant and non-pregnant patients. A non-significant ($p>0.05$) reduction in the haemoglobin of non-pregnant patients was observed while in pregnant patients, there was a non-significant ($p>0.05$) increase. MCHC was significantly ($p<0.05$) elevated in both pregnant and non-pregnant patients as compared to the controls. Non-significant increase in the values of total white blood cell counts, lymphocytes, eosinophils and basophils was observed in both patients as compared to the controls while neutrophils were decreased significantly ($p<0.05$).

Table 4: Serum Glucose and Lipid Levels in the General Population of Malaria Patients.

Parameters	Control	Patient
Glucose (mmol/l)	5.23 ± 0.12^{c}	5.01 ± 0.06^{c}
Cholesterol (mmol/l)	2.24 ± 0.10^{b}	2.59 ± 0.07^{c}
HDL (mmol/l)	0.24 ± 0.01^{b}	0.26 ± 0.01^{b}
LDL (mmol/l)	1.56 ± 0.09^{b}	1.91 ± 0.07^{c}
Triglycerides (mmol/l)	0.95 ± 0.09^{b}	0.93 ± 0.04^{b}

Values are mean ± standard error of mean (SEM).
Test values with the same superscript as control are not significantly different ($p>0.05$).

Significant ($p<0.05$) variations existed in potassium, chloride and creatinine in pregnant and non-pregnant patients (Table 6) except creatinine in pregnant patients that showed non-significant ($p>0.05$) reduction. Non-significant decrease in both patients' sodium level and increase in pregnant patient urea level were observed.

The liver function parameters in pregnant and non pregnant patients (Table 7) showed ALT activity, direct bilirubin and albumin levels to be non-significantly ($p>0.05$) reduced as compared to the controls whereas alkaline phosphatase and total protein were increased non-significantly ($p>0.05$). There was significant ($p<0.05$) elevation in AST activity in both patients but the elevation was not significant between the patients.

Serum glucose was reduced non-significantly ($p>0.05$) in both patients (Figure 1). Cholesterol and LDL were elevated non-significantly in pregnant subjects but the elevation was significant ($p<0.05$) in non-pregnant ones. HDL level in pregnant patients showed non-significant increase while in non-pregnant ones, a significant ($p<0.05$) reduction was observed. Triglyceride levels were significantly reduced in both patients.

Discussion

Malarial infection causes changes in haematological and biochemical parameters of affected individuals to varying degrees resulting in changes in body physiology [4,17]. Haemoglobin is the major biomolecule that is metabolized by the parasite. Reduction in haemoglobin concentration in this study was not however significant, probably because infection was not severe. The depreciation of haematocrit levels than haemoglobin shows that the red blood cell invasion

Table 5: Haematological Parameters in Pregnant and Non-Pregnant Malaria Patients.

	Parameters	Haematocrit (PCV) (%)	Haemoglobin (g/dl)	MCHC (g/dl)	Total WBC (X10^{9}/l)	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
Controls	Pregnant	35.50 ± 0.96^{bc}	11.56 ± 0.24^{b}	32.65 ± 0.46^{b}	6.26 ± 0.47^{b}	64.20 ± 1.44^{c}	31.80 ± 1.42^{b}	3.70 ± 0.30^{bc}	0.30 ± 0.15^{b}	0.00 ± 0.00^{b}
	Non-Pregnant	38.40 ± 1.17^{c}	12.18 ± 0.48^{b}	31.68 ± 0.59^{b}	6.22 ± 0.41^{b}	64.30 ± 1.17^{c}	31.00 ± 1.34^{b}	4.40 ± 0.42^{c}	0.30 ± 0.15^{b}	0.00 ± 0.00^{b}
Patients	Pregnant	32.36 ± 1.09^{b}	11.57 ± 0.37^{b}	36.30 ± 1.29^{c}	6.92 ± 0.37^{b}	58.68 ± 1.73^{b}	37.86 ± 1.58^{c}	3.09 ± 0.24^{b}	0.31 ± 0.13^{b}	0.04 ± 0.04^{b}
	Non-Pregnant	34.79 ± 0.72^{bc}	11.49 ± 0.29^{b}	33.05 ± 0.57^{b}	6.44 ± 0.19^{b}	59.38 ± 0.86^{b}	37.09 ± 0.86^{c}	3.12 ± 0.15^{b}	0.35 ± 0.09^{b}	0.03 ± 0.02^{b}

Values are mean ± standard error of mean (SEM).
Values with the same superscript are not significantly different ($p>0.05$)

Table 6: Renal Function Parameters in Pregnant and Non-Pregnant Malaria Patients.

