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Changes in liver enzymes and lipid profile of pregnant women with malaria in Owerri, Nigeria

Nwosu, D.C.[1], Nwanjo, H.U.[1], Obeagu, Emmanuel Ifeanyi[2*], Ibebuike, J.E.[3], Ezeama, M.C.[3] and Ihekireh, D.I.[4]

[1]Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Nigeria
[2]Diagnostic Laboratory Unit, University Health Services, Michael Opkara University of Agriculture, Umudike, Abia State, Nigeria
[3]Department of Nursing Science, Imo State University, Owerri, Nigeria
[4]Department of Optometry, Faculty of Health Sciences, Imo State University, Owerri, Nigeria
Corresponding author

KEYWORDS

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A B S T R A C T

Study on changes in lipid profile and liver enzymes of pregnant women with malaria was carried out using 40 subjects grouped into two of test and control, Total Cholesterol (TC),High Density Lipoprotein (HDL), low Density Lipoprotein (LDL),Triglycerides (TG) and liver enzymes such as Aspartate Aminotrasferase (AST), Alanine Aminotrasferase (ALT), and alkaline phosphatase (ALP), were investigated. In the lipid profile TC and TG shows significant increase in pregnant women talking with malaria (Test) with mean value- of 170,9±5,6 mg /dl and 106±6,0mg /dl (P<0,05) when compared with those without malaria (control) 116±6.7 mg/dl and 83.3±10.0mg /dl. Significant increase was observed in HDL and LDL with mean value of 51.0±3.5 mg/dl and 91.0±16.4 mg/dl (P<0.05) when compared with the control (45.2±4.4mg/dl and 54.7±5.1mg/dl). For the liver enzymes activities ALT and AST shows significant increase in tests with mean values of 18.6±1.6 IU/L and 27.6±2.2 IU/L (p<0.05) when compared with the control (13.0±2.4 IU/L and 16.6±3.5 IU/L) while the ALP increased in test with mean value of 39.0±2.1 IU/L (P<0.05) when compared with the control (24.6±4.4) IU/L. Malaria aggravate the hyperlipidemia associated with pregnancy.

Introduction

Malaria has become a major global problem affecting more than 2,000 million populations and causing 1.5 million deaths annually (Mishra and Mohanty, 2003).

During pregnancy, it poses a significant threat to both the mother and the unborn child, for the mother, it increases the risk of illness, severe Aneamia and death, and for

the unborn child it increases the risk of intra-uterine growth retardation and low birth weight (Recker *et al.,* 2009).

According to Ochie (2000) transmission of the disease to humans occurs when an infected female Anopheline mosquito injects the sporozoites while taking its blood meal, the disease can also be transmitted through blood transfusion, contaminated hypodermic needles as a congenital infection.

The four species of malaria parasites that infect human are plasmodium *falciparum, plasmodium vivax, P, malariae* and *P. ovale* (Nosten *et al.,* 1999). Parasites densities are higher in pregnant women than in non pregnant adults because they are more attractive to mosquitoes (Lindsay et al.,2000).

The pathology of malaria during pregnancy and its evaluation are highly dependent on the particular epidemiological setting due to differences in acquired immunity in women reaching child-bearing age (Recker *et al.,2009).*According to Cheesbrough (2005) the main symptom of malaria are fever, severe headache, back and joint pains vomiting, diarrhea, anaemia, Jaundice, splenomegally.

Placental malaria specifically affects pregnant women and is caused by placenta! sequestration of *P. Falciparium* parasitized red cells (Nosten *et al.,* 1999), Cerebral Malaria is the commonest cause of coma and death in falciparium malaria, particularly in children and non-immune adults. Many parasitized cells can be found in the capillaries of the brain and other organs (Cheesbrough, 2005). Pregnancy is associated with normal physiological changes that assist the nurturing and survival of the foetus. Biochemical parameters reflect these adaptive changes, and are clearly distinct from the non-pregnant state. (Trail,2005) lipids are heterogenous groups of fats, and fat like substances characterized by being water insoluble and soluble in non-polar solvent. (Teitz, 2008).

Chemical structures of the four main forms of lipids present in plasma are fatty acids triacylglycerols phospholipids and cholesterol.

According to Baker et al.(2001) the liver helps in synthesis of cholesterol phospholipids,endogenous triglycerides and lipoproteins.

