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Alterations in some Haematological Parameters, Ascorbic Acid and Pantothenic Acid levels among Pregnant Women with Plasmodium parasitaemia in Sokoto, Nigeria

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Abstract

Malaria in pregnancy is a major contributor to adverse maternal and prenatal outcome. In hyper endemic areas like ours, it is a common cause of anaemia and the haematological abnormalities. This case-control study evaluated the full blood count, ascorbic acid and pantothenic acid level of sixty (60) malaria parasitized pregnant women. Participants were recruited from Antenatal Clinic of Specialist Hospital Sokoto. A structured interviewer-administered questionnaire was used to obtain some socio-demographic characteristic and blood samples were collected and examine for malaria parasite, some haematological parameters (HCT, HGB, WBC, RBC and platelet count) were measured using the HA6000 auto-haematology analyzer (Perlong Medical Equipment Company). Ascorbic acid levels were assayed by a standard chemical method and pantothenic acid levels was analyzed using the ID-VIT pantothenic acid ELISA kit. Malaria parasitaemia and parasite load was confirmed by the examination of a Giemsa-stained thin blood film. Data generated was analyzed using SPSS 22.0 statistical package. A p-value $\leq$ 0.05 was considered significant in all statistical comparisons. The HCT, HGB RBC and Platelet counts of the parasitized subjects were significantly lower compared with the controls ($p<0.05$). Also, the ascorbic acid level of parasitized subjects was significantly lower compared to that of controls. There was a strong correlation between ascorbic acid and platelet count ($r= 0.413$ and $p= 0.00$). The mean parasite count among the parasitized subjects was $5.93 \pm 2.72 \times 10^3/\mu L$. Findings of this study has shown that malaria in pregnancy causes decrease in vitamin C and some of the haematological parameters. There is need to routinely monitor the vitamin C and full blood count should among parasitized pregnant women. We advocate that vitamin C and pantothenic acid be included in the guidelines of multivitamin supplementation particularly among plasmodium parasitized pregnant women.

Introduction

An estimated 30 million women living in malaria endemic areas of Africa become pregnant each year. Pregnant women are particularly vulnerable to malaria because pregnancy reduces immunity to malaria. Malaria infection increases susceptibility to severe anaemia and increased the risk of death among pregnant women. Malaria in pregnancy results in adverse pregnancy outcomes, such as spontaneous abortion, neonatal death, and low birth weight. Chronic anaemia, due to malaria may also affect a child's growth and intellectual development (WHO, 2006). It is estimated that at least 1 million people die of malaria particularly pregnant women and children less than 5 years of age, more than 80% of the deaths worldwide occur in sub-Saharan Africa (WHO Malaria Report, 2005).

Ascorbic acid is a natural water-soluble vitamin (Vitamin C). Ascorbic acid is a potent reducing and antioxidant agent that functions in fighting bacterial infections, in detoxifying reactions, and in the formation of collagen in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries. Found in citrus and other fruits, and in vegetables. Vitamin C cannot be produced or stored by humans and must be obtained in the diet (Hiten and Paula, 2011). Deficiency of Vitamin C may affect immune system as well as

causing anaemia which are involved in resistance to malaria.

Vitamin B5 is also known as pantothenic acid, or Pantothenate. It is a water-soluble B-complex vitamin (Horvath and Vecsei, 2009). It was discovered in 1931 by chemist Roger J. Williams (1893-1988) during his studies on the vitamin B complex (Kumar *et al.,* 2007). Vitamin B5 has many important functions including; converting food into glucose, synthesizing cholesterol, forming sex and stress-related hormones and forming red blood cells. Deficiency of Vitamin B5 deficiency is extremely rare as it is commonly found in nearly all foods.

Full blood count (FBC) is also known as Complete Blood Count (CBC). It is one of the most common laboratory tests performed today. It gives information about the production of all blood cells and identifies the patient's oxygen carrying capacity through the evaluation of red blood cells (RBC) indices, haemoglobin and haematocrit. It also provides information about the immune system through the evaluation of white blood cells (WBC) count with differential. This test is helpful in the diagnosis of anaemia, certain cancers, infection, acute haemorrhagic states allergies and immune deficiencies as well as monitoring of side effects of certain drug that causes dyscrasias (George-Gay and Parker, 2003).

