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Full Length Research Paper

Comparative biochemical and hematological analyses of malaria patients and normal human subjects of the Federal Medical Centre Owerri, Nigeria

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The aim of the study was to investigate the efficacy of the use of biochemical, and hematological indices as diagnostic parameters of malaria parasitemia. Twenty (20) normal human subjects and twenty (20) malaria patients were subjected to malaria parasite density, total serum protein (P), serum albumin (A), serum globulin (G), aspartate amino transferase (AST), conjugated bilirubin (CB), total bilirubin (TB), white blood cell total (WBC_{Total}), packed cell volume (PCV%), plasma triglyceride (TG), and plasma cholesterol (C) tests. Thirteen (13) malaria patients had the blood genotype AA and seven (7) malaria patients had the blood genotype AS. Fourteen (14) malaria patients suffered from *plasmodium falciparum* infection and six (6) malaria patients suffered from *plasmodium vivax* infection, of varying degrees of severity. Results recorded of the normal human subjects and malaria patients, expressed as mean ± standard error (S.E) (unit) were as follows : P (3.52±0.153) and (3.31±0.11) (g/dl), A (2.16±0.075) and (1.89±0.155) (g/dl), G (1.55±0.103) and (1.35±0.123) (g/dl), AST (5.8±0.772) and (8.8±0.909) (U/l), CB (0.23±0.037) and (0.56±0.045) (mg/dl), TB (0.55±0.069) and (1.04±0.07) (mg/dl), WBC_{Total} (5120±292.8) and (4320±440.66) (mm), PCV (38.3±1.274) and (34.2±0.879) (%), TG (125.47±5.316) and (156±6.734) (mg/dl), and C (116.51±0.417) and (117.76±0.215) (mg/dl), respectively. The mean values of G, PCV%, and WBC_{Total} were significantly lower (p<0.05), but those of AST, TG, TB, and CB were significantly higher (p<0.05) in malaria patients compared with normal human subjects. The statistical regression and correlation between PCV% and AST (U/l) of malaria patients were significant (p<0.05). Incidence of malaria parasitemia correlated positively and significantly (p<0.05) with significant decrease (p<0.05) in G, PCV% and WBC_{Total}, and significant increase (p<0.05) in AST, TG, TB and CB. Significant differences/alterations in the values of G, PCV%, WBC_{Total}, TG, TB, CB, and AST (in comparism with normal human subjects), could be used as effective criteria/yardstick for the diagnosis of malaria parasitemia.

Key words: Malaria parasitemia, plasma, serum, plasmodium, bilirubin, cholesterol.

INTRODUCTION

Half of the world's population is at risk of malaria. An estimated 243 million cases led to an estimated 863,000 deaths in 2008. Administration of fake malarial drugs has been implicated as a major causative factor of proliferation of drug-resistant malarial parasites leading to large scale deaths, due to malarial infections (Basco, 2004). In addition, there has been over-reliance on both quinoline compounds (that is, quinine, chloroquine, amodiaquine, mefloquine and primaquine) and antifolate drugs (that is, sulfonamides, pyrimethamine, proguanil and chlorproguanil), with consequent encouragement of cross-resistance among these compounds (Mutabingwa *et al*., 2005). The RBM partnership is a global network for co-ordinated action against malaria, launched in 1998 by WHO, UNICEF, UNDP and World Bank.

Artemisinin, (a sesquiterpene lactone), is derived from a herb, *Artemisia annua*, and is used as a drug to treat multi-drug resistant strains of falciparum malaria (Parker *et al*., 1999). *Saccharomyces cerevisiae* microbes can pro-

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duce the precursor artemisinic acid, by a technique of synthetic biology, via the mevalonate pathway (Covello *et al*., 2007). Artemisinin itself has physical properties such as poor bioavailability that limit its effectiveness. Semi-synthetic derivatives and analogues of artemisinin, such as Artesunate, Artemether, Artelinic acid, Artenimol, and Artemotil, with more efficient bio-availabity have been developed. Artemisinin and its derivatives are fast-acting, but other drugs are often required to clear the body of all parasites and prevent recrudescence. For this reason, artemisinin is administered together with other antimalarial drugs, unrelated to the artemisinin family, in what is known as artemisinin combination therapies (ACTs), The artemisinin derivative, artemether, is typically administered, in simultaneous combination with lumefantrine. The plasmodium parasite consumes hemoglobin and liberates free heme, an iron-porphyrin complex- moiety, during plasmodium infection of the red blood cells. The complex, produced, reacts with artemisinin to produce reactive oxygen radicals which destroy the parasite leading to its death. (Obimba, 2012).

