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*Original Article*

# Renal function status of Nigerian patients infected with Hepatitis B virus

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ABSTRACT: In Hepatitis B virus (HBV) infection is an important cause of morbidity and mortality in liver disease and hepatocellular carcinoma. It has also been implicated in a variety of renal diseases. This relationship was investigated in hepatitis B virus patients in Nigeria where the infection is highly endemic. Fifty hepatitis B positive patients (aged 27.3 ± 7.5 years) and 50 control subjects (aged 25.1 ± 3.6 years) were recruited in this study. Random blood sample was drawn from all subjects. Serum electrolyte, urea and creatinine were measured using standard laboratory methods. Serum potassium concentration was higher (5.16 ± 2.30, $p<0.05$) in hepatitis B carriers compared to controls (4.25 ± 0.5). There was an inverse correlation between bicarbonate and chloride ($r = -0.67$, $p<0.01$) in HBV patients. Results from this study showed a possible relationship between hepatitis B infection and insufficient renal function.

KEYWORDS: *Hepatitis B virus, renal disease, electrolytes*



## INTRODUCTION

The kidneys are responsible for urine production as well as a number of other homeostatic functions. These include regulation of electrolytes, acid-base balance and blood pressure, excretion of wastes such as urea and ammonium; reabsorption of glucose and amino acids; and production of hormones. Diseases of the kidney are diverse. Common clinical presentations include the nephritic and nephrotic syndromes, acute kidney failure, chronic kidney disease, urinary tract infection, nephrolithiasis and urinary tract obstruction (Cotran *et al*., 2005).

Hepatitis is inflammation of the liver, usually from a viral infection, but sometimes from toxic agents. Hepatitis B virus is highly infectious and easy to transmit from one infected person to another and has relatively higher prevalence in the tropics (Finlayson *et al*., 1999). Results from laboratory studies on the serum carrier rate of the surface antigen of hepatitis B virus (HBsAg) showed that infection by the virus is prevalent in Nigeria (Mustapha & Jibrin, 2004) and that HBV is also the major aetiological factor for the occurrence of liver cirrhosis and hepatocellular carcinoma in Nigerians (Mustapha & Jibrin, 2004).

The first symptoms of acute hepatitis B may be non-specific including fever, skin rash and joint pain and inflammation. Other symptoms may include fatigue, loss of appetite, nausea and jaundice (Ryder & Beckingham, 2004). About 10-20% of people with chronic hepatitis B develop complications in other organs and tissues outside the liver, vascular inflammation and kidney diseases are the two most common complications (Yim & Lock, 2006). Renal function tests include measurement of serum urea, creatinine, creatinine clearance, uric acid, calcium, and phosphorus, electrolytes (sodium, potassium, bicarbonate, lithium and chloride). Hepatitis B infection is usually complicated with nephritic syndrome. During acute hepatitis B infection serum creatinine, urea and some electrolytes are usually elevated, this effects are a signal of on-going kidney disease particularly glomerulonephritis (Nippo & Gakkai, 2006).

It was suggested that hepatitis B virus antigenemia may play a significant role in the development of specific forms of glomerulonephritis and this can run an indolent but relentless progressive clinical course (Kar & Michael, 1986). Some workers reported that hepatitis B virus can lead to significant changes in renal function and subsequently renal disease including membranous glomerulonephritis, polyateritis nodosa and membranoproliferative glomerulonephritis (Lai & Lai, 1991; Johnson & Couser, 1990). In addition, widespread use of hepatitis B vaccination has been observed to decrease incidence of HBV-related renal diseases in children thereby providing evidence of the probable pathogenic role of HBV (Xu *et al*., 2003).

The clinical causes of hepatitis B virus infection are complex and are influenced by several factors such as: age at acquisition of infection, immune status and concurrent infection with other hepatotropic virus and alcohol intake. Nigeria is classified among the group of countries highly endemic for HBV infection (Sirisena *et al*., 2002). In a prevalence study carried out in Nigeria, out of the 25 donors that were seropositive for HBsAg, 14.6% were males while 12.9% were females with the higher preponderance observed in donors aged 20-30 years than those aged 31-40 years (Uneke *et al*., 2005). The possibility of the transmission of HBV is increased in Nigeria as a result of greater occurrence of road accidents, pregnancy related haemorrhage armed robbery attacks and through transmission of contaminated blood (UNSN, 2001). Individuals with chronic hepatitis B develop complications in other tissues outside the liver with vascular inflammation and kidney disease been the two most common complications (Yim & Lock, 2006).

Biochemical parameters are often key factors to be considered in monitoring progression of renal disease. This study is aimed at investigating renal function in hepatitis B positive patients through laboratory analysis of serum creatinine, urea and electrolytes concentrations.