Pregnant women are particularly vulnerable to malaria, which is a major cause of prenatal mortality, low birth weight and maternal anaemia. It accounts for 40% of public health expenditure, 30 - 50% of in-patient admissions and up to 50 % of out-patient visits in areas with high malaria transmission (WHO, 2005b). In Nigeria, malaria is endemic and stable, being a major cause of morbidity and mortality, resulting in 25% infant and 30% childhood mortality (FMH, 2005a). Tragically, the health status of children under the age of five and pregnant women has remained a major barrier to Nigeria's development. Among pregnant women, malaria is responsible for more than one in 10 deaths and accounts for considerable proportion of low-birth-weight babies born to these mothers. These babies born with low birth weight are usually at higher risk of dying from infant and childhood illnesses (RBM, 2005).

In Sokoto, there had been a number of researches on malaria (Elueze *et al.,* 1990; Jiya, 2001) but there is paucity of published information on full blood count, ascorbic acid and pantothenic acid among pregnant women in the area. The aim of this study is to analyse the ascorbic acid, pantothenic acid and full blood count of plasmodium parasitized pregnant women in Sokoto attending Specialist Hospital Sokoto.

Materials and Methods

Study Area

This study was carried out in the Antenatal Clinic of Specialist Hospital, Sokoto, North–Western Nigeria. Specialist Hospital Sokoto is a tertiary institution located within the Sokoto metropolis. Sokoto is the capital city of Sokoto State of Nigeria. The State is located in the extreme Northwest of Nigeria, near to the confluence of the Sokoto River and the Rima River. The State is in the dry Sahel, surrounded by sandy savannah and isolated hills, with an annual average temperature of 28.3°c (82.9°F). Sokoto is, on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under 40°c (104.0°F) and the dryness makes the heat bearable. The warmest months are February to April when day time temperature can exceed 45°c (113.0 °F). The rainy season is from June to October during which shower are a daily occurrence. Sokoto city is a major commerce center in leather crafts and agricultural products. As at 2006, the state has a population of 3.6 million (NPC/FGN, 2006).

Study Population

The study population for this study includes 60 malaria- infected pregnant women (subject) and 30 age- matched healthy pregnant women without plasmodium infection, which were monitored as controls. Both subjects and controls ware recruited in the Antenatal Clinic Specialist Hospital, Sokoto, Sokoto North-Western Nigeria.

Study Subjects/ Selection

Inclusion and Exclusion Criteria

Women who meet the following inclusion criteria were recruited in the study; pregnant women parasitized with plasmodium attending Antenatal Clinic Specialist Hospital, Sokoto,

Nigeria, women who gave written informed consent in their clinic and agreed to be included in the study. The following were excluded from participating as subjects in the study; non-pregnant women parasitized with plasmodium, healthy pregnant women that are not parasitized with plasmodium and plasmodium parasitized pregnant women who did not offer an informed consent to be included in the study.

Study Design

The research was a case-control study to assess the level of some ascorbic acid and pantothenic acid along with complete blood count parameters of 60 Plasmodium parasitized pregnant women and 30 age and gender-matched healthy non-parasitized pregnant women were monitored as controls visiting the Antenatal Clinic Specialist Hospital, Sokoto. Blood sample were collected (from both subjects and controls) and tested for complete blood count, Vitamin B5 and Vitamin C.

Sample Size Determination

The sample size was determined using the standard formula for calculation of minimum sample size:

($n = z^2 pq/d^2$)

n = minimum sample size

z = standard normal deviation and probability.

p = prevalence of value to be estimated from previous studies.

q = Proportion of failure (= 1 - p)

d = precision, tolerance limit, the minimum is 0.05.

Therefore $n = z^2 pq/d^2$

Where Z = 95% (1.96)

P = 4.8% (0.048) (Isah *et al.,* 2011).

q = 1 - 0.048 (=0.952)

d = 5% (0.05)

Therefore $n = (1.96)^2 (0.048)(0.952)/(0.05)^2$

$n = 70$

Questionnaire

A semi- structured interviewer-administered questionnaire was administered to all consenting participants in order to obtain information on their socio-demographic, nutritional and medical history.

Informed Consent

Written informed consent was obtained from all the study participants (subjects and controls).

