IJBPAS, January, 2013, 2(1): 125-137

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ASSESSMENT OF SOME ENZYME AND HAEMATOLOGICAL PARAMETERS IN MALARIA PATIENTS RESIDENT IN MINNA, NIGERIA

JIGAM AA* AND NIWOYE AA

Department of Biochemistry, Federal University of Technology, Minna, Nigeria

***Corresponding Author: E Mail- alijigam@yahoo.com, Ph.: +2348036136862**

ABSTRACT

The serum activities of Glutamate Pyruvate Transaminase (SGPT), Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase(LDH) and Glucose -6-Phosphate Dehydrogenase (G6PD) were assayed in ninety eight (98) malaria patients attending Minna, General Hospital. Sixty eight (68) healthy volunteers were randomly selected and screened for these enzymes. Packed cell volume (PCV) levels of the patients and healthy individuals were also determined. Results obtained showed SGPT (36.92 ±2.11 IU/L), SGOT (35.04±2.23 IU/L), ALP (45.71±1.79 IU/L) and LDH (398.80±2.11 IU/L) except G6PD (7.13±1.24 U/gHb) were significantly ($p<0.05$) elevated in malaria patients in comparison to the healthy volunteers. When males alone were compared, a similar trend to the general population was obtained in the patients except LDH (304.95±31.38 IU/L) and G6PD (4.99±1.06 U/gHb) which were not significantly ($p>0.05$) different from the activities in the controls. SGPT (35.66±3.03 IU/L) and G6PD (8.69±1.02 U/gHb) were also significantly ($p<0.05$) elevated in male malaria patients compared to their female counterparts. Male patients had consistently higher enzyme activities in comparison to female patients with most values within the specified normal ranges except LDH which was elevated in both sexes. Packed cell volume was generally higher in the control group compared to malaria patients and this was irrespective of gender.

Keywords: Transaminase, Phosphatase, Malaria , Assay, Dehydrogenase

INTRODUCTION

Morbidity and mortality due to malaria have remained unabated primarily as a result of the unavailability of suitable vaccines and the spread and intensification of drug resistant *Plasmodium* parasites **[1]**. Despite the increased availability of new antimalarials such as fixed dose artemisinin combination therapy, significant gaps remain for medicines blocking transmission, preventing relapse, and long–duration molecules for chemo-protection **[2]**. This has led to a frightening scenario in which it is estimated that annually, 350-500 million people are infected by the parasites with 1-3 million deaths globally **[3, 4]**. The most vulnerable groups to the disease are children under five years of age, pregnant women and adults who have lost or never acquired immunity **[5]**. Apart from vector control and chemotherapy, early and proper diagnosis employing novel analytes such as serum enzymes have been suggested as part of an alternative and likely viable strategy in the management of malaria. Plasmodial life cycle is a complex multi-stage phenomenon which includes a sporogonic (in mosquitoes), erythrocytic and exo-erythrocytic(in the liver and other tissues) phases. During such phases the Parasites interfere with the metabolism and affect also cellular membrane intergrity resulting in the release of enzymes raising their levels in serum of patients. Pathological damage to a tissue embraces a wide spectrum of effects. Thus a mild reversible viral inflammation of the liver is likely to increase only the permeability of the cell membrane and allow cytoplasmic enzymes to leak into the blood. However, a severe attack causing necrosis also disrupts the mitochondrial membrane and both cytoplasmic and mitochondrial enzymes are detected in blood.

The selection of which enzyme to measure in serum for diagnostic or prognostic purposes depends on its distribution among various tissues **[6]**. Such enzymes of diagnostic utility are diverse including; amylase, Cholinesterase, Trypsin, 5'-Nucleotidase, Lipase, creatine kinase etc.

