International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR |Volume 4 | Issue 3 | July - Sep - 2016
www.ijamscr.com

Research article *Medical research*

Renal Biomarkers in Plasmodium Infected Hepatitis B Surface Antigen (Hbsag) Sero-Positive Patients

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ABSTRACT

Background

While co-infection with HBV and malaria is a major public health problem in large areas of the world, the mutual interactions between the two pathogens are poorly understood.

Aim and Objective

This study is designed to investigate the renal biomarkers in plasmodium infected HBsAg seropositive patients.

Method

Semi structured and pre tested questionnaires were administered to 100 participants to obtain their demographic information. From each individual subject, 5 ml of blood sample was obtained via venopuncture from the subjects using vacoutainer needle which was used for parasitological analysis and to obtain the serum for the serological detection of the HbsAg, serum creatinine, uric acid and GST.

Result

There was a significant difference ($P < 0.05$) in mean values of GST, uric acid and serum creatinine when control subjects was compared among plasmodium falciparum infected patients (PM), hepatitis B virus infected patients and patients co-infected with both organisms.

Conclusion

With regards to the following result, there is a significant alteration in serum level of creatinine, uric acid and glutathione s transferase among patients co-infected with malaria and hepatitis B virus.

INTRODUCTION

Malaria has been described as entirely preventable and treatable blood-borne mosquitos' transmittable disease [1]. However, despite continuous global efforts at all levels of health care to achieve global control, it still remains endemic in tropical and subtropical region, though with decreasing trend. Malaria remains a wide spread health threat to humanity, affecting more than halve of the entire humanity, and it was estimated that more than 50% of the population in endemic African region experience at least one episode of malaria yearly [1].

Hepatitis B virus (HBV) infection is also a preventable viral infection that affects the liver and can cause both acute and chronic liver diseases. It is endemic in regions of the world, including Sub Saharan Africa. The importance of the disease is

stressed by the ample reservoir of carriers seen in human population globally, which are estimated to be 320-350 million. Malaria and HBV infections are co-endemic throughout much of the tropical and sub-Saharan Africa, and they both present major threats to public health. Coinfection of Malaria and HBV may occur in areas where both infections are endemic and because of their geographical coincidence [2]. These two infections share some of their developmental stages within the liver which may cause impaired clearance of the liver stages of the malaria parasite due to hepatocytes damage in HBV infection. Therefore, coinfection with Plasmodium parasite and HBV virus in an individual may possibly influence further pathogenic progression of both agents resulting in severe morbidity, complications and increased mortality [2].

Renal parameters are of utmost importance in (chronic hepatitis B) CHB patients for renal dysfunction impacts clinical outcomes [3]. In a prospective study, including patients with HBV infection, the authors showed that an elevated serum creatinine at baseline was significantly associated with mortality rates at 6 months in multivariate analysis, with a hazard ratio (HR) for death of 5.23, almost as high as that of detectable HBVDNA. HBV infection increases the risk of occurrence of kidney disease in Chinese diabetic patients [3]. Finally, in such patients with both kidney disease and HBV infection, besides the impact of kidney disease by itself on their prognosis, the therapeutic management of HBV infection is essential, while antiviral drugs, dosages must be adjusted to renal function and potential renal toxicity of antivirals may further damage the kidneys and lead to clinical complications [4].

The level of severity of malaria infection can be determined by both renal and hepatic malfunction [4]. The clinical manifestation of renal involvement is associated with infection by *P. falciparum* and *Plasmodium malariae* and may be responsible for an immune complex mediated glomerular disease leading to nephrotic syndrome. Other implications range from urinary sediment abnormalities, mild proteinuria and electrolyte changes to acute renal failure with metabolic acidosis [5]. In addition, renal tubular changes have been reported to be associated to P. falciparum infection more than glomerular changes, and complication may range from minor to acute tubular necrosis and acute renal failure (ARF) accompanied by frequent oliguria and hyper catabolism. ARF can be diagnosed by the presence of oliguria and increased serum creatinine and blood urea nitrogen [5]. Malaria has been reported to be one of the factors responsible for acute renal failure among children in malaria endemic areas, and this adverse effect of malaria parasite on the kidney could lead to an increase in blood urea, hyper-nareamia, hyper-kalaemia, low urine specific gravity, metabolic acidosis and low ratio of urinary to blood urea [5]. The sudden increase in the urea level and an imbalance in the electrolyte level, such as sodium, potassium, bicarbonate, and chloride in malaria infected people could serve as indicators for kidney dysfunction [4].