Sample Collection

Whole blood was collected via venipuncture, using BD vacutainer system into K_3 EDTA anticoagulated and plain tube under strict aseptic techniques. The EDTA anticoagulated blood sample was used to analyze complete blood count while sample from the plain tubes was allowed to clot. The clotted blood sample was centrifuged at 3000 rpm for ten minutes on a bench-top centrifuge. The serum obtain was transferred into sterile plastic tube and stored immediately until ready to be analyzed. These samples were tested in the Pathology Laboratory of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Nigeria. The following laboratory investigations were carried out on K^3EDTA anticoagulated blood (FBC) and serum (ascorbic acid and pantothenic acid).

Analytical Method

Diagnosis of Malaria

Giemsa-stained blood film was examined for the presence of malaria parasites. The slides were examined under the microscope using 40× and 100× (oil immersion) objectives (Cheesbrough, 2005). The full blood count was carried out using the HA6000 Auto Haematology Analyzer (Perlong Medical Equipment Company, China). The analyser determines haematological parameters which include: Red Blood Cell Count, Haemoglobin (HGB) Haematocrit (HCT), Total White Cell Count (TWBC) and Platelet Count.

Estimation of Pantothenic Acid Level (Vitamin B5) and Ascorbic Acid

Serum samples were tested for pantothenic acid level (vitamin B5) was carried out using the Immunodiagnostic (Germany) ID-VIT pantothenic acid ELISA kit. Serum samples were diluted and added into the microtitre plate wells coated with lactobacillus plantarum which metabolizes pantothenic acid. The presence of pantothenic acid both in standards (STD) and samples gives a pantothenic acid-dependent growth response. After incubation at 37 ^{0}C for 24 hours, the growth of lactobacillus plantarum is

measured turbidimetrically at 610 nm-630 nm using the Neoeldex 4700 ELISA-reader (Monobind Inc., USA). Serum vitamin C (ascorbic acid) was assayed using chemical method by Natelson (1971). Ascorbic acid is oxidized by copper II ion to form dehydroascorbic acid, which reacts with acidic 2, 4-dinitrophenylhydrazine to form a red bis-hydrazone which is measured spectrophotometrically at 520nm.

Statistical Analysis

Data obtained was entered into a statistical package (such as SPSS version 22) on a computer to define the nature of the distribution of data for each group. Statistical differences of data were analyzed using series of statistical analysis such as mean, standard deviation, Chi –square, student's t-test, ANOVA depending on the nature (categorical or continuous) and distribution of data (normal or non-normal). Pearson's correlation was used to determine the relationship between sets of data. Probability ($p \leq 0.05$) was used to determine the level of significant for all statistical analysis.

Ethical Consideration

Ethical approval for this study will be obtained from the Ethical Committee of Specialist Hospital Sokoto.

Results

The result of 60 malaria parasitized pregnant women (subjects) recruited from the Antenatal Unit of Specialist Hospital Sokoto, and 30 apparently healthy pregnant women (controls) were analyzed. The mean parasite count among the parasitized subjects was $5.93 \pm 2.72 \times 10^3/\mu L$. Table 1 presents the socio-demographic characteristics of the Malaria parasitized pregnant subjects and controls. A proportional comparison of, those that use mosquito net and those that are on medication showed statistically significant difference ($p<0.05$). Age, gravidity, education status, fruit intake, occupation, ethnicity and residence show no statistical difference ($p>0.05$). The mean parasite count among the parasitized subjects was $5.93 \pm 2.72 \times 10^3/\mu L$. Table 2 indicates that RBC, HGB, HCT and Platelet levels were significantly lower among malaria parasitized pregnant subjects as compared to controls ($p<0.05$). However, there was no statistically significant difference in the WBC of malaria parasitized pregnant subjects and that of the controls ($p>0.05$). Table 3 revealed that ascorbic acid levels was significantly lower among malaria parasitized pregnant women subjects as compared to controls ($p<0.05$). There were no statistically significant differences in the pantothenic acid levels between parasitized subjects and the non-parasitized controls ($p>0.05$). Table 4 presents the correlation between the pattern of full blood count and some biochemical parameters. Only platelet count showed a significant positive correlation with ascorbic acid levels ($p<0.05$) and there was no significant correlation between platelet count and pantothenic acid level, HCT, HGB, WBC and RBC counts. Similarly, here was no significant correlation between ascorbic acid and pantothenic acid levels ($p>0.05$).