The enzymes selected for analysis in the present study have appreciable activities in the liver and erythrocytes which are tissues involved with *plasmodium* life cycle. Aminotransferases catalyse the interconversion of amino acids to 2-oxo-acids by transfer of amino groups. GOT (Aspartate amino transferase E.C. 2.6.1.1) and more specific GPT (Alanine aminotransferase E.C. 2.6.1.2) are elevated in the serum mainly due to liver disease. GOT activity in serum is stable for up to 48 hours at $4^{\circ}C$ while SGPT activity is lost at room temperature, $4^{\circ}C$ and -

25^0C. SGPT activity is better maintained at -70^0C.

Alkaline phosphatase (E.C 3.1.3.1., Orthophosphoric – monoester phosphohydrolase (Alkaline optimum); ALP) catalyzes the alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates. ALP activity is prevalent in most organs including liver, bones etc. and especially associated with membrane and cell surfaces. The specific metabolic rate of ALP is obscure but is associated with lipid transport in the intestine and bone calcification. Elevations in serum ALP activity commonly originate from the liver and bone **[7]**.

Lactate dehydrogenase (E.C 1.1.1.27; L-lactate: NAD^+ oxidoreductase; LDH) is a hydrogen transfer enzyme that catalyzes the oxidation of L-Lactate to pyruvate with the mediation of NAD^+ as hydrogen acceptor. LDH is a tetrameric protein composed of 5 isoenzymes: LD1 (H_4), LD-2(H_3M), LD-3(H_2M_2), LD-4(HM_3), LD-5 (M_4) with a molecular weight 134 KDa. Due to its wide distribution in all tissues, serum LDH elevations occur in myocardial infarction, haemolysis and disorder of the muscle, kidneys, lungs and the liver. Serum is the preferred specimen for measuring LDH activity. Plasma samples may be contaminated with platelets, which contain high concentrations of LDH. Haemolysed serum cannot also be used because erythrocytes contain 150 times more LDH activity (particularly LD-1 and LD-2) than serum **[8]**.

Glucose – 6- phosphate dehydrogenase (E.C 1.1.1.49; D-Glucose-6-phosphate: $NADP^+$ oxidoreductase; G6PD) is expressed in all cells and catalyzes the first step in the hexose monophosphate pathway, the conversion of Glc-6-p to 6-phosphogluconate generating NADPH. The enzyme is a dimer (predominantly) or tetramer (pH dependent) in the active form composed of several identical sub-units 515 amino acids long and weighs about 59KDa **[9]**. The monitoring of G6PD is critical during malaria due to the obligatory association of *Plasmodium* parasites with erythrocytes. The organisms utilize oxygen, NADPH, reduced glutathione and other metabolites much needed by red cells for survival. Majority of the G6PD-deficient individuals develop haemolysis only when oxidative stress occurs, as with infections and after ingestion of certain drugs or fava beans. Outside these periods, they are usually asymptomatic. However, mild to severe chronic haemolysis, exacerbated by oxidative stress can occur **[10]**.

The present study is thus intended to appraise the relevance of these enzymes in malaria after other diseases with hepatic and erythrocytic involvement have been precluded. The results will provide necessary information in a bid to ensure thorough diagnostic evaluation of malaria patients.

MATERIALS AND METHODS

Blood Samples

Blood samples were obtained from malaria patients and healthy volunteers by the kind permission of the authorities of General Hospital Minna and University Health Services Unit, Federal University of Technology Minna. This was in accordance with relevant codes of conduct for such researches at the two institutions.

Fresh un-haemolyzed blood samples were collected in sterile tubes containing appropriate anticoagulants from ninety eight (98) volunteer malaria patients i.e. individuals who tested positive for mixed infections with the parasites with apparent acute clinical features of the disease and no evidence of other forms of infection. The patients comprised 53 males and 45 females. Control samples were similarly collected from 68 apparently healthy i.e. individuals without evidence of any form of infection or disease. This group comprised of 30 males and 38 females. The blood samples were centrifuged and the sera collected but fresh whole blood was used for G6PD determination. Analysis was carried out on each day of sample collection.