STATEMENT OF THE PROBLEM

While co-infection with HBV and malaria is a major public health problem in large areas of the world, the mutual interactions between the two pathogens are poorly understood. Because serum creatinine increases in both infections, there are no clear evidence that co-infection with HBV and malaria could alter renal biomarkers [5]. Therefore, this study is designed to investigate the possible changes in renal biomarkers in Plasmodium infected HBsAg seropositive patients.

JUSTIFICATION OF THE STUDY

There may be possible changes in renal biomarkers in plasmodium infected HBsAg seropositive patients in Owo metropolis. Thus, this study is aimed at determining the prevalence of possible changes in renal biomarkers in plasmodium infected HBsAg seropositive patients in Owo metropolis, Nigeria.

AIM OF THE STUDY

This study is designed to investigate the renal biomarkers in Plasmodium infected HBsAg seropositive patients.

OBJECTIVES OF THE STUDY

- To determine the pattern of renal biomarkers in Plasmodium infected HBsAg seropositive patients.

- To determine if co-infection of Plasmodium parasites with HBV may possibly increase the progression of both renal biomarkers.

With regards to the following result, there is a significant alteration in serum level of creatinine, uric acid and glutathione s transferase among patients co-infected with malaria and hepatitis B virus.

MATERIALS AND METHODS

Study area

This study was conducted among patients presented with hepatitis B viral infection in Federal Medical centre in Owo metropolis, Ondo State. Owo is a town in Ondo State, situated in the Southern-Western Nigeria, latitude 7.19620 and longitude 5.586810 at an elevation/altitude of meters. It is at the southern edge of the Yoruba hills, and at the intersection of roads from Akure, Kabba and Benin City [7].

Ethical clearance

Ethical clearance was obtained from the research ethics committee of Federal Medical Centre in Owo metropolis. Permission was sought from the Management of the Clinic and informed consent was also sought and obtained from the participants.

Study population

One hundred informed and consented patients within the age group of 15-64years was recruited for the study. Subjects with an established clinical condition other than malaria and/or HBV infection such as obstructive jaundice, cirrhosis, renal diseases, hypertension, diabetes mellitus, sickle cell disease, pregnancy, cancer and patients already on course of chemotherapy or who had it in the last two weeks for treatment of an earlier diagnosed illnesses were excluded from the study.

Sample size

The sample size was determined by the formula

$N = Z^2 X P (1-P) / d^2$ [6]

Where, N is the minimum sample size,

P = Prevalence rate of disease = 7% = 0.07 [7]

d = Desired level of significance = 0.05 (5%)

Z = Confidence interval = 1.96 (95% Confidence interval)

$N = Z^2 X P (1-P) / d^2$

$N = (1.96)^2 x 0.07 (1-0.07) / (0.05)^2$

$= 3.8416 x 0.07 x 0.93 / 0.0025$

$= 0.2505 / 0.0025$

$= 100.19$

Approximately, N = 100

The sample size (N) was 100, however a total of 100 samples was used for this research project.

Data and sample collection

Semi structured and pre tested questionnaires were administered to the participants to obtain information on the age, occupation, and knowledge on the diseases under study. From each individual subject, 5 ml of blood sample was obtained via venopuncture from the subjects using vacoutainer needle. Two milliliters of the blood were placed in ethylenediethyltetra acetic acid (EDTA) bottles for parasitological analysis. The remaining 3 ml was placed in a lithium heparin bottle and centrifuged at 3000rpm for 5 minutes to obtain the serum for the serological detection of the HbsAg, serum creatinine, uric acid and GST.

Processing of specimen

Plasma was separated from the red cells by centrifuging the blood samples at 1500rpm for 15mins. The plasma was stored in the refrigerator at 2 - 4˚C prior to analysis.

Parasitological examination

Incidence of malaria parasites in the blood samples was established using the gold standard microscopic procedure using Giemsa staining technique on thin and thick film smears was made from the 2ml collected above for specie determination and the level of parasiteamia.

Hepatitis B serology

Hepatitis B infection was diagnosed using serology. The 3ml portion of the blood sample was used for the detection of HBsAg. The blood was centrifuged and the serum obtained from which HBsAg was detected using Micropoint ELISA commercial Kits technique following the manufacturer's instructions.