Table 1: The socio-demographic characteristics of subjects and controls

Variables	Patients (n=60)	Controls (n=30)	X^2	p-value
Age group				
15-20	7 (11.7%)	5 (16.7%)	t=1.2884	0.751
21-25	27 (45.0%)	10 (33.3%)		
26-30	14 (23.3%)	8 (26.7%)		
31-35	9 (15.0%)	4 (13.3%)		
36-40	3 (5.00%)	3 (10.0%)		
Use of mosquito net				
Yes	15 (25.0%)	26 (86.7%)	30.665	0.000*
No	45 (75.0%)	4(13.3%)		

Ethnicity				
Hausa	53 (88.3%)	25 (83.3%)	2.352	0.707
Yoruba	2 (3.3%)	3 (10.3%)		
Igbo	2 (3.3%)	1 (3.3%)		
Fulani	2 (3.3%)	1 (3.3%)		
Others	1(1.7%)	Nil		
Gravidity				
Primigravidea	20 (33.3%)	5 (16.7%)	3.513	0.173
Multigravidea	34 (56.7%)	23 (76.7%)		
Grand multipara	6 (10.0%)	10 (6.7%)		
Educational Status				
No Formal Education	25 (41.7%)	10 (33.3%)	1.158	0.763
Primary	10 (16.7%)	4 (13.3%)		
Secondary	15 (25.0%)	9 (30.0%)		
Tertiary	10 (16.7%)	7 (23.3%)		
On Medication				
Yes	30 (50.0%)	Nil	22.500	0.000*
No	30 (50.0%)	30 (100%)		
Frequent Fruit intake				
Yes	41 (68.3%)	27 (90%)	5.084	0.24
No	19 (31.7%)	3 (10%)		
Place of Residence				
Rural	13 (21.7%)	5 (16.7%)	0.313	0.576
Urban	47 (78.3%)	25 (83.3)		
Occupation				
House wife	47 (78.3%)	21 (70.0%)	3.032	0.354
Business	6 (10.0%)	3 (10.0%)		
Civil Servant	2 (3.3%)	4 (13.3%)		
Teacher	5 (8.3%)	2 (6.7%)		

*Data are presented as mean ± SEM for age and percentages for others. Figures in brackets are percentages of total. Key: t = t-test, x^2 = chi-square, * = statistically significant.*

Table 2: Mean comparison of some Haematological parameters for the subjects and controls

Parameters	Patients	Controls	t-test	p-value
WBC ($\times 10^9$/l)	7.403±0.2650	6.630±0.3824	1.674	0.098
RBC ($\times 10^9$/l)	3.5072±0.4082	3.7350±0.5410	-3.354	0.001*
HGB (g/dl)	9.370±0.1758	10.141±0.1035	-2.979	0.004*
HCT (%)	28.378±0.3146	29.840±0.2426	-3.060	0.003*
PLT ($\times 10^9$/l)	194.13±7.722	288.50±7.103	-7.840	0.000*

Data are presented as mean ± SEM. ***Key:*** *RBC= Red Blood Cells, HCT = Haematocrit (Packed cell volume), HGB= Haemoglobin, WBC = white cell, PLT = platelet, * = statistically significant.*

Table 2 shows the difference in haematological parameters of the subjects and control, there is a statistically significant decrease in HCT, HGB and Platelet of the subject compared to the controls. However, the WBC count is within the normal reference range.

Table 3: Mean comparison of some Biochemical parameters for subjects and controls

Parameters	Patients	Controls	t-test	p-value
Vit B_5 (µg/dl)	0.3257± 0.2466	0.2994± 0.2316	0.681	0.498
Vit C (mg/l)	0.2524± 0.1775	0.4208± 0.1928	-5.893	0.000*

Data are presented as mean ± SEM. ***Key:*** *Vit C = vitamin C (Asorbic acid), Vit B_5 = vitamin B5 (pantothenic acid), *=statistically significant.*

Table 3 Shows the difference in ascorbic acid and pantothenic acid of the subjects and controls. The ascorbic acid level was significantly lower among the subjects compared to the controls. There was no significant difference in *pantothenic acid* levels between subjects and controls.