Determination of Packed Cell Volume (PCV)

Packed Cell Volume (PCV) was determined using the microhaematocrit method **[11]**. An un-caliberated capillary tube was filled 2/3 of the volume with blood by capillary action and one end sealed with crystal seal. The tubes were transferred to the haematocrit centrifuge and allowed to spin at 12,000rpm for 5 minutes. PCV was then obtained using the haematocrit measuring guage. Haemoglobin values were calculated as a third of the PCV.

Determination of Serum Transaminase Activity

The test principle for the determination of SGPT is based on the oxidation of NADH to NAD^+ in the conversion of alanine to pyruvate, the resulting decrease in absorbance at 340nm being proportional to the activity of the enzyme in the sample. This is a modified formulation for the assay of SGPT, as recommended by the International Federation of Clinical Chemistry (IFCC). The IFCC reference method includes pyridoxal phosphate (PP) which functions as a co-factor in amino acid transamination. It increases SGPT activity. It also avoids falsely low values in samples containing insufficient

endogenous pyridoxal phosphate as in patients with myocardial infarction, liver disease and intensive care patients **[12]**.

The laboratory procedure involved taking a 1000ul of working reagent, 100ul of serum, which were mixed and incubated at 37^{o}C for 1 minute. The change in absorbance (A/min) was read at 340nm in a glass cuvette of 1cm path length within 3 minutes. SGPT activity (U/L) was obtained by A/min x 1768.

The determination of SGOT is similar to that of SGPT but only differed in the substrate, aspartate. The oxidation of NADH to NAD^{+} and resultant decrease in absorbance at 340nm being proportional to the activity of SGOT in the sample **[8]**.

Determination of Serum Alkaline Phosphatase (ALP) Activity

Serum alkaline phosphatase (ALP) was determined on the basis of the conversion of p-nitrophenol to its intensely yellow coloured derivative, 4-nitrophenoxide **[7]**. The laboratory method involved setting up tubes for serum sample, serum blank, standard and reagent blanks. 2mls of disodium phenyl phosphate carbonate bicarbonate buffer was added to all the tubes and incubated for five minutes at 37^{o}C. 50µl of phenol (standard reagent) was added to the standard test tube only and 50µl of serum to sample tubes, all tubes were incubated for exactly 15 minutes at 37^{o}C and the blocking reagent (4-amino antipyridine) was then added to all the tubes in a quantity of 0.5ml each.0.5ml of sodium arsenate buffer (pH 10) was added to all the tubes and properly mixed. 50µl of distilled water was added to the serum sample and reagent blank tubes only. They were mixed, allowed to stand for 10 minutes in the dark and the colour intensity measured at 510nm.

This solution was stable for 45 minutes. Activity was calculated using the absorbance of serum blank and serum samples in relation to the optical density of the standard using a factor of 142.

Determination of Glucose-6-Phosphate Dehydrogenase (G6PD) Activity

The test Principle of G6PD activity in red blood cells (RBC) involved releasing the enzyme by a lysing agent present in the reagent. The G6PD released catalyses the oxidation of glucose 6-phosphate with the reduction of $NADP^{+}$ to NADPH. The rate of reduction of NADP to NADPH is measured as an increase in absorbance which is proportional to the G6PD activity in the sample.

The laboratory procedure involved pipetting 1ml of G6PD working reagent in a clean dried test tube with 0.01ml of fresh whole blood, mixed well and incubated for 5-10 minutes at room temperature (26^{o}C) after which 2.0ml of

starter reagent was added. They were mixed again and incubated at 37^0C for 5 minutes. The change in absorbance was read after 1, 2 and 3 minutes at a wavelength of 340nm. G6PD activity was obtained from A x 4778 and expressed as:**U/gHb/Hb (g/dl)**

Where Hb = PCV/3

Determination of Serum Lactate Dehydrogenase (LDH) Activity

The quantitative diagnostic determination of LDH involved a kinetic ultraviolet method based on the catalysis of pyruvate and NADH to produce NAD^+ and L – lactate.

The initial rate of the NADH oxidation is directly proportional to the catalytic LDH activity and was determined by measuring the decrease in absorbance at 340nm.

