

## SHORT COMMUNICATION

## Glycohaemoglobin Concentration and Hepatocellular Enzymes Activities in Malaria Patients in Owerri, Nigeria

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**Abstract:** *Objectives:* The study aims to estimate the level of glycohaemoglobin concentration and activities of hepatocellular enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in patients with malaria. *Background:* Malaria is a public health problem in Nigeria and a major hinderance to economic development. There is growing evidence that malaria affects the hepatic cells. *Method:* 100 confirmed malaria patients age ranged between 10-20 years attending General Hospital Owerri were selected for the study. 100 normal subjects age 10-20 years free from malaria were used as a control group. Patients with infections, catarrh and cough were excluded. *Results:* The result showed that the glycohaemoglobin significantly decreased ( $4.80 \pm 1.6\%$ )  $P < 0.05$  in malaria subjects when compared with the control ( $5.92 \pm 0.42\%$ ). Serum activities of aspartate aminotransferase ( $29.90 \pm 5.82$ iu/l) and alanine aminotransferase ( $19.64 \pm 3.11$ iu/l) were significantly higher  $P < 0.05$  in malaria subjects when compared with the control ( $19.71 \pm 2.41$ iu/l) and ( $13.24 \pm 2.16$ iu/l) respectively. Alkaline phosphatase serum activity was not significantly different when compared with the control. *Conclusion:* This shows that malaria patients could probably be prone to low glycohaemoglobin which would indicate a likelihood of hypoglycaemia, and some hepatic enzymes impairment.

**Key words:** Glycohaemoglobin, hepatocellular enzymes, Malaria, Owerri, Nigeria

### Introduction

Malaria is a vector borne infectious disease caused by plasmodium. It is widespread in the tropics and subtropical region. There are approximately 350 to 500 million cases of malaria killing between one and three million people yearly [1]. The majority are mostly children in sub Sahara Africa. Malaria is a major hindrance to economic development and it is associated with poverty. It is one of the most common infectious diseases and a great public health problem [2]. The most serious forms of this disease is caused by *Plasmodium falciparum* usually, people get malaria by being bitten by an infective female anopheles mosquito [3]. Malaria transmission can be reduced by preventing mosquito bites with mosquito nets and insect repellents. The classic symptom of malaria is cyclical occurrence of sudden coldness followed by vigor and then fever and sweating lasting 4 to 6 hours [4-5]. Malaria has been found to cause cognitive impairment especially in children. Neurologic damage can occur resulting from cerebral malaria to which children are more vulnerable [6]. In the other hand, the malaria affects the liver in one way or the other. Liver is the largest solid organ in the body. An initial step towards detecting liver damages is the determination of the presence of certain enzymes in the blood. These enzymes resides within the cells of the liver. However when hepatocellular damage for any reason, these enzymes are spilled into the blood stream raising the enzymes activities in the blood.

Among the most widely used liver enzymes are aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase. Alanine aminotransferases catalyze reactions in which the building block of protein (amino acid) is transferred from a donor molecules to a recipient molecule. It is found largely in the liver. Hence, it serves as a slightly marker of liver while Aspartate aminotransferase is found in a diversity of tissues including liver, muscle, heart, kidney, and brain. It is increased when any of these tissues is injured. Hence it is not a highly specific indicator of liver damage [7]. Also, alkaline phosphatase is found mainly in the bone as well as liver and placenta. It is more of specific marker to bones than liver. However, it is fairly used as an indicator in liver damage, though, it is not specific. Alkaline phosphatase is an enzyme in the cells of lining the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction. The liver also helps in the regulation of glucose level in the blood. The average glucose concentration attached to haemoglobin could be referred as glycohaemoglobin [8]. It is characterized by haemoglobin that is irreversibly glycosylated to an N-terminal valine of the  $\beta$ -chain [9]. This process which is non-enzymatic, reflects the average exposure of haemoglobin to glucose over an extended period. In this present study, glycohaemoglobin concentration and hepatocellular enzymes (Aspartate aminotransferase (AST), alanine amino transferase (ALT) and Alkaline phosphate activities will be assayed with a view to provide information on their changes in malaria subjects in Owerri, Nigeria.