Azadirachta indica, Gossypium hirsutum, Phyllanthus amarus, Carica papaya, Cinchona pubescens, Alstonia congensis , Ageratum conyzoides, Tetrapleura tetraptera (Igwe *et al*., 2012), *and Psidium guajava* are anti-malarial plants, used in alternative medicine for the treatment of malaria patients.

Severe and acute *P. falciparum* infections cause hepatocellular damage which leads to alterations in plasma lipid and lipoprotein patterns (Sibmooh *et al*., 2004). Serum LDL and HDL of malaria patients were significantly lower ($p<0.05$) than those of normal human subjects, of age bracket 11-20 years (Chikezie and Opara, 2013). Reduced serum levels and oxidative modification of HDL-C is associated with the pathophysiology of malaria in children (Ogbodo *et al*., 2008).

Hyperbilirubinemia, and increased plasma levels of aspartate transferase (AST) and alanine transferase (ALT) activities suggest hepatocytic dysfunction in patients with P. falciparum infection (Onyesom and Onyemakonor, 2011). Significant increases ($p<0.05$) in aspartate amino transferase, alanine aminotransferase and alkaline phosphatase were observed of malarial patients compared with normal human subjects, and a positive/significant correlation ($p<0.0001$) between these enzyme activities and malaria parasitemia (Ignatius *et al*., 2008).

Malaria patients who were pregnant had significantly higher ($p<0.05$) levels of AST, ALT, total bilirubin and indirect bilirubin than normal pregnant women, but significantly lower ($p<0.05$) levels of serum hemoglobin (Hb) concentration, serum total protein, serum albumin and globulin than normal pregnant women. A significant positive correlation ($p < 0.05$) was observed between liver enzymes, age, hemoglobin concentration and bilirubin levels (Elbadawi *et al*., 2012).

The populations in greatest need of iron supplementation are also those at greatest risk of malaria: pregnant women and young children. Iron supplementation has been shown to increase malaria risk in these groups in numerous studies. Conversely, the risk of anemia is increased by malaria infections and preventive measures against malaria decrease anemia prevalence in susceptible populations without iron supplementation (Spottiswoode *et al*., 2012).

Mean values of red blood cell counts, hemoglobin concentrations, lymphocyte count, platelet counts, total white cell count and CD4 counts between malaria parasitaemia positive and negative people living with HIV/AIDS in Douala suggests no significant differences, indicating that malaria may not be the primary cause or correlate of hematological disorders in these patients (Tchinda *et al*., 2012).

Malaria parasite reduced significantly ($p<0.05$), red blood cell count, packed cell volume and Hemoglobin concentration, in malaria patients in comparism with normal human subjects (control) (Ovuapkoraye, 2011). Significant reduction ($p<0.05$) of hemoglobin concentration and packed cell volume were observed of patients with malaria parasitemia (George and Ewelike-Ezeani, 2011).

The range of values of normal levels of some diagnostic indices of malaria are shown in Table 1.

The aim of the study was to investigate the efficacy of the use of biochemical, and hematological indices [total serum protein (P), serum albumin (A), serum globulin (G), aspartate amino transferase (AST), conjugated bilirubin (CB), total bilirubin (TB), white blood cell total (WBC_{Total}), packed cell volume (PCV%), plasma triglyceride (TG), and plasma cholesterol (C)] as diagnostic parameters of malaria parasitemia.