Table 4: Correlation between Full Blood Count parameters and some Biochemical Parameters Malaria Parasitized

BIOCHEMICAL PARAMETERS	WBC ($\times 10^9$/l)	RBC ($\times 10^9$/l)	HGB (g/dl)	HCT (%)	PLT ($\times 10^9$/l)
Vit B_5 (µg/dl)	r = -0.058	r = 0.008	r = 0.031	r = -0.112	r = -0.143
	p = 0.588	p = 0.938	p = 0.771	p = 0.292	p = 0.178
Vit C (mg/l)	r = -0.099	r = 0.149	r = 0.130	r = 0.117	r = 0.413
	p = 0.353	p = 0.161	p = 0.221	p = 0.273	p = 0.000*

Key: *r = Pearson correlation, PCV= Packed cell volume, WBC= White blood cell, PLT= Platelet, Vit C= Vitamin C (Ascorbic acid), Vit B5= Vitamin B5 (Pantothenic acid), * = statistically significant.*

Table 4.4 shows correlation between ascorbic acid, pantothenic acid and haematological parameters. There is a positive correlation between platelet count and ascorbic acid where as there was no correlation between all the other parameters.

Discussion

Malaria is a major public health problem in sub-Sahara Africa including Nigeria, where it accounts for more cases of infection and death than other countries in the world. The aim of this study was to investigate the effect of malaria on some haematological and biochemical parameters in pregnant women attending antenatal clinic in Specialist Hospital Sokoto. A total of 90 pregnant women participated in this study. The subjects were aged 15-40 years. Our finding is consistent with a previous report (Sa'idu *et al,* 2015) in Sokoto which indicated that, young maternal age contributed to the seroprevalence of malaria parasitaemia among pregnant women.

We observed that younger women in the age group 21-25 years constituted a significant number of the subjects (45%) compared to older age group 36-40 (6%). This finding is consistent with a previous report of Panti and Colleagues (2010) who reported that majority of the asymptomatic malaria positive pregnant women (84%) were aged between 20 and 34 years. Uneke and Colleagues (2007) in Southern Nigeria also reported that individuals of age group 20-24 have the highest prevalence of maternal malaria (52%) while the least was recorded among those > 40 years. Susceptibility to plasmodium parasitaemia has been linked to the level of antibodies to placental sequestrated parasites (Elliot et al., 2005). This may be attributed to the fact that majority of the younger women are likely to be primigravidae and are expected to have higher malaria parasitaemia. This also supports the existing knowledge that high prevalence at lower age is due to the existence of low natural immunity to infectious diseases including malaria at that age.

This study has also found that use of insecticide-treated mosquito net has great influence in preventing malaria, and is significant ($p<0.05$) as 75% of the study subjects happens not to be using mosquito net whereas 86.7% of the controls are users of mosquito net. This confirms the report of World Health Organization (WHO 2011) and Ntonifor and Veyufambom, 2016) on the use of insecticide treated mosquito nets as a means to reduce the lethal impact of malaria.

In this study, the level of education was found to have influence on prevention of malaria in pregnancy. Majority of the study subjects has no formal education (41%), this is followed by those who attained secondary level education (25%) while women educated to primary and tertiary level each constituted 16% of the subjects. This finding is consistent with previous reports (Hamidu et al., 2003; Ali et al., 2005) in Karachi, India and Maiduguri Nigeria respectively. This is suggestive that the level of education can play a role in preventing malaria infection.

This study indicated that the HGB, HCT, RBC and Platelet count was significantly lower ($p<0.05$) in malaria parasitized pregnant women. The mean values of WBC count in both infected and non-infected women were within normal reference range. For continued survival and reproduction, plasmodium parasites need to infect the red blood cells of their human host. Consequently, changes in the red blood cell indices are some of the commonest observations seen in malaria. Anaemia, which is a fall in haemoglobin level below the normal range for age, gender, race, or pregnancy status, is the most frequent outward manifestation of such changes. Malaria is the most common cause of severe anaemia in endemic areas (Abdalla 2004). Our findings compared favorably well with the findings of other workers in Nigeria (Onwukeme and Uguru, 1990; Akingbola *et al*., 2006) which indicated that infected patients tended to have significantly lower platelets, haemoglobin and red blood cell count. The lower HCT HGB and RBC may reflect anaemia which is mainly due to mechanical destruction of parasitized red blood cells as well as splenic clearance of parasitized and defected red cells. The reduced platelet count in malaria is said to be due to platelet activation, splenic pooling and decrease platelet life span (Beale *et al.,* 1972; Abdulla, 2004). In normal pregnancy, the physiological change in haemoglobin concentration (Hb) and platelet count during pregnancy are well known phenomena (Yip, 2000). Anaemia due to Malaria in pregnancy is an important public health concern in developing countries (more pronounced in primigravidae than in multigravidae). However, anaemia due to malaria can be more severe in pregnant women