Principle of the Jaffe-Slot alkaline picrate creatinine method

Creatinine reacts with picric acid in an alkaline medium. The absorbance of the yellow-red colour produced is measured in a colorimeter using a

bluegreen filter 490nm or in a spectrophotometer at 510nm wavelength. The amount of complex formed is directly proportional to the creatinine concentration. A number of other compounds similarly react with picric acid giving artificially high results for creatinine if one simply measures the total yellow-red colour produced. A second reading was therefore made after making the solution acidic. The colour produced by creatinine was quickly destroyed by acid whereas that given by non-creatinine chromogens was destroyed more slowly. By subtracting the second reading which was due to non-creatinine substances from the first reading (due to creatinine and noncreatinine substances), the colour produced by the true creatinine can be obtained.

The Uricase method for Serum uric acid

This method is based on the use of the enzyme uricase. Uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidises 3.5-Dichloro- 2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound.

Enzyme assay for Glutathione s transferase

Glutathione S Transferase (GST) is an enzyme involved in the detoxification of a wide range of compounds and is involved in reducing free radical damage in red blood cells. The enzyme is easily purified by affinity chromatography and has been used as a fusion partner for many recombinant proteins. Identification of the GST was done by western blotting or more easily by enzymatic assay.

Enzyme Reaction

Glutathione –SH + CDNB -> Glutathione –S-CDNB

The reaction is measured by observing the conjugation of 1-chloro, 2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH). This is done by watching an increase in absorbance at 340nm. One unit of enzyme will conjugate 10.0 nmol of CDNB with reduced glutathione per minute at 25oC [8].

STATISTICAL ANALYSIS

A statistical package for social science (SPSS) 19 and analysis of variance was used for the analysis of the data approximately. Continuous variable were display as means and standard deviation (SD) and correlation between the parameters were displayed in the table. The level of significant difference $P < 0.05$ was considered significant.

RESULTS

Table 1: Mean SD of Glutathione-S-transferases (GST), creatinine and uric acid among infected patients *with P. falciparum* (PM), hepatitis B virus, patients co-infected with both organisms and the control subjects.

Parameters	**HbsAg Mean ± SD**	**PM Mean ± SD**	**PM + HbsAg Mean ± SD**	**Control Mean ± SD**
GST (µmol/ml/min)	129.2 ± 102.39*	134 ± 34.33*	112 ± 49.73*	190± 123.67
Creatinine (mg/dl)	2.10 ± 2.00*	1.13 ± 0.66*	1.06± 0.30*	0.88 ± 0.32
Uric acid (mg/dl)	7.15 ± 1.55*	7.14 ± 5.11*	7.34 ± 0.98*	6.89 ± 1.9

* = $P < 0.05$

Table 1 shows that there was a significant difference ($P < 0.05$) in mean values of GST, uric acid and serum creatinine when control subjects was compared among the three infected groups.

Table 2: Mean SD of GST, creatinine and uric acid between *P. falciparum* infected patients and hepatitis B virus infected patients.

Parameters	HbsAg Mean ± SD	PM Mean ± SD	P value
GST (µmol/ml/min)	129.2 ± 102.39	134 ± 34.33	0.98
Creatinine (mg/dl)	2.10 ± 2.00	1.13 ± 0.66	0.11
Uric acid (mg/dl)	7.15 ± 1.55	7.14 ± 5.11	0.56

Table 2 shows no significant difference in mean values of Glutathione S-transferases (GST), serum creatinine and uric acid when *Plasmodium falciparum* infected patients was compared with hepatitis B virus infected patients.

Table 3: Mean SD of GST, creatinine and uric acid between *Plasmodium falciparum* infected patients and patients co-infected with both organisms.

Parameters	PM + HbsAg Mean ± SD	PM Mean ± SD	P value
GST (µmol/ml/min)	112 ± 49.73	134 ± 34.33	0.87
Creatinine (mg/dl)	1.06± 0.30	1.13 ± 0.66	0.12
Uric acid (mg/dl)	7.34 ± 0.98	7.14 ± 5.11	0.07

Table 3 shows no significant difference in mean values of Glutathione S-transferases (GST), serum creatinine and uric acid when *Plasmodium falciparum* infected patients was compared with patients co-infected with both organisms.