The laboratory procedure involved pipetting into a cuvette at 37°C a working reagent (1ml) and sample of (20µl) which was mixed. The initial absorbance was read after 30 seconds and was read again after 1, 2 and 3 minutes. The mean absorbance change per minute (A/min) was determined and multiplied by a factor of 8095.

Statistical Analysis

Results are expressed as mean ± Standard error of the mean. Students T-test was used to test for differences between groups using statistical package for social sciences (SPSS) version 16. A value of $p<0.05$ was accepted as significant.

RESULTS AND DISCUSSION

Values obtained for enzyme activities in malaria patients and healthy controls are given in **Table 1**. It shows significant ($p<0.05$) elevations in the activities of SGPT, SGOT, ALP and LDH in the test group compared with the controls. These values are also within the IFCC upper reference limit for both sexes. G6PD activities were high but also within the reference values of 8-14 U/gHb and not significantly ($p>0.05$) different between test and control groups.

Table 2 indicates enzyme activities of male subjects alone. The activities of SGPT, SGOT and ALP are significantly ($p<0.05$) higher in test males compared to the controls. LDH and G6PD levels are however not appreciably varied between the two groups.

The levels of SGPT, SGOT, LDH and G6PD significantly ($p<0.05$) varied between healthy and malaria infected females **(Table 3)**. ALP activity in this category remained unchanged.

Table 4 represents the comparison of the activities of the enzymes between male and female patients. Transaminases (SGPT and SGOT) are not significantly ($p>0.05$) different but ALP, LDH and G6PD values were significant ($p<0.05$). Packed cell volume **(Figure 1, 2)** were significantly ($p<0.05$) higher in healthy individuals than malaria patients irrespective of gender.

Table 1: Serum Enzyme Activity in Malaria Patients and Healthy Subjects

	Mean + S.E.M (IU/L)	
Enzymes	**Control**	**Test**
SGPT	**27.52 ± 2.63**	**36.92 ± 2.11[a]**
SGOT	**21.93 ± 1.35**	**35.04 ± 2.23[a]**
ALP	**35.55 ± 2.52**	**45.71 ± 1.79[a]**
LDH	**329.24 ± 27.61**	**398.88 ± 22.44[a]**
G-6-PD (U/gHb)	**4.69 ±1.11**	**7.13 ± 1.24[b]**

NOTE: [a] = p < 0.05; [b] = p > 0.05

Table 2: Serum Enzyme Activity in Male Malaria Patients and Healthy Male Subjects

	Mean ± SEM (IU/L)	
Enzymes	**Male Control**	**Male Test**
SGPT	**27.92 ± 3.86**	**38.40 ± 2.92[a]**
SGOT	**21.93 ± 1.85**	**38.91 ± 3.23[a]**
ALP	**33.92 ± 5.18**	**49.67 ± 2.34[a]**
LDH	**312.21 ± 36.14**	**304.95± 21.38[b]**
G-6-PD (U/gHb)	**4.12 ± 1.58**	**4.99 ± 1.06[b]**

NOTE: [a] = p < 0.05; [b] = p > 0.05

Table 3: Serum Enzyme Activity in Female Malaria Patients and Healthy Female Subjects

	Mean ± SEM (IU/L)	
Enzymes	**Female Control**	**Female Test**
SGPT	**27.01 ± 3.62**	**35.6 ± 3.0[a]**
SGOT	**21.90 ± 2.05**	**31.2 ± 2.9[a]**
ALP	**36.53 ± 2.82**	**41.9 ± 2.3[b]**
LDH	**344.08 ± 42.51**	**492.7 ± 28.3[a]**
G-6-PD (U/gHb)	**5.76 ± 1.13**	**8.69 ± 1.02[a]**

NOTE: [a] = p < 0.05 **[b] = p > 0.05**

Table 4: Comparison of Serum Enzyme Activities Between Male and Female Malaria Patients