MATERIALS AND METHODS

Experimental Design

The experimental design used in the present study is a single factor completely randomized design (CRD) whose linear equation is

$\hat{Y} = \mu + T_i + \epsilon_{ij}$

$\hat{Y}$ = individual observation

μ = overall mean

T_i = ith type of infection, and is significant of malaria parasitemia.

ϵ_{ij} = error which is independently, randomly and normally distributed with zero mean and constant variance.

SPSS for windows (version 17.0, SPSS, Chicago, IL, USA) was used to perform the statistical analyses. The significance level was p value<0.05.

Selection of human subjects: Twenty (n = 20) clinically confirmed (WHO, 2008) malaria male patients, of age bracket 18-30 years and twenty normal or healthy sub-

Table 1. Range of values of normal levels of some diagnostic indices of malaria.

Diagnostic index	Range of normal values
Serum globulin	2.5 – 3.0 g/dl
PCV%	40 – 53 %
WBC $_{Total}$	4,500 – 10, 000 mcl
Conjugated bilirubin	0 – 0.3 mg/dl
Total bilirubin	0.3 – 1.9mg/dl
Plasma triglyceride	< 150mg/dl
Aspartate aminotransferase	6- 40 I.U/l

Source: Fairbanks and Tefferi (2000), Nyblom *et al*. (2006), Higuera (2012), Dugdale (2013a), Dugdale (2013b).

jects (n=20) of the same age bracket, voluntarily participated in this study, at Federal Medical Centre Owerri, Imo State. Nigeria. The subjects were randomly selected between September and October 2014. Exclusion criteria included: gastrointestinal tract infection, protein energy malnutrition, renal diseases, cirrhosis, hepatitis, obstructive jaundice, cancer, diabetes mellitus, hypertension, obesity, smoking, alcoholism, persons living with HIV, patients taking anti-malaria drugs and vitamin supplements, and patients who have treated malaria in the past 2 months, consistent with the methods of Onyesom and Onyemakonor (2011), Idonije *et al*.(2011), Chikezie and Opara (2013).

Blood was obtained by veni-puncture carried out by a Phlebotomist nurse.

The method described by Thavasu *et al*. (1992) was used in obtaining the serum. Whole blood was collected in a covered test tube, and allowed to clot by leaving it undisturbed for 15-30 minutes at room temperature. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge, to obtain the blood serum.

Citrate phosphate dextrose-adenine 1 (CPDA-1) stored whole blood was used for whole blood analysis.

Determination of Haemoglobin Genotype

The technique for haemoglobin electrophoresis described by John and Lewis (1986) and Tidi *et al*. (2013) was employed.

Fifty micro-liters of washed cells were added into khan tubes containing 50 micro-liter of 0.1% white saponin and were mixed thoroughly (haemolysate). The haemolysate was centrifuged to remove any debris. The supernatant was used for the test.

Cellulose acetate papers were soaked and blotted. Haemoglogin genotype controls used include: HbA, HbF, HbS and HbC.

One hundred ml of the Tris-EDTA and boric acid buffer was introduced into each of the outer section of the electrophoresis chamber. One micro-liter of each haemolysate sample (tests and controls) was transferred into the well plate. Using an applicator, 0.5 micro-liter of the haemolysate (samples and controls) was applied onto the cellulose acetate paper leaving about 0.5 cm gap for each sample.

The cellulose paper was placed on a cathode bridge of the electrophoresis chamber containing Tris-EDTA and boric acid buffer. Two hundred voltages were applied for 15 minutes, and the results recorded.

Malaria Parasite Density Test

Measurement of parasite density of peripheral blood smear was by Giemsa stained techniques. The films were examined microscopically using ×100 objective under oil immersion (Cheesbrough, 1998). Level of parasitemia or degree of severity of malaria infection could be graded as low+ (1 to 999 /µL), moderate++ (1000 to 9999 /µL) and severe+++ (>10,000 /µL) (Idonije *et al*., 2011).

Malaria Rapid Diagnostic Test: *In Vitro* Qualitative Test for Malaria Parasites.