The liver enzymes include the Amino transferases which are aspartate Amino transferase (AST) and Alanine Aminotraiisferases .T) ALT).They are concern with Amino acid metabolism. Alkaline phosphatase is present in most organs of the body and is especially associated with membrane and cell surfaces. An increase of up to two-three times the upper reference limit is observed in women in the third trimester of pregnancy with the additional enzyme being of placental origin (Tietz, 2008). However, little or no study was done, correlating to liver enzymes in pregnant malarious patients hence the work.

The main aim of this study includes to determine the lipid profile level in pregnant women with malaria. To determine the level of liver enzymes, AST ALT and ALP in pregnant women with malaria. And also to compare the lipid profile level and liver enzymes activities of pregnant women with malaria and those without malaria.

Materials and Methods

Study area

The study was conducted at General Hospital Umuguma, Owerri, a state owned hospital.

Study population

The subjects consist of 40 pregnant women,20 malaria women as test subjects and 20 non-malaria as control attending the General Hospital Umuguma Owerri. Malaria diagnosis was confirmed by demonstrating malaria parasites in the blood of the subjects by Giemsa staining technique.

Selection criteria

The selection was based on the following:-

a.Subject's (control) did not present with any clinical signs of ill health i.e. they were apparently healthy by clinical standards.
b.Subjects (Test) are not on antimalaria drug and exclude those who had typhoid infection.
c.Those from whom informed consent was obtained.

Exclusion criteria

Those with known underlyning choice illness such as typhoid, liver disease, were excluded.

Patients who presented with clinical symptom such as fever, headache, body weakness and nausea but without parasites obtained in blood smears were excluded. Patients on drugs that affect lipid metabolism and liver enzymes were also excluded as well as those who refused consent.

Sample collection

Using a sterile 5ml syringe, about 5mls of blood sample was collected from each subjects by clean venipulture from the anticubital vein into plain tubes and allowed to clot at room temperature, sera were obtained after centrifugation using surgien field centrifuge (Model SM 800D) at 3000 revolution per minute for 10 minutes. The sera got were then used for analysis of Lipid profile and Liver enzymes under study.

Laboratory procedures

All reagents were commercial purchased and the manufacturers Standard Operating Procedure (SOP) were strictly followed. LDL cholesterol was, calculated using the empirical equation of Friedewalt *et al.*,(1972).

A. Serum total cholesterol determination

The biosystem total cholesterol reagent kit was used - COD number 11505 (Catalog Number)

Principle

In the presence of excess acid such as phosphoric acid and ferric ions (fe^{++}), cholesterol is oxidized to disulphoric acid which is reddish purple in colour. It is read colorimetrically at 56.0nm (green yellow filter).

Procedure

Test tubes were arranged into test, standard and blank. 1ml of cholesterol reagent was added to all tubes. Then 10ml of the sample cholesterol standard and distilled water was added to the test tube respectively. It was thoroughly mixed and incubated for 10 minutes of room temperature.
The absorbance of sample and standard was read against the blank at 500nm.

B. Serum HDL-cholesterol determination

The Biosystem HDL cholesterol reagent Kit was used with catalogue number 1-1523.

Chylomicrous VLDL and LDL are precipitated by phosphqtungstic acid in the presence of magnesium ions, leaving HDL in solution the cholesterol content of the supernatant can determined by the usual method. About 0.5ml of plasma, 0.5ml of phosphotungstic acid reagent and 0.02ml of magnesium chloride reagents were pipetted into a centrifuge tube, mixed well and centrifuge as 5000rpm *for* 20minutes, using a pasture pipetted for drawing it out separated the supernatant.

Three test tubes were dispensed and labeled test (T) standard (S) and blank (B) respective. 0.1ml of supernatant was added to the test tubes and 0.1ml of distilled water added to the test tube (B).2.5ml of cholesterol reagents 1 was added into 3 test tubes and they were thoroughly mixed and kept in water bath at room temperature. Absorbance of the test and the standard were read against blank at 575nm.

C. Triglycerides estimation

The Randox triglyceride reagent kit with catalogue number TR 210 was used.