	Mean ± SEM (IU/L)	
Enzymes	**Male test**	**Female Test**
SGPT	**38.40 ± 2.92**	**35.6 ± 3.03[b]**
SGOT	**38.91 ± 3.23**	**31.24 ± 2.91[b]**
ALP	**49.61 ± 2.34**	**41.92 ± 2.33[a]**
LDH	**304.95 ± 21.38**	**492.74 ± 8.35[a]**
G-6-PD (U/gHb)	**4.99 ± 1.06**	**8.69 ± 1.02[a]**

NOTE: [a] = p < 0.05 **[b] = p > 0.05**

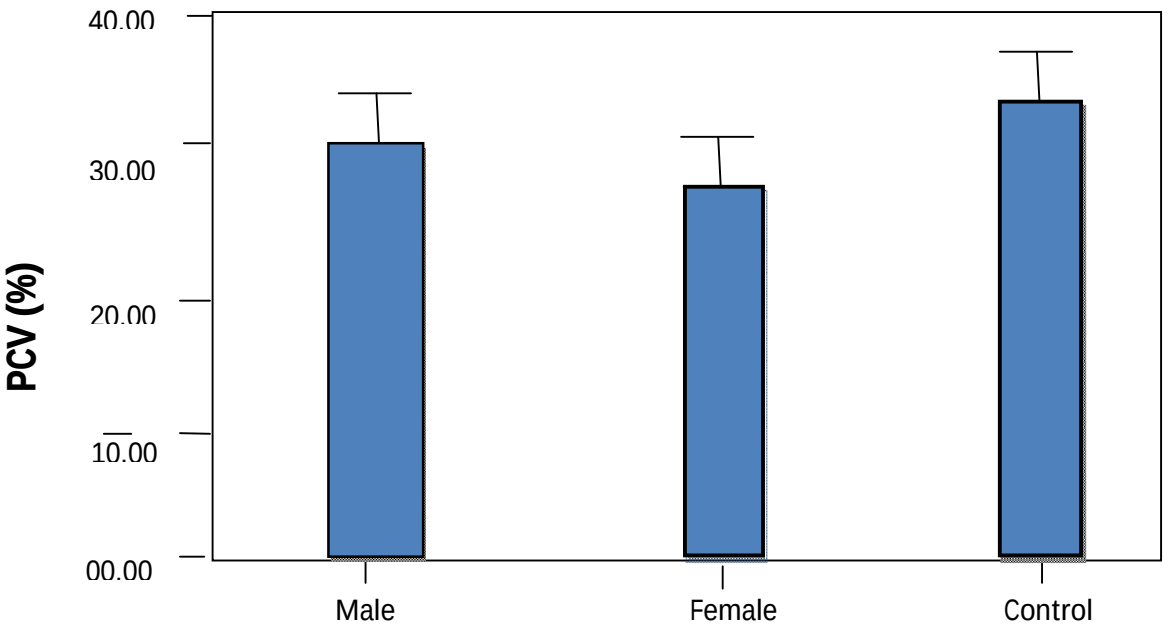

Figure 1: Packed Cell Volume Levels in Malaria Patients and Controls

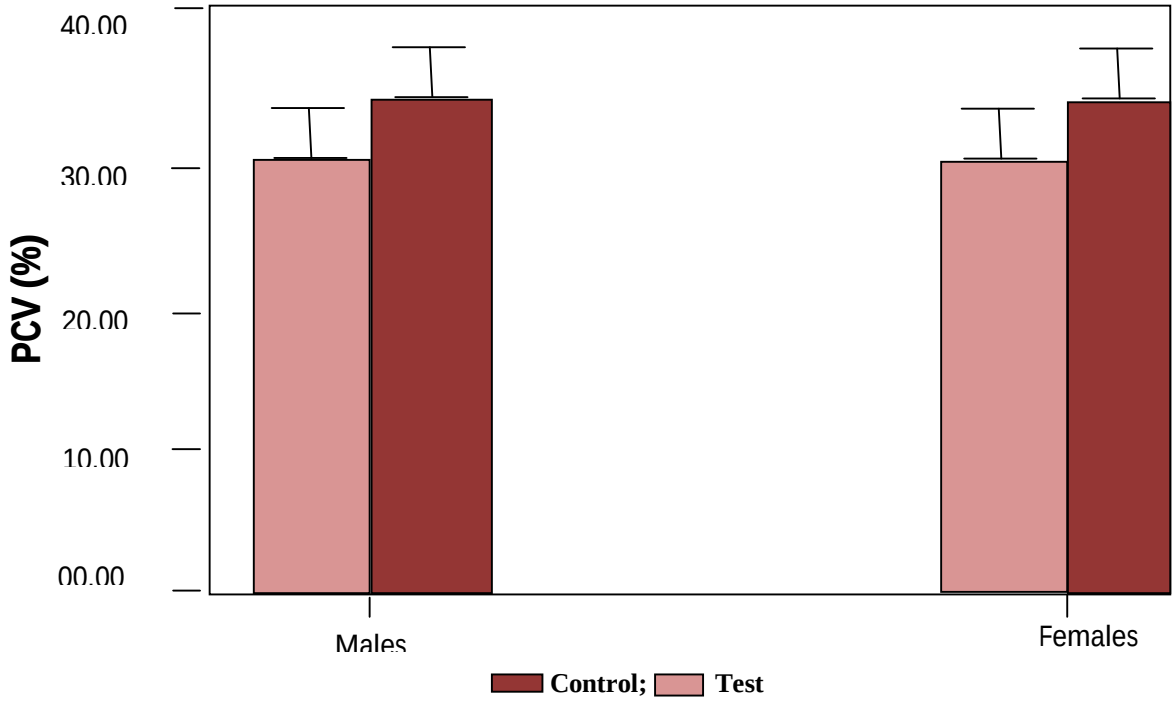

Figure 2: Packed Cell Volume Levels in Males and Females

DISCUSSION

Investigations into the effects of *Plasmodium* parasites on the levels of serum enzymes have gained recognition as an important area of research in the pathogenesis of malaria **[13]**. Hepatic involvement in the disease is common especially in severe cases which may manifest as jaundice i.e. raised serum bilirubin, hepatomegaly, elevated liver enzymes like aspartate and alanine transaminases **[14]**. There may also be low and falling serum albumin and prothrombin time may be prolonged **[15]**.

Plasmodium falciparum malaria is the most common and virulent form of the disease in tropical Africa.It predisposes to kidney or liver failure, coma and death **[16]**. Infections by the other species though causing less severe illness often result in recrudescence i.e. parasites (hypnozoites) remaining in the liver causing reappearance of symptoms months or years later **[17]**. It has been postulated that the factors involved in hepatic dysfunction in acute *Plasmodium falciparum* infection involves a synergy between local circulatory failure, centriolobular liver damage and the destruction of the host red blood cells consequent to erythrocytic merogony. The elevation in SGPT, SGOT, ALP and G6PD activities in patients over the healthy subjects is indicative of the hepatic stage of the parasites with significant perturbation and destruction of liver membrane parenchyma **[18]**.