#### Material and Method

*Subjects:* 100 confirmed malaria patients (50 males and 50 females) aged 20-50 years diagnosed by Giemsa staining method [10] were selected for the study. Patients with infections, catarrh and cough were excluded. 100 normal subjects free from malaria were used as control. Informed consent was obtained from all the subjects verbally. *Blood Sample:* In all subjects, 5ml of veinous blood was collected. 3ml of blood was transferred into non-anticoagulated tubes and 2ml was put into EDTA container. The sample in non anticoagulant tubes was spun in a Wisperfuge (model 684) centrifuge at 1000g for 10minutes and the serum collected into a clean dry bijou bottle. The serum activities of AST, ALT and ALP were estimated within 2 hours of collection while sample in EDTA was used for the estimation of glycohaemoglobin.

*Estimation of glycohaemoglobin:* The glycosylated haemoglobin was measured by the colorimetric method using Teco Diagnostics kits GS540: 04/03. In brief, a haemolysed preparation of the whole blood is mixed continuously for 5minutes with a weak binding cation-exchanged resin. During this time, non-glycohaemoglobin binds to the resin. After the mixing period, a filter is used to separate the supernatant containing the glycohaemoglobin from the resin. The percentage glycosylated haemoglobin is determined by measuring the absorbance at 415nm of the glycohaemoglobin fraction and total haemoglobin fraction. The ratio of the two absorbances gives the percent glycohaemoglobin Estimation of aspartate aminotransferase and alanine aminotranferase activites were done using Reitman and Frankel method [11]. While estimation of alkaline phosphatase activities was done by using king and king [12].

## Result

Table-1 shows the mean glycohaemoglobin concentration and aspartate aminotranferase, alanine aminotranferase, in control and malaria infected subjects. The glycoslated haemoglobin concentration decreased from  $5.92 \pm 1.6\%$  in control group to  $4.80 \pm 0.42\%$  in malaria subjects. This was statistically significant ( $P < 0.05$ ). The serum activities of aspartate aminotranferase ( $29.90 \pm 5.82$ iu/L) and alanine aminotranferase ( $19.64 \pm 3.11$  iu/L) in malaria subjects were significantly higher when compared with the control ( $19.71 \pm 2.41$ iul) and ( $13.24 \pm 2.16$ iu/L) respectively ( $p > 0.05$ ). Also, the serum alkaline phosphatase showed no significant increase in malaria subjects ( $61.67 \pm 4.06$ iu/L) when compared with the control ( $60.42 \pm 4.25$ iu/L)  $P > 0.05$ .

Parameter	Control	Malaria subjects
Aspartate aminotranferase (iu/L)	$19.71 \pm 2.41$	$29.90 \pm 5.82^*$
Alanine aminotranferase(iu/L)	$13.24 \pm 2.16$	$19.64 \pm 3.11^*$
Alkaline phosphatase (iu/L)	$60.42 \pm 4.25$	$61.67 \pm 4.06$
Glycohaem. %	$5.92 \pm 0.42$	$4.80 \pm 1.6^*$
*Significantly different from control		

## Discussion

Aspartate aminotranferase (AST), alanine aminotranferase (ALT) and alkaline phosphate activities are used as markers in hepatic diagnosis while, glycosylated haemoglobin could be used as a valuable adjunct to blood glucose estimation in the diagnosis of diabetes. In this study, the increase in the levels of AST and ALT in malaria subjects reflects an impairment in the liver. When the liver is impaired the liver cells release the enzymes into the blood raising the enzymes levels. This is in line with the work of Nyblom *et al* [7] in which the levels of liver enzymes were increased in cirrhosis patients. This is consistent with the work of Maduka *et al* [13] in which the level of AST and ALT were elevated in HIV positive patients. The results are also in agreement with the works of Adam *et al* [14] and Price [15]. The elevation of the serum enzymes indigenous to the liver is an indication of hepatic dysfunction. The liver damage may have been caused by the free radicals generated by *Plasmodium falciparum* malaria. The deleterious effects were considered to be caused by free radicals produced during peroxide formation. Really, the levels of hydroxyl and peroxide radicals induced by *Plasmodium falciparum* parasites may be responsible for the changes in AST and ALT levels. Transaminases are the important markers of hepatocellular damage. The alanine aminotranferase is more specific to the liver damage because it is predominantly produced within the liver cells [14]. Furthermore, the level of alkaline phosphatase showed no significant increase. This may be probably that alkaline phosphatase are not specific to the liver. In the other hand, the study showed decreased glycohaemoglobin in malaria subjects when compared with the control.