A blood specimen collected from the patient was applied to the sample pad on the test card along with certain reagents /dipstick was smeared with blood obtained from venous punture. After 15 minutes, the presence of specific bands in the test card window/presence or absence of colored stripes on the dipstick, served to indicate the type of *Plasmodium* parasite that infected the patient.

Dipstick tests could be used to distinguish between all five different species of human malaria parasites, because of antigenic differences between their plasmodium lactate dehydrogenase isoenzymes (Pattanasin *et al*., 2003).

Packed Cell Volume (PCV%)

Analysis of packed cell volume (PCV%) was carried out according to the method described by Ovuakporaye (2011). A plain capillary tube was filled with whole blood in an EDTA container by capillary action. It was sealed using plasticine or bunsen burner flame and placed in the haematocrit centrifuge for 10mins and the value of PCV% was obtained using haematocrit reader.

Lipid Profile Assays

Plasma cholesterol (C), and plasma triacylglycerol (TG) were determined using commercial kits (Randox Laboratory Ltd., UK), in conformity with the methods employed by Ibegbulem and Chikezie (2012); Chikezie and Okpara (2013).

Quantitative *in Vitro* Analysis of Aspartate Amino Transferase (AST)

Quantitative *in vitro* determination of serum aspartate amino transferase (AST) was carried out using the method employed by Reitman and Frankel (1957). The test based on the reaction in which l-aspartate and α-ketoglutarate are converted to l-glutamate and oxaloacetate by the catalytic activity of AST. The oxaloacetate so formed, forms a complex known as oxaloacetate hydrazone with 2,4-dinitrophenyl hydrazine. The intensity of the colour of the hydrazone, which is measurable with a colorimeter at 578nm is directly proportional to the AST enzyme activity.

Quantitative *in Vitro* Determination of Serum Albumin

Quantitative *in vitro* determination of serum albumin was carried out using the method described by Qureshi and Qureshi (2001) and Huang and Fraker (2003). Serum albumin was determined using human albumin standards and sigma diagnostics albumin reagent (Sigma, St. Louis, MO) containing bromocresol green. The absorbance of the mixture of the reagent and serum albumin was measured at 578 nm against a reagent blank.

Quantitative *in Vitro* Determination of Serum Globulin

Quantitative *in vitro* determination of serum globulin was carried out using the method described by Woodward et al. (1972). Serum was mixed with a reagent of *p*-dimethylaminobenzaldehyde to form a coloured derivative. Tryptophan in globulin present in the serum reacted with the *p*-dimethylaminobenzaldehyde reagent to form the coloured derivative which absorbed white light maximally at 578 nm, in a spectrophotometer. The absorbance of the coloured derivative is directly proportional to the concentration of the serum globulin (by Beer-Lamberts law).

Quantitative *in Vitro* Determination of Total Serum Protein.

The methods described by Flack and Woollen (1984) were used for the assay of total serum protein. The peptide bonds of protein in blood serum were made to react with the copper II ions in alkaline solution to form a blue-violet complex (the biuret reaction), each copper ion complexing with 5 or 6 peptide bonds. Tartrate was added as a stabilizer whilst iodide was used to prevent auto-reduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and was measured at 520-560 nm.

White Blood Cell total (WBC_{Total}) Assay

The white blood cell total count (mcl) was determined according to the method described by Annan and Plahar (1995). Blood samples (0.02 ml) were mixed with sequesterine and diluted in 0.38 ml diluting fluid (1.5 ml glacial acetic acid, 0.5 ml malachite green, 98.0 ml water). The diluted blood was mounted on a counting chamber, and white blood cells were counted.

Bilirubin Assays

Total and conjugated bilirubin assays were carried out consistent with the methods described by Simmons (1968). In the determination of total bilirubin, the serum was added to a caffeine reagent, which acted as an accelerator, and then mixed with combined diazo reagent. The diazo reaction was terminated by the addition of ascorbic acid which destroyed the excess diazo reagent, the azo bilirubin was made alkaline by the addition of a tartrate buffer, and the intensity of the colour (absorbance) was read at 600 nm. In the determination of the conjugated bilirubin, the serum was mixed with water and diazo reagent. The reaction was terminated by ascorbic acid and then caffeine was added. Again the azo-bilirubin was made alkaline with tartrate buffer. When blanks were run, the serum was mixed with water and ascorbic acid. Diazo reagent, caffeine, and tartrate reagent were added subsequently.