Table 4: Portrays the Mean SD of GST, creatinine and uric acid between Hepatitis B virus infected patients and patients co-infected with both organisms.

Parameters	PM + HbsAg Mean ± SD	HbsAg Mean ± SD	P value
GST (µmol/ml/min)	112 ± 49.73	129.2 ± 102.39	0.455
Creatinine (mg/dl)	1.06± 0.30	2.10 ± 2.00	0.083
Uric acid (mg/dl)	7.34 ± 0.98	7.15 ± 1.55	0.601

Table 4 shows no significant difference in mean values of Glutathione S-transferases (GST), serum creatinine and uric acid when hepatitis B virus infected patients was compared with patients co-infected with both organisms.

DISCUSSION

While co-infection with HBV and malaria is a major public health problem in large areas of the world, the mutual interactions between the two pathogens are poorly understood. It was suggested that Serum creatinine increases in both infections, there are no clear evidence that co-infection with HBV and malaria could alter renal biomarkers [5]. Therefore, this study is designed to investigate the possible changes in renal biomarkers in Plasmodium infected HBsAg seropositive patients.

A decline in GFR has been ascertained to be related to an increase serum creatinine and uric acid. Unfortunately, both are an unreliable indicator of acute renal injury because they are both influenced by multiple non-renal factors such as age, gender, exercise and diet [9].

Malaria and hepatitis B virus infection is related to acute renal failure. Oliguria and increased serum creatinine are interesting clinical features of acute renal failure (ARF). Kim [10] and his group suggested that baseline serum uric acid level might be

a good clinical marker for patients with acute kidney failure.

In this study it was observed that there was a significant increase in serum level of creatinine and uric acid in patients with *Plasmodium falciparum*, patients with hepatitis B virus infection and subjects who are co-infected with both organisms when compared with the control subjects. However, no significant difference was seen when the mean values of both serum parameters were compared between *Plasmodium falciparum* infected patients with hepatitis B virus infected patients, *Plasmodium falciparum* infected patients with patients co-infected with both organisms, and Hepatitis B virus infected patients with patients co-infected with both organisms. This could be as a result of sequestration of the malaria parasite into the renal microvasculature bed which may lead to renal ischemia [11] or through the deposition of immune complexes on the glomerulus which is due to the presence of the viral components (Kim *et al.*, 2009).

Although, this study was unable to estimate urinary Glutathione s transferase (GST) which is used to quantify and localise renal tubular injury in transplantation, nephrotoxicity and ischaemic injury, however it is of interest to note that there was a significant increase in serum level of Glutathione s transferase in patients with *Plasmodium falciparum,* patients with hepatitis B virus infection and subjects who are co-infected with both organism when compared with the control subjects. However, no significant difference was seen when the mean values of the serum uric acid were compared between *Plasmodium falciparum* infected patients with hepatitis B virus infected patients, *Plasmodium falciparum* infected patients with patients co-infected with both organisms, and Hepatitis B virus infected patients with patients co-infected with both organisms. Intriguingly, hepatocytes contain high levels of alpha GST and serum alpha GST has been found to be an indicator of hepatocyte injury in transplantation, toxicity and viral infections [12, 13]. Similarly, in humans, renal proximal tubular cells contain high concentrations of alpha GST, while distal tubular cells contain pi GST [14]. However, our inability to measure urinary GST makes it impossible to illustrate the reason for an increase in this parameter since both organisms share some of their developmental stages within the liver which may elicit impaired clearance of the liver stages of the malaria parasite due to hepatocytes damage in HBV infection.

CONCLUSION

Finally, although these changes in renal markers in association with malaria and hepatitis B virus coinfection are not novel, our findings have added more information, hitherto the limited knowledge and sparsely reports on changes in renal profile of malaria infected, hepatitis B virus infected and coinfected individual habitat in Owo Municipality.

Recommendation

It is important that serum uric acid and creatinine are considered as part of the differential diagnosis in patient co-infected with both organisms in order to avoid severe renal damage.

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How to cite this article: Mathew Folaranmi OLANIYAN and Tomisin Emily LAWAL. Renal Biomarkers in Plasmodium Infected Hepatitis B Surface Antigen (Hbsag) Sero-Positive Patients. Int J of Allied Med Sci and Clin Res 2016; 4(3): 522-528.

Source of Support: Nil. **Conflict of Interest:** None declared.