(McCrae *et al.*, 2011). Anaemia due to malaria infection results from the obligatory destruction of parasitized erythrocytes, the accelerated destruction of normal erythrocytes, and variable dyserythropoiesis. Anaemia due to malaria infection can be assessed by the measurement of packed cell volume (haematocrit) and the measurement of Haemoglobin concentration (James *et al.*, 2008). Pregnancy outcome is influenced by many factors some of which include culture, environment, socioeconomic status and access to medical care. The haematological profile of pregnant women also has an impact on pregnancy and the outcome of the pregnancy (Burrows *et al.*, 2009). The most common haematological indices are the indicators of haemoglobin concentration. Low haemoglobin in the blood (anaemia) is widely identified as a haematologic abnormality and it is associated with adverse pregnancy outcome (Miltchel *et al.*, 2006). Anaemia in malaria is believed to occur due to haemolysis of parasitized and non-parasitized RBCs, peripheral removal/sequestration of RBCs, and ineffective erythropoiesis (due to high circulating tissue necrotic factor (TNFα)) (Akhtar *et al.,*2012). In malaria endemic areas, the prevalence and severity of anaemia are usually determined by a number of interacting factors. These include, among others, the parasite species, level of parasitaemia, age of host, host genetic factors (e.g., coexisting RBC polymorphisms like haemoglobinopathies, G6PD), and nonmalarial causes of anaemia (e.g., infections, malnutrition) (Abdalla, 2004). As observed elsewhere (Chandra and Chandra, 2013), the mean red blood cell indices (Hb, MCV, MCH, MCHC, and RDW) of patients with acute uncomplicated malaria in this study were normal. This could probably have been because uncomplicated malaria is associated with milder biochemical changes, for example, a lower production of cytokines, less endothelial cell activation, milder changes in the coagulation profile, less sequestration, and less haemolysis as opposed to complicated/severe malaria.

Malaria infected patients tended to have significantly lower platelets, WBCs, lymphocytes, eosinophils, RBCs and Hb level, while monocyte and neutrophil counts were significantly higher in comparison to non-malaria infected patients (Bakhubaira, 2013). Previous report indicated that parasitized patients have higher WBCs count compared with community controls (Ladhani, 2002). The most common complication during malaria infection is thrombocytopenia (Erhart *et al.,*2004). Persons with platelet counts < 150,000/μL were 12-15 times more likely to have malaria infection than persons with platelet counts > 150,000/Ml (Erhart *et al.,*2004). Leukocytes play a vital role in the defense against malaria. Leukocyte changes in malaria are variable and depend on many factors such as acuteness of infection, parasitaemia, disease severity, state of the host immunity to malaria, and concurrent infections (Abdalla, 2004). Commonly, majority of patients with acute uncomplicated *P. falciparum* malaria usually have their mean total leukocyte count (TLC) within the normal range (Haroon *et al.,*2013). However, in some cases, a mild leucopenia may occur, especially in non-immune adults or in cases of complicated malaria (Reiley and Barett, 1971). The mean Total leucocyte count (TLC) in parasitaemic patients in this study was $5.93 \pm 2.72 \times 10^3/\mu$L, which is in agreement with results from prior studies (Tailor *et al.,*2008). Nevertheless, despite the fact that the mean TLC in parasitaemic patients was normal, an increase in the level of parasitaemia in this study was associated with a decrease in the number of leukocytes. These findings are similar to those from a retrospective study done in a malaria endemic Indian province involving 334 patients with acute malaria caused by *P. vivax*, *P. falciparum*, or dual infection in which a significant decrease in the mean TLC in the parasitaemia group was also observed (Chandra and Chandra, 2013).

In addition, according to previous studies, leukopenia does not appear to be parasite specific as exemplified in a study on patients with *P. vivax* infection in Panama (Reiley and Barrett, 1971), in Turkey (*P. vivax*) (Koltas *et al.,*2007), and in another study on 404 American service men from Vietnam with *P. vivax*, or *P. vivax*, and *P. falciparum* dual infection (Goldstein, 1968) where a leukopenia was observed in majority of patients that had malaria caused by several different malaria parasite species.