## MATERIALS AND METHODS

### Study population

A total of 100 human subjects (62 males and 38 females) between the ages of eighteen and fifty years were randomly selected and enrolled in this study. Fifty hepatitis B carriers (aged 27.3 ± 7.5 years), comprising of 32 males and 18 females who were positive for hepatitis B surface antigen were recruited from Ladoke Akintola University of Technology and Obafemi Awolowo University Teaching Hospitals in Osun State, Nigeria respectively. Fifty apparently healthy individuals (aged 25.1 ± 3.6 years) and comprising of 30 males and 20 females, who tested negative for HBsAg and other viral infections served as control subjects.

Hepatitis B carriers and control subjects were screened for HBsAg using Dipstick technology to confirm their seroconversive state. Patients with serology positive for HBsAg, hepatitis B core antibody (HBcAb) and hepatitis Be antigen (HBeAg) were confirmed as hepatitis B virus positive. Informed consent was obtained from each of the subjects prior to commencement of the study.

### Blood sample collection

In order to determine renal function parameters, 5 ml of random blood sample was collected by venepuncture without venous stasis and dispensed into a plain separating bottle. The blood was allowed to clot and spun at 3000 rpm for 5 minutes. Serum was separated into a sterile plain bottle and stored at -20°C until analysis.

### Determination of renal function parameters

Serum creatinine was determined by spectrophotometric analysis, using the modified Jaffe method (Jaffe, 1886). Briefly, picric acid reacts with creatinine in free filtrate resulting in the production of an acid tautometer of creatinine picrate in the presence of alkaline solution which was read at an absorbance of 520 nm. Serum urea was analysed by enzymatic method involving urease. Urea was decomposed to form ammonium carbonate which reacts with phenol in the presence of hypochloride to form indophenol which was measured at 578 nm using a spectrophotometer (Patton & Crouch, 1997). Bicarbonate was estimated by titrimetic method using neutral red as indicator and a fixed amount of hydrochloric acid (Louis, 1999). Serum chloride was determined using mercuric nitrate method with diphenyl-1-carbazone as indicator (Schales & Schales, 1941). Serum sodium and potassium were estimated by flame photometry using CORNING Clinical Flame Photometer. Quality control samples were included in each batch of the test to ensure the reliability of test results.

### Data analysis

The SPSS package version 15.0 was used in statistical analysis. Values are expressed as mean ± standard deviation when compared using Student's t test. Correlation among the variables was determined using Pearson's product moment correlation coefficient.

## RESULTS

Table 1 showed that the mean serum potassium level was significantly increased in hepatitis B carriers when compared with the value in the control subjects. There were no significant differences in serum concentrations of creatinine, urea, sodium, chloride and bicarbonate in patients and control. All statistical analysis was carried out at 95% confidence interval.

The mean serum concentrations of creatinine, urea and electrolytes except potassium did not vary between male and female hepatitis B carriers. Potassium was significantly higher in the males than in female patients (Table 2).

From Table 3, the relationship among the different biochemical parameters analysed was determined using Pearson product moment correlation analysis. An inverse correlation was observed between bicarbonate and chloride whereas a positive correlation was observed between serum potassium and creatinine, sodium and urea and sodium and chloride.

## DISCUSSION

The objective of this study was to evaluate the status of renal function parameters in hepatitis B carriers with respect to renal complications of viral hepatitis B infection. Results from this study showed an increase in mean serum potassium level in hepatitis B carriers compared to control subjects. This could be due to damage caused by the hepatitis B virus which then brought about changes in the glomerular filtration pattern since potassium is actively filtered through the glomerular basement membrane and reabsorbed in the tubules (Han, 2004). Some viral infections of the liver, including hepatitis B and C can also lead to inflammation of the glomerular membrane (Philipneri & Bastani, 2001).

Hyperkalaemia is a common feature of renal tubular acidosis type 4 which results from a defect in hydrogen and potassium secretion in the distal tubules rather than from aldosterone deficiency (Battle *et al*., 1981). This could explain the reason for the

TABLE 1 Estimated parameters in patients with hepatitis B virus and control subjects (mean ± SD)

| PARAMETERS | PATIENTS (n = 50) | CONTROL (n = 50) | t-value | p-value |
|---|---|---|---|---|
| Age (years) | 27.3 ± 7.49 | 25.6 ± 3.55 | 1.71 | 0.07 |
| Creatinine (µmol/L) | 88.9 ± 30.67 | 80.9 ± 29.36 | 1.52 | 0.11 |
| Urea (mg/dL) | 32.0 ± 9.42 | 30.9 ± 10.03 | 0.45 | 0.62 |
| $Na^+$ (mmol/L) | 134.4 ± 8.37 | 135.6 ± 3.70 | -0.99 | 0.33 |
| $K^+$ (mmol/L) | 5.2 ± 1.94 | 4.3 ± 0.48 | 3.19 | 0.00* |
| $HCO_3^-$ (mmol/L) | 27.1 ± 6.34 | 27.8 ± 5.08 | -0.71 | 0.49 |
| $Cl^-$ (mmol/L) | 98.1 ± 11.35 | 97.0 ± 6.21 | 0.57 | 0.56 |