The research was given Ethical approval from the Department of Biochemistry, School of Science, Federal University of Technology Owerri, because it was carried out in compliance with the Declaration on the Right of the Patient (WMA, 2000).

RESULTS

Table 2 shows the results on the blood genotype, malaria parasite density and type tests carried out using the blood samples of twenty individuals infected with plasmodium parasites. The twenty normal human subjects tested negative to the malaria parasite density test. There were more malaria patients of genotype AA, in each category of infection viz : +, ++, and +++, than there were of malaria patients of the genotype AS. Thirteen (13) malaria patients had the blood genotype AA and seven (7) malaria patients had the blood genotype

Table 2. Results on the blood genotype, malaria parasite density and type tests.

Blood genotype	low+ (1 to 999/µL)		moderate++ (1000 to 9999 /µL)		severe+++ (>10,000 /µL)	
AA	2	1	3	3	3	1
AS	1	-	3	1	2	-
	P. falciparum Infection	*P. vivax* infection	*P. falciparum* infection	*P. vivax* infection	*P. falciparum* infection	*P. vivax* infection

(n = 20).
Values indicate no. of malaria patients that fall under intersecting classifications of: degree of infection, blood genotype and type of infection.

Table 3. Results on the biochemical indices: Total serum protein, serum albumin, serum globulin and white blood cell (total) of the normal human subjects and malaria patients.

	Total serum protein (g/dl)	Serum albumin(g/dl)	Serum globulin (g/dl)	White blood cell total (WBC_{Total}) mcl
Normal human subjects	3.52±0.153[a]	2.16±0.075[a]	1.55±0.103[a]	5120±292.8[a]
Malaria patients	3.31±0.11[a]	1.89±0.155[a]	1.35±0.123[b]	4320±440.66[b]

Results are expressed as mean ± standard error (S.E) (unit) (n = 20).
Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

Table 4. Results on the biochemical indices : Aspartate amino transferase, total bilirubin and conjugated bilirubin, and the hematological index: Packed cell volume % of the normal human subjects and malaria patients.

	Aspartate amino transferase (U/l)	Total bilirubin (mg/dl)	Conjugated bilirubin (mg/dl)	Packed cell volume (PCV%)
Normal human subjects	5.8±0.772[a]	0.55±0.069[a]	0.23±0.037[a]	38.3±1.274[a]
Malaria patients	8.8±0.909[b]	1.04±0.07[b]	0.56±0.045[b]	34.2±0.879[b]