The damage may have been caused by free radicals generated by malarial parasites **[19, 20]**. The consistently higher activities of SGPT and SGOT in males compared to females are in conformity with IFCC established upper reference limits for these enzymes.

ALP, SGOT and more specifically SGPT become elevated whenever disease processes affect liver cell intergrity. SGPT levels rarely increase in conditions other then liver disease. Moreover, elevations of in SGPT activity persists longer than that of SGOT **[9]**.

The increased ALP activity in the patients support the relevance of transaminases in malaria. This is because elevations in ALP activity commonly originate from the liver due to hepatobiliary disease. It could also originate from bone disease associated with increased osteoblastic activity, from the placenta in the third trimester of pregnancy, in patients with malignant disease etc. If the later group is precluded, as was the case in the present study, the liver is thus assumed to be the primary source in the elevation of ALP **[21]**. Glucose-6-phosphate dehydrogenase was generally high in the patients and this can be attributed to oxidative stress due to malaria

and other infections. Paradoxically, G6PD deficiency trait protect against *falciparum* malaria which accounts for its high frequency in malaria infested regions of the world. *Plasmodium* requires reduced gluthathione and the products of the pentose phosphate pathway for optimal growth. This advantage to heterozygotes is neither shared by normal homozygotes nor sickle cell homogzygotes **[22]**.