Overall, the changes seen in the total lymphocyte count in malaria parasitaemic patients are usually attributed either to an increase or a decrease in the differential white blood cell (WBC) lines. In consideration of lymphocytes for instance, there have been varying reports from different studies on whether the differential lymphocyte count remains normal, increased, or decreased in an acute malaria infection. Pre-1970s literature had indicated that lymphocyte count remains normal during an acute malaria infection (Abdalla, 2004). However, most recent literature shows that lymphopenia, which is sometimes profound but transient or temporary, is a common finding in acute malaria in nonimmune adults (Richards *et al.,* 1998) as well as in children found in malaria endemic areas (Maina *et al.,* 2010). Monocytosis has been one of the most consistent observations reported from prior studies done on the haematological changes that characterize malaria (Abdalla, 1988). These findings are in agreement with our study, where a significant (p = 0.01) mild monocytosis was observed in parasitaemic patients (10.89 ±6.23%) compared to the non-parasitaemic patients (8.98 ±5.02%).

The mean neutrophil count was normal for both parasitaemic (46.28 ±18.30 %) and non-parasitaemic patients (42.87 ±15.77%) in this study. These findings are similar to those from two studies: one involving 400 cases in a malaria endemic region of India, in which about 85% of the patients had normal neutrophil counts (Akhtar *et al.,*2012) and another in Singapore where majority of the adults with acute uncomplicated malaria had normal neutrophil counts (Kueh and Yeo, 1982). In contrast, though, some earlier studies had reported neutropenia (Dale and Wolff, 1973) or neutrophilia (Abdalla, 2004) among malaria cases, especially in the paediatric patients (Maina *et al.,* 2010). The mechanism of neutropenia in malaria has been postulated to involve increased margination and sequestration of neutrophils (Dale and Wolff, 1973) as a result of the increased expression of cell adhesion molecules (ICAM-1 and VCAM-1) that occurs in malaria (Clark *et al.,*2006).

The eosinophil count was not significantly different between the parasitemic and non-parasitemic patients in this study (P = 0.72). A few other studies that looked at eosinophils in malaria found low levels (eosinopenia) in majority of patients (Davis *et al.,*1991), although the significance of these findings was unknown. However, follow-up of these patients' days or weeks after treatment surprisingly revealed a marked elevation in the eosinophil count (Davis *et al.,*1991), a feature that the researchers attributed to the rebound eosinophilic response that resulted from of an enhanced T helper-2 response that occurred during the malaria recovery period.

Thrombocytopenia is a major complication of malaria (Khan *et al.,* 2012), the magnitude of which is dependent on the parasite species or disease severity. In light of the above, *P. vivax* malaria infection and severe malaria have been associated with a more heightened and severe thrombocytopenia than *P. falciparum* infection and uncomplicated malaria. In this study, although the mean platelet count in parasitaemic patients (172.43 ±80.41 × 10^3/μL) was normal, it was significantly (p = 0.00) lower than that of the non-parasitaemic group (217.82 ±95.96 × 10^3/μL). This only reiterates the fact that acute uncomplicated malaria is not associated with a marked reduction in platelets, as compared to severe malaria. In an attempt to compensate for the low absolute platelet count, the bone marrow increases the formation of megakaryocytes, which usually escape from the bone marrow as mega platelets during an acute malaria infection. Evidence to support this hypothesis comes from a study by Kreil *et al*. (2000), that found a marked elevation in the level of thrombopoietin, a key platelet growth factor in patients with malaria. Because of an increase in the number of mega platelets, the mean platelet volume is increased during an acute malaria infection (Maina *et al.,* 2010). In contrast, the mean platelet volume (MPV) of parasitaemic patients in this study was normal. These findings may suggest that uncomplicated malaria is associated with mild or nonsignificant changes in the platelet profile. The pathogenesis of thrombocytopenia is thought to involve a constellation of processes, some of which include splenic pooling of platelets, antibody (IgG) mediated platelet destruction, adenosine diphosphate (ADP)

release following the hemolysis of parasitized RBCs, dysmegakaryopoiesis, platelet aggregation and activation, parasite invasion of platelets, platelet phagocytosis, platelet adhesion to erythrocytes, and oxidative stress (Lacerda *et al.,*2011). The relatively diverse causative pathophysiological mechanisms could probably explain why changes in platelet homeostasis are more prominent than in other blood cell lines. Nevertheless, thrombocytopenia in malaria is observed to improve with disease resolution, and a normal platelet count is usually reported within 7 days after the initiation of antimalarial treatment (Moulin *et al.,* 2003).