	Parameters	Urea (mmol/l)	Creatinine (mg/dl)	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)
Controls	Pregnant	2.46 ± 0.41^{b}	0.71 ± 0.03^{b}	134.82 ± 18.28^{b}	4.27 ± 0.18^{c}	97.10 ± 3.94^{c}
	Non-Pregnant	3.03 ± 0.34^{b}	0.87 ± 0.05^{c}	129.00 ± 7.90^{b}	3.69 ± 0.49^{b}	96.73 ± 3.14^{c}
patients	Pregnant	2.59 ± 0.39^{b}	0.60 ± 0.03^{b}	117.67 ± 5.14^{b}	3.94 ± 0.11^{bc}	73.97 ± 5.50^{b}
	Non-Pregnant	3.03 ± 0.32^{b}	0.69 ± 0.02^{b}	120.46 ± 3.74^{b}	4.04 ± 0.08^{bc}	82.69 ± 3.79^{bc}

Values are mean ± standard error of mean (SEM).
Test values with the same superscript as control are not significantly different ($p>0.05$).

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Table 7: Liver Function Parameters in Pregnant and Non-Pregnant Malaria Patients.

	Parameters	AST (U/l)	ALT (U/l)	Alkaline Phosphatase (U/l)	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Controls	Pregnant	12.77 ± 1.96^{b}	15.16 ± 2.86^{b}	147.38 ± 24.34^{b}	4.68 ± 0.87^{b}	4.25 ± 0.48^{bc}	0.84 ± 0.34^{c}	0.15 ± 0.03^{b}
	Non-Pregnant	13.21 ± 2.36^{b}	14.95 ± 2.78^{b}	232.39 ± 22.27^{b}	4.68 ± 0.97^{b}	4.66 ± 0.32^{c}	0.41 ± 0.04^{b}	0.12 ± 0.02^{b}
Patients	Pregnant	21.47 ± 1.66^{c}	14.76 ± 1.13^{b}	177.14 ± 31.58^{b}	5.08 ± 0.40^{b}	3.43 ± 0.24^{b}	0.33 ± 0.05^{b}	0.14 ± 0.02^{b}
	Non-Pregnant	19.21 ± 0.97^{c}	17.59 ± 1.46^{b}	184.87 ± 13.50^{b}	5.42 ± 0.36^{b}	3.42 ± 0.22^{b}	0.41 ± 0.03^{b}	0.11 ± 0.01^{b}

Values are mean ± standard error of mean (SEM).
Test values with the same superscript as control are not significantly different (p>0.05).

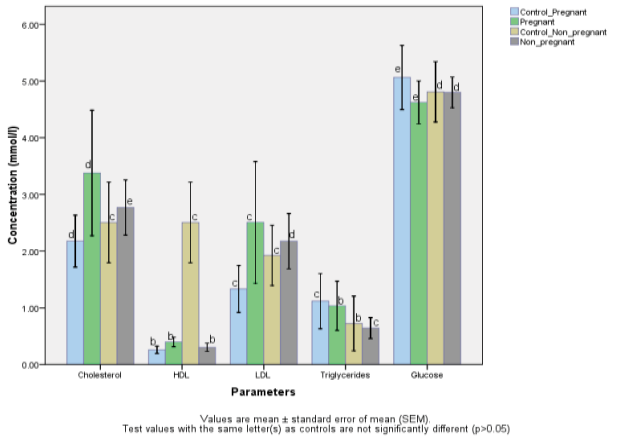

Figure 1: Serum Glucose and Lipid Levels in Pregnant and Non-Pregnant Malaria Patients.