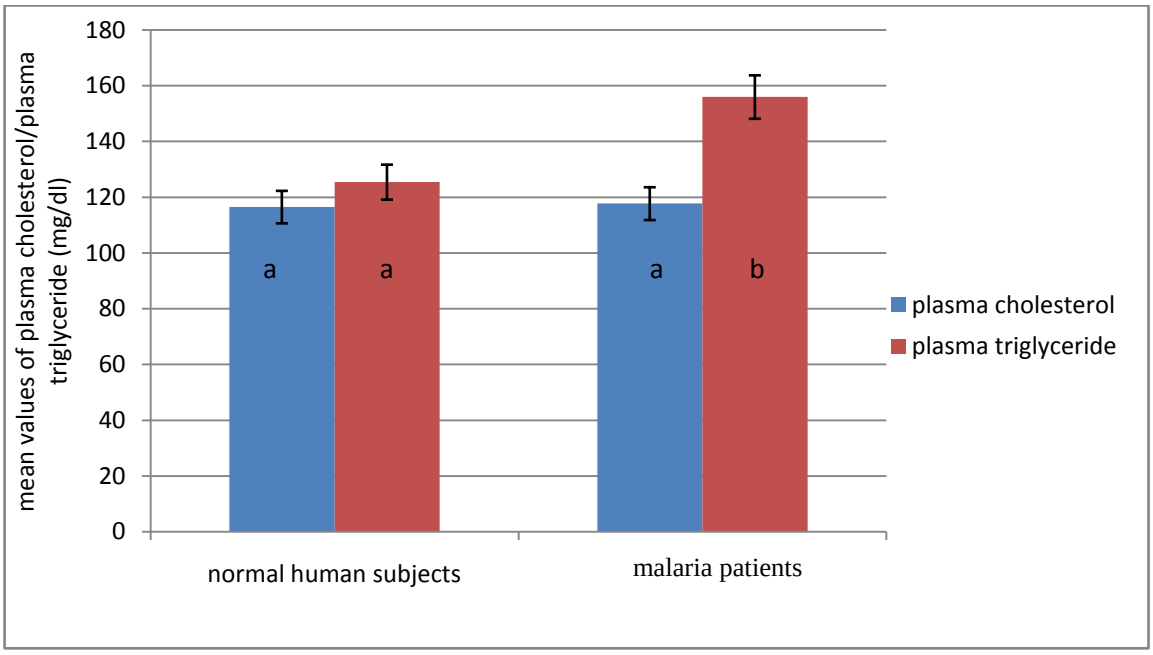
Results are expressed as mean ± standard error (S.E) (unit) (n = 20).
Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

AS. Fourteen (14) malaria patients suffered from *plasmodium falciparum* infection, and six (6) malaria patients suffered from *plasmodium vivax* infection. The intersecting classifications of degree of infection, blood genotype and type of infection show among others, that three (3) individuals suffered from severe *P falciparum* malaria infections.

Table 3 shows the results on the biochemical indices : total serum protein, serum albumin, serum globulin and white blood cell (total) of the normal human subjects and malaria patients. The mean values of the total serum protein and serum albumin observed of the malaria patients were numerically lower than (but not significantly different (p<0.05) from), the corresponding values observed of the normal human subjects. The mean values of the serum globulin and the white blood cell (total) observed of the malaria patients were significantly lower (p<0.05) than the corresponding values observed of the normal human subjects.

Table 4 shows the results on the biochemical indices: aspartate amino transferase, total bilirubin and conjugated bilirubin, and the hematological index: packed cell volume % of the normal human subjects and malaria patients. The mean values of the aspartate amino transferase, total bilirubin and conjugated bilirubin of the normal human subjects were significantly lower (p<0.05) than the corresponding values observed of the malaria patients. The mean value of the packed cell volume % observed of the normal human subjects was significantly higher (p<0.05) than the corresponding value observed of the malaria patients.

Figure 1 shows the results on the biochemical indices: plasma cholesterol and plasma triglyceride of the normal human subjects and malaria patients. The mean value of the plasma cholesterol observed of the malaria patients were numerically higher than (but not significantly different (p<0.05) from), the corresponding value observed of the normal human subjects. The mean value of the plasma triglyceride observed of the malaria patients was significantly higher (p<0.05) than the corresponding value observed of the normal human subjects.



Statistical results are expressed as mean ± standard error (mg/dl) (n = 20). Error bars represent values of standard error (0.215 - 6.734mg/dl). Corresponding bars labelled with the same letters represent mean values of plasma cholesterol or plasma triglyceride which are not significantly different ($p<0.05$).
Figure 1. Graphical results on the biochemical indices : plasma cholesterol and plasma triglyceride of the normal human subjects and malaria patients.