Triglycerides are hydrolysed by the enzyme lipase to produce glycerol and fatty acids. The enzyme glycerol kinase act on glycerol in the presence of ATP to form glycerol -3-phosphate and ADP, glycerol 3 phosphate is oxidized by glycerol phosphate oxidase to dihydroxyacetone and hydrogen peroxide oxygen is released from the H2O2 in the presence of peroxidase, which oxidizes p-chloropherol chromagen to form a coloured compound, which is measured colorimetrically.

Procedure

Test tubes were arranged into test, standard and blank. Then lOjil of sample, triglyceride marked 'test' 'standard' and *'blank'* respectively. It was mixed and incubated for 10 minutes at room temperature. The absorbance of the sample and standard was read against the blank.

D. Serum low density lipoprotein determination

The emperical equation of Friedewalt *et al.(* 1972) was used LDL-C = Total cholesterol - Triglyceride + HDL

E. Determination of aspartate aminotransferases (AST)

The Randox reagent kit with catalogue number AS 100 was used. The method used was Reitman and Frankel method reported by Cheesbrough (2007). Aspartate aminotransferase catalyzes the transfer of the amino group from ketoglutarate, thereby farming oxaloacetate and glutamate. The oxalpacetate reacts with 2,4- dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone which in alkaline medium gives a red-brown colour. The absorbance of the colour was red at 505iim and is equivalent to the AST present. The equation for reaction is thus; a-Oxoglutarate + L-Aspartate -glutamate + Oxoacetate

Test tubes were, arranged into reagent blank, and sample. Exactly 0.5ml of AST buffer was added to all the test tubes. Then, O.lnil Of serum and distilled water was added to sample test tubes and reagent blank test tubes respectively. This was mixed and incubated at 37C for 30 minutes; then 0.5ml of AST 2, 4-dinitrophenylhdrazine was added to all test tubes. It was mixed and incubated at room temperature for 20 minutes. 5,0ml of sodium hydroxide was added to all test tubes. This was mixed and the absorbance of the sample read against

the reagent blank after 5 minutes at 505nm.

F. Alanine aminotransferase (ALT)

The Randox reagent kit with catalogue number AL100 was used. The method used was Reitman and Frankel method reported by Cheesbrough (2005).

Alanine aminotranferase catalyzes the transfer of the amino group from alanine to ketoglutarate. The pyruvate reacts with 2,4- dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone whichgives a reddish brown colour in an alkaline medium. The absorbaiice was reacting at 505nm.

The equation for reaction is
a-Oxoglutarate + L-alaline – L-glutamate Pyruvate

Test tubes were arranged into reagent blank and sample. Exactly 0.5ml of ALI buffer was added to all the test tubes. Then O.lml of serum and distilled water was added to sampled test tubes and reagents blank test tubes respectively. This was mixed and incubated at 37C for 30 minutes then 0.5ml of ALT 2, 4-dinitrophenylhydrazine was added to all test tubes, it was mixed and incubated at room temperature for 20 minutes. 5.0ml of sodium hydroxide was added to all the test tubes. This was mixed and the reagent blank after 5 minutes at 505nm.

F. Alkaline phosphatase (ALP)

The Teco reagent kit with catalogue number A506 was used.

The modified King Armstrong method reported by Nwanjo (2006) was used.

The alkaline phosphatase acts upon the AMP buffered sodium thymolpthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen which is measured photometrically at 510nm wavelength. 0.5ml of alkaline phosphatase substrate was dispensed into test tubes labeled test, standard and blank. At time intervals, 0.05ml of each sample's serum was added to the test tubes labeled test alongside the standard and the distilled water respectively. This was mixed and incubated for 10 minutes at 37C, then 2.5ml of alkaline phosphatase colour developer and mixed.

The absorbance of the sample against the blank was read.

Statistical analysis

Statistical evaluation of data was performed using the population of mean and standard deviation. The test of significance was done using students' T-test.Level of significance was set at $p<0.05$

Results and Discussion

The table below shows the mean values of serum lipid profile, and liver enzymes level of control, test and level of significance as it is observed in the research:

In the table 1 above TC shows significant increase in test with the mean value of 170.9±5.6 mg/dl ($P<0.05$) when compared with the control (116±6.7 mg/dl). There was significant increase in test of TG with the mean value of 106.0+ 6.0 mg/dl ($P<0.05$) when compared with the control (88.3+10.0 mg/dl). LDL shows significant increase in test with the mean value of 91.0±16.4mg/dl9p<0.05) when compared with the control(54.7±5.1 mg/dl).There was also significant increase in test of HDL with the mean value of 51.0±3.5 mg/dl ($P<0.5$) when compared with the control (45.2±4.4) mg/dl.