and rupture is more affected than haemoglobin consumption as indicated by increase in the level of MCHC. This trend is in agreement with that reported by Kotepui et al., 2015 and Kotepui *et al.*, 2014 [18,19]. Since MCHC is an anaemia index, its significant elevation in pregnant patients implies that red blood cell concentration is greatly affected. This can be attributed to both malaria and reduced iron and folate levels during pregnancy [20], which are inimical to erythropoiesis. During the intraerythrocytic stage, the infection leads to the rupture of red blood cells [21], leading to the reduction in the red cell number and thus affects the haematocrit value. Effect of malaria infection on haemoglobin and red blood cells can cause reduction in the amount of oxygen carried to the body tissues and amount of carbondioxide removed as the number of haemoglobin and red blood cells are reduced. This can result into fatigue, causing muscle pain and convulsions. In the pregnant and non-pregnant subject the increased levels of eosinophils has shown response to parasitic infection [22] and the lymphocytes has shown an indication for immune response. Electrolytes are substances that ionize in solution and are involved in electric signal conductance and osmotic balance [23]. Alterations from the normal levels of these ions causes disturbance to body cellular function. Some electrolytes such as sodium and chloride are lost in the sweat. Reduction in plasma electrolytes is due to renal and extrarenal losses [24]. Sweating elicited by malaria infection due to fever and urinary loss may account for the decrease in the levels of electrolytes. The decrease in electrolyte, urea and creatinine levels found in malaria patients in this study was in consistence with the study of Sharma et al. [25], who reported the same trend with the exception of urea that was elevated. In normal pregnancy, there is a reduced level of plasma electrolytes which may be due to increased plasma levels of aldosterone and other mineralocorticoids [26]. Malaria infection causes increase in membrane porosity of the red blood cells which result in the influx of nutrients into the cell that are used for parasite growth. This can further affect plasma levels of the electrolytes. These two phenomena can contribute to the reduction in plasma electrolytes in pregnant malaria subjects which is shown by the lower levels of electrolyte reduction in pregnant subjects than in the non-pregnant ones.

Clarence and Samuel (2014) reported elevated activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and reduced levels of albumin. Whereas in this study, the activities of AST, alkaline phosphatase (ALP) and total protein were elevated in general population of malaria patients with a reduction in ALT, albumin and total bilirubin. Increase in permeability of the membrane in malaria infection [27] may be attributed to the increase in levels of AST in the patients. These combined effects may be the cause of the different degrees of alterations in the biochemical parameters.

Glucose utilization and physiological stress induced by malaria parasite may account for the reduction of glucose in the patients. The general decrease in blood glucose level in malaria patients was also reported by Esan (2015). During pregnancy, the foetus relies on the mother for nutritional need. These combined effects can lead to more reduction of glucose level in pregnant women than in non-pregnant subjects. Mohapatra et al. [28], reported that hypoglycemia was observed in pregnant women in their study. The use of cholesterol and phospholipids by malaria parasites was hypothesized and was thought to result in a decrease in the level of serum HDL. Changes in serum lipid profile during malaria infection and the underlying biological mechanisms remain unclear [29]. Red blood cell (RBC) membrane is composed of a complex mixture of lipids and proteins with cholesterol and distinct phospholipids making the core of the membrane which are arranged in a lipid bilayer [30]. With these phenomena, the rupture of red blood cells during malaria infection can contribute to the elevation of plasma lipid levels. Close monitoring of the elevated levels of lipid and nutritional guide should therefore be of concern so as to prevent the affected patient from developing other infections secondary to malaria.

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Appropriate measures to restore the normal physiological state of the patients are therefore needed to be taken to ensure restoration of normal body function. However, caution must be taken in the administration of treatment drugs. This could include other laboratory investigations such as 24 hour urine, as conclusion cannot be made on only serum parameters [31].

Conclusion

Malaria infection has affected haematological and biochemical parameters to varying degrees. The use of these parameters can be indices for monitoring the extent of infection and providing appropriate solution to the affected patients during management.

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