Since LDH exists in appreciable amounts in the liver and red blood cells, the clinically significant levels in malaria patients represents a pathophysiological combination of acute hepatocellular injury and red blood cell haemolysis induced by invading plasmodial merozoites **[18]**.

Also, the observed significant ($p<0.05$) LDH activity in female than males could additionally be linked to menstrual cycles in the former. LDH elevation and changes in the ratio of its isoenzymes represent some type of tissue damage due to its widespread distribution. Usually LDH levels will rise as the cellular destruction begins, peak after some period of time and then begin to fall. It could thus be concluded that the increase in LDH activity is indicative of a stress adaptive response as in the case of parasite invasion **[23]**.

The significant difference in haematocrit and haemoglobin levels signify anaemia a common manifestation of *falciparum* malaria which developes from profound haemolysis the degree of which correlates with parasitaemia and schizontaemia **[24]**. Anaemia in malaria is multufactorial, the causes of which include obligatory destruction of red blood cells at merogony, accelerated destruction of non-parasitized red cells, which is a major contributor in severe malaria, inhibition of erythropoiesis and dysfunction that can persist for weeks, shortened red cell survival and increased spleenic clearance.*Plasmodium* protozoan preferentially attack immature red blood cells but *Plasmodium falciparum* invades RBC of all ages. *Plasmodium* oxidizes red blood cell NADPH from the pentose phosphate pathway for its metabolic requirements. This results in the deficiency of red blood cell GSH, a condition that is most severe in G6PD deficient individuals leading to peroxide-induced haemolysis which also curtails the development of the parasite. After several cell cycles, the *plasmodium* can adapt to produce its own GSH, reducing the adaptive benefit of G6PD deficiency; this could be responsible for its lower activity in malaria patients **[22]**.

CONCLUSION

The assessment of SGPT, SGOT, ALP and LDH in malaria patients could represent additional and useful parameters in determining the clinical and prognostic aspects of the disease. This is because malaria is a disease whose pathogenesis is not clearly defined as it is specie-specific and of geographical variability. These findings have necessitated that cases of elevation in the enzymes analysed be referred for malaria parasite screening as the increased enzyme activity may be as a result of malarial parasites rather than primary liver damage, hepatitis or jaundice.

ACKNOWLEDGEMENT

The authors are grateful to the laboratory staff of the General Hospital Minna and University Health Services Unit of the Federal University of Technology Minna, Nigeria who assisted with the collection of samples and other technical issues. The SCIENCE AND TECHNOLOGY POST BASIC (STEP-B) programme of the Federal University of Technology, Minna provided some financial assistance which is also appreciated.

REFERENCES

[1] Dell'Agli M, Sanna C, Rubiolo P and Bosisio E, Antiplasmodial and insecticidal activities of the essential oils of aromatic plants growing in the Mediterranean area, Malar J.S, 11, 2012, 219

[2] Anthony MP, Burrows JN, Dupari S, Moehrle J and Well TNC, The global pipeline of new medicines for the control of malaria, Malar. J., 11, 2012, 316

[3] Soni S and Gupta S, *In vitro* Anti-plasmodial activity of *Eicostemma littorale*, Amer. J. Inf. Dis., 5, 2009, 259-262

[4] Jigam AA, Usman TA, Rukkaya SS and Patience SK, Effects of sub-chronic administration of *Diospyros mespiliformis Hoscht (Ebenaceae)* root extracts on some biochemical parameters in mice, J. App. Pharm. Sci., 2(5), 2012, 158-162.

[5] Eze KC and Mazeli FO, Radiological Manifestations of Malaria, The Resident Doctor, 5(1), 2001, 41.