In the analysis of table 2 ALT shows, significant difference when comparing the mean value of test with that of control (39.0+2. Hu/L and 24.6±4.4Iu/L). There was significant increase in test, of AST with the mean value of 27.6± 2.2Iu/L (P<0.05) when compared with the control (16.6±3.5Iu/]L). Significant increase was observed in test of ALP with mean value of 39.0±2.1Iu/L (P < 0.05) when compared with the control (24.6±4.4)*lu/*

In this study ability to synthesize different lipid moieties and their distribution through plasma to all the body tissues seems to be altered in pregnancy with malaria. In this present study, the level of total cholesterol and triglycerides show significant increase in test when compared with the control this correlates with the work done by Eteng *et al.*(2010).and is also in agreement with the work done by Parola *et al.* (2004).

Table.1 Mean value of total- cholesterol (TC) High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Triglyceride (TG) of test and control

PARAMETERS	TEST	CONTROL
TC (mg/dl)	170.9+5.6	116.0±6.7
HDL (mg/dl)	51.0±3.5-	45.2±4.4
LDL (mg/dl)	91.0+16.4	54.7±5.1
TG (mg/dl)	"l06.0±6.0	83.3±10.0

Lengend: X - Not significant when P>0.05 XX = Significant different P<0.05

Table.2 Mean value of Serum AspartatG Aminotransferas (AST), Alanine Aminotransferase (ALT) and Alkaline phosphatase (ALP) of test and Control as it was observed in the research

PARAMETERS	TEST	CONTROL
AST (lu/L)	27.6±2.2	16.60±3.5
ALT *(lu/L)*	18.6±1.6	13.0±2.4
ALP(Iu/L)	39.0±2.1	24.6±4.4

Lengend: X = Not significant when P>0.05; XX - Significant different P<0.05

It is well known that serum cholesterol and triglycerides increases in pregnancy as a result of changes in sex hormones, genetic and environmental factors. In this present study it was seen that malaria worsens lipid status in pregnancy. In terms of pathogenesis, the liver of the host is among the organs affected in early stage of malaria Falciparum Malaria leading to significant alteration in host hepatocyte physiology and morphology. Malaria involvement with the liver is indicated by abdominal pain, nausea, vomiting with bile or blood on it, thus triglyceride and cholesterol are increased in liver disease.

From the result analysis LDL and HDL value also showed significant increase in test when compared with the control. In this present study, it was seen that markers of liver function increased, there was a significant increase in AST ALT and ALP value of the test when compared with the control. However, it is known that in

pregnancy all markers of liver function decrease due to expansion of extracellular fluid except alkaline phosphatase which is elevated due to ALP of placenta origin. The picture appears to be different in the presence of malaria as there was a significant increase in ALT, AST and ALP. This increase in the activity of enzymes especially ALP may be an indication of the hepatic stage of the parasite life cycle in its human host and is accompanied by a significant perturbation in the hepatocytes membrane leading to leakage of this enzymes out of the liver cell. This is in concordat with the work done by Garba and Gregory (2005). It is also in agreement with the work of Maegraith *et al. (1981).* Changes in serum ALP activity can be used as a potential biomarker in assessing the integrity of the hepatic drainage system during acute malaria infection *(Falciparum).*

Conclusion

In conclusion,it shows that liver disease as a result of malaria parasite infection can result to alteration in lipid metabolism. The result of study suggests that malaria aggravate the hyperlipidermia associated with pregnancy. In this present work it also shows that liver enzymes AST ALT and ALP increases in pregnancy with malaria as a result of perturbation in the hepatocyte membrane of the host leading to leakage of these enzymes out of the host liver cell. In pregnancy, malaria tends to induce labour, abortion and occasionally still birth. To ensure accurate conclusion, it is necessary to rule out all other possible reasons of hepatic enzyme increase in pregnancy such as those related to intrahepatic cholestasis of pregnancy, acute fatty liver enzymes of pregnancy.

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