DISCUSSION

The life cycle of the plasmodium parasite in the human host starts with a bite by a malaria-infected female Anopheles mosquito, during a blood meal, in which sporozoites are inoculated into the blood stream of the human host, and migrate to the liver. They infect liver cells, where they multiply into merozoites, rupture the liver cells, and return to the bloodstream. Then the merozoites infect red blood cells, where they develop into ring forms, trophozoites and schizonts that in turn produce further merozoites. Sexual forms are also produced, which, if taken up by a mosquito, infect the insect and continue the life cycle. Various malaria drugs target pathogenic blood stages in humans. Artemisinin combination therapies are effective in reducing the gametocyte or sexual forms of the parasite (Bousema *et al*., 2010). Effective diagnosis of malaria infection is fundamental to the efficiency of the drug therapy of the disease. The number of malaria patients of genotype AA, shown in Table 2, is significantly higher ($p<0.05$) than the number of malaria patients of genotype AS, and corroborates the findings of Yahaya *et al*. (2013), who observed that malaria parasitemia was significantly ($p<0.05$) prevalent in individuals of genotype AA compared with individuals of any other hemoglobin genotype. A total serum protein test measures the total amount of protein in the blood. It also measures the amounts of two major groups of proteins in the blood: albumin and globulin. Albumin is made, mainly in the liver. It helps keep the blood from leaking out of blood vessels. Albumin also helps carry some medicines and other substances through the blood and is important for tissue growth and healing. Globulin is made up of different proteins called alpha, beta, and gamma types. Some globulins are made by the liver, while others are made by the immune system. Certain globulins bind with hemoglobin. Other globulins transport metals, such as iron, in the blood and help fight infection (Thompson *et al*., 2013).

It is recorded in Table 3 that the mean values of the serum globulin and the white blood cell (total) observed of the malaria patients were significantly lower ($p<0.05$) than the corresponding values observed of the normal human subjects. The latter finding is consistent with the postulates of McKenzie *et al*. (2005) and Adebisi *et al*. (2002). Significant reductions observed of the white blood cell total is due to a phenomenon that is widely thought to reflect localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis. Significant decrease in total globulin levels, in severe cases of malaria is as a result of immunity-supression that is caused by acute *P falciparum* infection (Benten *et al*., 1992).

Table 4 shows that the mean values of the aspartate amino transferase, total bilirubin and conjugated bilirubin, of the normal human subjects were significantly lower ($p<$

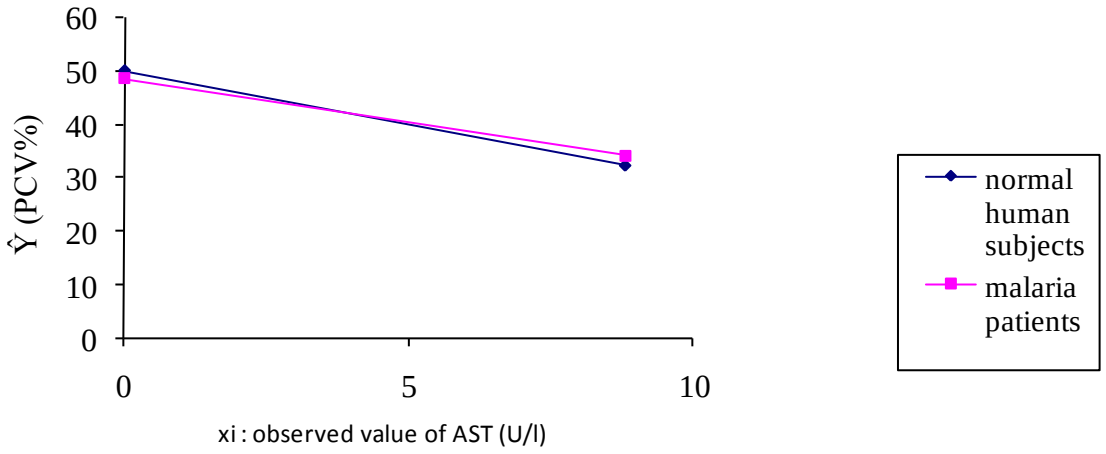


Figure 2: Regression curve of PCV% and AST(U/l).
(normal human subjects): $\hat{Y} = -2.03xi + 50.1\%$
(malaria patients): $\hat{Y} = -1.625xi + 48.5\%$.