This is in line with the work of Atabani *et al* [8] which stated that glycohaemoglobin are decreased in sickle cell anaemia. This could be associated with the destruction of red blood cell by the parasite, *Plasmodium falciparum* in malaria. Throughout the circulatory life of the red blood cell, glycohaemoglobin is formed continuously by the addition of glucose to N-terminal of the haemoglobin beta-chain. This process reflects the average exposure of haemoglobin to glucose over an extended period. Hence, the invasion of the red blood cell by *Plasmodium falciparum* results in decreased glycohaemoglobin. A low concentration of glycohaemoglobin is an indication of hypoglycaemia [16].

These observations showed that malaria patients could be prone to hypoglycaemia as well some hepatocellular damage or dysfunction.

#### References

1. Snow RW, Guerra CA, Noor AM, Myint HY and Tay SI. The global distribution of clinical episodes of *plasmodium falciparum* malaria. *Nature* 2005; 432:214-217.
2. Boivin MJ. Effects of early cerebral malaria on cognitive ability in Senegalese children. *J Dev Behav Paediatr* 2002; 23(5): 353-364.
3. Muller I, Zimmerman PA and Reader JC. *Plasmodium malariae* and *plasmodium ovale*, the bashful malaria parasite. *Trends Parasitol* 2007; 23(6): 278-283.
4. Holding RA and Snow RW. Impact of *plasmodium falciparum* malaria on performance and learning. *Am J Trop med Hug* 2001; 64:68-75.
5. Maude RJ, Hassan MU and Bear NA. Severe vitinal whitening in an adult with cerebral malaria. *Am J Trop. Med. Hug.* 2009; 80(6):881-882.
6. Collins, WE and Barnwell JW. *Plasmodium knowlesi*: finally being Recognized. *J infect Dis.* 2009; 199(8):1107-1108.
7. Nyblom H, Bjornsson E, Simren M, Aldenborg F, Aldenborg F, Almer OS, and Olsson R. The AST/Alt ratio as an indicator of Cirrhosis in patients with PBC. *Liver Int* 2006; 26(7): 840-845.
8. Atabani GS, Hassan DA, Abdul R and Saeed BO. Glycosylated haemoglobin levels in Sudanese sickle cell anaemia. *Acta Haematologica.* 1989; 81:140-142.
9. Bry PC. Effects of haemoglobin variants and chemically modified derivatives of glycohaemoglobin. *Clin. Chem.* 2001; 47:153-163.
10. Sutherland CJ and Hallett R. Detecting malaria parasites in the blood. *J Infect Dis* 2009; 199(11):1561-1563.
11. Reitman S and Frankel S. Transaminases. *Am J Clin Pathol* 1957; 28:56.
12. King EJ and King PRN. Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrene *J Chem. Path* 1954; 7:322-326
13. Maduka CI, Neboh EE, Ikekpeazu EJ, Ureme SO, Umeh Cand Ejezie E. Assesment of liver enzymes in asymptomatic HIV seropositive patients. *Res. J Bio. Sci* 2009;4(3):360-362
14. Adam S, Alaquatrain AA and Alhag EA. Effects of various levels of dietary artificial leaves on rats. *Laboratory animals*, 2001; 34(93):307-312.
15. Price RN. (1999) Adverse effects in patients with acute *falciparum* malaria treated with artemisinin derivatives. *Am J Trop Med Hyg* 1999; 60(4):547-555.
16. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D and Heine RJ. Translating the A1C assay into estimated average glucose values. *Diabetes Care* 2008; 31(8):1473-1478.

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