*significant at p<0.05

TABLE 2 Serum creatinine, urea and electrolytes in male and female patients (mean ± SD)

| PARAMETERS | MALE (n = 32) | FEMALE (n = 18) | t-value | p-value |
|---|---|---|---|---|
| Creatinine (µmol/L) | 100.4 ± 29.5 | 68.6 ± 21.1 | 4.84 | 1.23 |
| Urea (mg/dL) | 34.7 ± 9.9 | 27.4 ± .5 | 1.34 | 0.19 |
| $Na^+$ (mmol/L) | 133.8 ± 10.0 | 135.4 ± 4.3 | -1.29 | 0.23 |
| $K^+$ (mmol/L) | 5.4 ± 2.3 | 4.7 ± 0.9 | 2.51 | 0.02* |
| $HCO_3^-$ (mmol/L) | 27.7 ± 6.3 | 25.8 ± 6.4 | 0.63 | 0.54 |
| $Cl^-$ (mmol/L) | 97.4 ± 13.1 | 99.3 ± 5.6 | -0.52 | 0.63 |

*significant at p<0.05

TABLE 3 Pearson product moment correlations between physical parameter and biochemical parameters in hepatitis B carriers

| | Age | Creatinine | Urea | $Na^+$ | $K^+$ | $HCO_3^-$ | $Cl^-$ |
|---|---|---|---|---|---|---|---|
| Age (years) | 1.00 | 0.08 | 0.14 | 0.05 | 0.12 | 0.09 | 0.02 |
| Creatinine (umol/L) | 0.08 | 1.00 | 0.25 | 0.07 | 0.49** | 0.17 | 0.03 |
| Urea (mg/dL) | 0.14 | 0.25 | 1.00 | 0.35* | 0.13 | 0.05 | 0.25 |
| $Na^+$ (mmol/L) | 0.05 | 0.07 | 0.35* | 1.00 | -0.03 | -0.05 | 0.72** |
| $K^+$ (mmol/L) | 0.12 | 0.49** | 0.13 | -0.03 | 1.00 | -0.09 | 0.25 |
| $HCO_3^-$ (mmol/L) | 0.09 | 0.17 | 0.05 | -0.05 | -0.09 | 1.00 | -0.67** |
| $Cl^-$ (mmol/L) | 0.02 | 0.03 | 0.25 | 0.72** | 0.25 | -0.67** | 1.00 |

**correlation significant at the 0.01 level

*correlation significant at the 0.05 level

increased serum potassium level in hepatitis B carriers. Acute tubular necrosis involves damage to the renal tubules and can be a complication in individuals with hepatitis B infection as a result of diminished reabsorption of potassium by the tubules (Gines & Arroyo, 1999). Consistent with this is the result of the study carried out by Lai & Lai (1991). They reported a diminution in glomerular filtration capacity as a renal complication of hepatitis B viral infection. Also, the DNA of the hepatitis B virus was found in the nearby renal tubules, where urine is concentrated. It was suggested that the virus replicates in the tubules of the kidney.

Hepatorenal syndrome is a type of kidney disease that affects individuals with liver cirrhosis or, less commonly, with fulminant liver failure (Han, 2004). The syndrome involves constriction of the blood vessels in the splanchnic circulation, which supplies the intestines (Gines & Arroyo, 1999). The classification of hepatorenal syndrome identifies two categories of renal failure, termed type 1 land type 2 HRS. In both categories, the deterioration in kidney function is investigated either by an elevation in creatinine level in the blood, or by decreased clearance of creatinine in the urine (Arroyo *et al*., 1996). However in this study, only a slight increase in serum creatinine level was observed in the patient group which suggests that the patients in this study probably do not suffer from HRS.

There were no distinct variations in serum creatinine, urea, sodium, bicarbonate and chloride in patients and control subjects. This observation may be explained based on an earlier report that recorded no change in plasma urea and creatinine levels in subjects suffering from renal tubular acidosis with no glomerular lesion (Mayne, 1996). A study by Ventataseshan *et al*. (1990) also observed that extra-hepatic complication of hepatitis B infection will only manifest in chronically ill patients, it is worthy of note that a large percentage of patients recruited for this study were acute hepatitis B carriers whose level of viral antigenaemia is minimal.

One of the findings from this study was that of an inverse correlation between serum bicarbonate and chloride levels. This implies that as the serum concentrations of bicarbonate increases, that of chloride decreases and vice versa. This observation can be explained based on the "chloride shift" mechanism which maintained the electrochemical neutrality of $CI^-$ in the opposite direction into cells when much of the $H^+$ generated from carbonic acid dissociation is buffered by haemoglobin and the $HCO_3^-$ diffuses out into the extracellular fluid along a concentration gradient (Martin, 2006).

It could be deduced from this study that impaired renal function may be a complication of hepatitis B viral infection. It is recommended that hepatitis B screening be encouraged in a larger population in this community and that renal function be effectively managed in subjects that are seropositive before the infection progresses to a chronic stage. In addition, polymerase chain reaction techniques could be employed to detect mRNA of the hepatitis virus in kidney biopsies from infected patients for prompt medical intervention.

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