The results of this study indicated a significant decrease in the ascorbic acid (vitamin C) level of malaria parasitized pregnant women when compared with control ($p<0.05$). This is in consonance with the finding of Boudová *et al*. (2014) who reported that the mean Vitamin C level were lower in malaria positive pregnant than malaria negative pregnant women. Malaria infection is associated with increase production of ROS by phagocytes, this change may play a vital role in host defense against malaria and it could also render host tissue such as erythrocytes more susceptible to oxidative damage (Vasquez *et al.,* 2003).

Ascorbic acid is potent water-soluble antioxidant and the biological system utilizes it in scavenging/neutralizing an array of Reactive Oxygen Species (ROS) which were produced at very high level because of increased activity of NADPH oxidase of immune cells (Une and Gupta, 2013). The decrease in vitamin C is therefore attributed to increase in its consumption as antioxidant vitamin in clearing the Reactive Oxygen Species (ROS). Vitamin C deficiency is prevalent particularly among low-income populations, pregnant women, pregnant smokers and pregnant women with type 1 diabetes (Madruga de Oliveira et al., 2004; Schleicher et al., 2009; Juhl et al., 2016). Previous report recommends regular vitamin C supplementation during pregnancy and that it reduces hospitalization (Hans and Edward, 2010). Vitamin C supplementation has been shown to reduce the risk of premature rupture of the membranes (Stuart *et al.,2005)* as well as reduce the risk of urinary tract infection in pregnancy (Ochoa-Brust et al.,2007). The recommended daily intake of Vitamin C among pregnancy in the EU (European Food Safety Authority (EFSA), 2017) and United States (Panel on Dietary Antioxidants and Related Compounds,2000) is 105 mg and 85 mg respectively.

In this study, the pantothenic acid levels among malaria parasitized pregnant women subjects showed no significant difference when compared with the controls. There seems a paucity of data on pantothenic acid levels among malaria parasitized pregnant women subjects. Pantothenic acid is a component of coenzyme A (CoA) and phosphopantetheine, both of which are involved in fatty acid metabolism. It is widely distributed in foods and the deficiency is only seen in individuals who are fed with synthetic diets (Fry *et al*., 1976) or in those fed an antagonist (Hodges *et al*., 1958). Our finding is consistent with a previous report which indicated that the average pantothenate blood level of the pregnant women was lower than that of non-pregnant controls (Song *et al*., 1985). Our finding is at variance with a previous report (Srinivasan and Belavady, 1976) which indicated that pregnant subjects had blood levels of the pantothenic acid comparable to those of the non-pregnant controls. Plasmodium parasitaemia may be a factor responsible in the reduced pantothenic acid among our cohort of parasitized pregnant women. Previous report indicated that it is important for dieticians, nutritionists, physicians, and other healthcare providers to be able to offer accurate and evidence-based advice on supplement use in pregnancy. Nutritional supplementation may be a safe and cost-effective way to reduce risk of outcomes among pregnant women.

In this study, we observed that there is no correlation between the haematological parameters and ascorbic acid and pantothenic acid except for a positive correlation between Platelet count and ascorbic acid level. There was no similar study in relation to these findings. This observation might be due to the fact that vitamin C functions to maintain healthy blood vessels which protect small vessels from damage and also helps in wound healing. It also prevents hardening of the vessels and blood clot (Iqbal *et al*., 2004) amongst others.

Conclusion and Recommendations

Conclusion

Findings of this study has shown that the HCT, HGB, RBC and Platelet count of malaria parasitized pregnant women subjects was significantly lower compared to non-parasitized contols. The subjects tended to have a normal WBC count, a normal pantothenic acid level and a lower ascorbic acid level compared to the controls. There was a significant positive correlation between the Platelet count and the ascorbic acid level among malaria parasitized pregnant subjects. Vitamin C should therefore be assayed in conjunction with haematological parameters in routine antenatal diagnosis and appropriate supplement should be given depending on the laboratory test result.

Recommendations

This study recommends that:

1. More effort should be invested to ensure the implementation of malaria prevention and control strategies (insecticide- treated mosquito nets and malaria prophylaxis) among pregnant women.
2. Vitamin C and pantothenic acid level along with haematological parameters should be routinely monitored particularly among plasmodium parasitized women.
3. We advocate that vitamin C and pantothenic acid be included in the guidelines of multivitamin supplementation particularly among plasmodium parasitized pregnant women

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