[6] Pantegini M and Renze B, Enzymes, In: Tietz Fundamentals of Clinical Chemistry, Burtis CA, Ashwood ER and Bruns DE Ed., 6th Ed., 2012, 140-154.

[7] Burtis CA, Ashwood ER and Bruns DE, Tietz Fundamentals of Clinical Chemistry, 6th Ed., 2012, 317 – 335.

[8] Schumann G, Bonora R, Ceriotti F, Ferard G, Ferrero CA and Franck

PFH, IFCC primary reference procedures for measurement of catalytic activity concentrations of enzymes at 37°C, part 4, Reference procedure for the measurement of catalytic concentration of alanine aminotransferase, Clin. Chem. Lab Med., 40, 2002, 718-24.

[9] Panteghini M, Ceriottif, Schumann G, and Siekman L, Establishing a reference system in clinical enzymology, Clin. Chem. Lab med., 39, 2001, 795-800.

[10] Devlin TM, Ed., Textbook of Biochemistry with clinical correlations 6th Ed., John Wiley and sons Inc. Hoboken, New Jersey, 2006, 640.

[11] Green JH, An Introduction to Human physiology, Oxford Medical Publications, London, 1976, 128-130.

[12] Rahman MF, Siddiqui MK and Jamil K, Biochemical Toxicolgy, Biology Division, Indian Institute of chemical Technology, Hyderabad, India, 2002

[13] Ells G, Goldbery D and Spooner R, Serum Enzyme Tests in Disease of the Liver and Biliary Tract, Amer. J. Clin. Path., 197, 70, 248-258.

[14] Jigam AA and Kaka AB, Serum levels of Aspartate and Alanine (AST and ALT) transaminases in malaria patients in Minna, Niger State, Nigeria, Proc. 2nd Ann. Conf. Sch. Sci. and Sci. Edu. Fed. Univ. Technol. Minna, 2006, 20-23.

[15] Anand AC, Ranji C and Narula AS, Malaria hepatitis, a heterogeneous syndrome, Nat. J. India, 5(2), 1992, 59 – 62.

[16] Mishra SK, Mohapatra S, Mohanty S and Mohapatra DN, Acute Renal Failure in *falciparum* malaria, J. Indian Acad. Clin. Med., 3(2), 2002, 141-147.

[17] Bruce-Chwatt LJ, Essential Malariology, 2nd Ed., William Heinemann Medical Books, London, 1980, 28-34.

[18] Jarike AE, Emuveyon EE and Idogun SF, Pitfalls in the Interpretation of Liver Parenchymal and Membranous Enzyme Result, Nig. Clin. Med., 10(2), 2002, 21-27.

[19] Price RN. Adverse effects in patients with acute *falciparum* malaria treated with artemisinin derivatives, Amer. J. Trop. Med. and Hyg., 1999, 60:547-355.

[20] Nyblom H, Bjornsson E, Simren M, Aldenborg F, Almer OS and Olsson R, The AST/Alt ratio as an indicator

of Cirrhosis in patients with PBC, Liver invest., 26(7), 2006, 840-845.

[21] Maduka CI, Neboh EE, Ikekpeazu EJ, Ureme SO, Umeh C and Ejezie E. Assessment of liver enzymes in asymptomatic HIV seropositive patients, Res. J. Bio. Sci., 4(3), 2009, 360-362.

[22] Berg JM, Tymoczko JL and Stryer L, Biochem., International Ed., 5th Ed., W.H Freeman and company, New York, 2002, 5.

[23] Pattanasins S, Proux S, Chompasuk D and Nosten F, Eradication of a new plasmodium Lactate Dehydrogenase Assay (Optimal-TT) for the Detection of Malaria, Transac. Royal Soc. Trop. Med., 97(6), 2003, 672-4.

[24] Cheesebrough M. Laboratory Diagnosis of Malaria Parasites. In: District Laboratory Practice in Tropical Countries, Cambridge University Press, 1998, 246-250.