0.05) than the corresponding values observed of the malaria patients, and is in conformity with the findings of Onyesom and Onyemakonor (2011), who observed hyperbilirubinemia and increased plasma levels of aspartate transferase (AST) and alanine transferase (ALT) activities in patients with *P. falciparum* infection: an evidence of hepatocytic dysfunction. The mean value of the packed cell volume % observed of the normal human subjects was significantly higher ($p<0.05$) than the corresponding value observed of the malaria patients, and is in keeping with the findings of George and Ewelike-Ezeani (2011), who reported significant reductions of hemoglobin concentration and packed cell volume (PCV%) of patients with malaria parasitemia.

Aspartate amino transferase functions in the biosynthesis of amino acids as well as the excretion of α-amino groups of amino acids. Bilirubin's main physiologic role is as a cellular antioxidant, and immuno-modulator (Liu *et al*., 2008).

Excessive removal of non-parasitized erythrocytes, immune destruction of parasitized red cells and impaired erythropoiesis as a result of bone marrow dysfunction are few of the different mechanisms by which malaria may cause anaemia, and therefore, a reduction of PCV% [Ekvall (2003), Adesina *et al*. (2009)].

Malaria parasite can cause congestion, sinusoidal blockage and cellular inflammation in three organs in the body, namely, brain, kidney and liver organ (Jarike *et al*., 2002), leading to the leakage of the parenchymal enzymes e.g aspartate amino transferase (AST) and membranous enzymes e.g alkaline phosphatase, into the blood circulation (Burtis *et al*., 2001).

Malaria parasitemia engenders increased red blood cell haemolysis, which is associated with increase in bilirubin biosynthesis, hepatocellular damage, biliary tract obstruction, haemolysis and jaundice (Yokoto and Calisei, 2006).

Figure 1 reveals that the mean value of the plasma triglyceride observed of the malaria patients was significantly higher ($p<0.05$) than the corresponding value observed of the normal human subjects, and is in consonance with the findings of Nilsson-Ehle and Nilsson-Ehle (2009), who reported moderately increased plasma triglyceride concentrations in sixteen (16) patients with acute malaria.

Significant increase in plasma triglycerides indicate impaired metabolism of chylomicrons (Mohanty *et al*., 1992). Hyperlipidemia is one of the indicators of malaria infection and could lead to depletion of natural antioxidants and facilitate the production of reactive oxygen species which is capable of reacting with all biological molecules in the body system and exert cytotoxic effects on cellular components (Akanbi, 2013).

The statistical regression and correlation between PCV% and AST (U/l) of malaria patients were significant ($p<0.05$), with a Pearson's product moment correlation coefficient of 0.985 as shown in figure 2. Observed values of AST (U/l) could be used with high precision, to predict the values of PCV% in malaria patients. The negative slope of the graph of equation: $\hat{Y} = -1.625xi + 48.5\%$ (figure 2), indicates that the value of PCV% decreases, as the corresponding value of AST (U/l) increases in malaria patients. Incidence of malaria parasitemia correlated positively and significantly ($p<0.05$) with significant decrease ($p<0.05$) in serum globulin (G), packed cell volume (PCV%), and white blood cell total (WBC_{Total}), and significant increase ($p<0.05$) in plasma triglyceride (TG), total bilirubin (TB),

Conjugated bilirubin (CB), and aspartate aminotransferase (AST), in comparism with normal human subjects.

CONCLUSION

Incidence of malaria parasitemia correlated positively and significantly ($p<0.05$) with significant decrease ($p<0.05$) in G, PCV%, and WBC_{Total} and significant increase ($p<0.05$) in TG, TB, CB and AST. Significant differences in the values of G, PCV%, WBC_{Total}, TG, TB, CB and AST (in comparism with normal human subjects), could be used as effective criteria/yardstick for the diagnosis of malaria parasitemia. Therefore, statistical significant decrease in values of G, PCV%, and WBC_{Total} and significant increase ($p<0.05$) in TG, TB, CB and AST are associated with malaria infection of patients. For the reason that some of the diagnostic indices measured of the malaria patients were in the range of values of normal levels, significant differences/alterations ($p<0.05$) in the values of the diagnostic indices, between normal human subjects and malaria patients, rather than use of the range of values of normal levels, are more efficient criteria for the diagnosis of malaria parasitemia.

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