Original Research Article

CORRELATION OF SMALL DENSE LOW DENSITY LIPOPROTEIN, TUMOUR NECROSIS FACTOR-ALPHA WITH LIVER ENZYMES IN CHRONIC HEPATITIS B PATIENTS.

ABSTRACT

Aim: This study investigated the relationship between small dense low density lipoprotein (sdLDL), tumour necrosis factor-alpha (TNF-α), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in chronic hepatitis B patients.

Subjects and methods: Sixty (60) participants were recruited for this cross sectional study. They comprise thirty (30) clinically diagnosed chronic hepatitis B virus (HBV) infected patients attending clinic at a tertiary hospital in Osogbo, Osun sta,country name. Thirty (30) apparently healthy volunteers were recruited as control subjects after fulfilling the inclusion criteria. Anthropometric measurements were performed using standard method. About 6mL of venous blood was collected from each study participants and serum was extracted and kept at -80^{o}C until time of analysis. Small dense LDL, TNF-α, AST, ALT and ALP were determined using enzyme linked immunosorbent assay and colorimetric method as appropriate. Data analysis was done obtained using Student's t-test for comparison of variables and Pearson's correlation was used to determine the relationship between variables. *P*–value less than 0.05 was considered significant.

Results: SdLDL, TNF-α, AST and ALT were significantly elevated in HBV patients when compared with the control subjects (P<0.05). SdLDL had a significant positive correlation with TNF-α (P=0.03), AST (P=0.01), ALT (P=0.00). TNF-α had a significant positive correlation with AST (P=0.02) and ALT (P=0.00).

Conclusion: This study revealed a noteworthy positive relationship between sdLDL, TNF-α and hepatic aminotransferases in chronic hepatitis B patients.

1. INTRODUCTION

Viral hepatitis is now recognized as a major public health challenge that requires an urgent response (1). In 2015, about 325 million people were living with chronic hepatitis infections worldwide and it was reported that approximately 1.34 million people died of hepatitis globally (2). This global mortality is comparable to deaths caused by tuberculosis and human immunodeficiency virus (HIV). While deaths resulting from tuberculosis and HIV appear to be declining, deaths from hepatitis are on the increase.

Hepatitis B viral infection has been described as one of the leading causes of mortality worldwide with about 650,000 annual deaths (2). Hepatitis B viral infection poses a major threat to human health and it is highly prevalent in developing countries (3). The prevalence of Hepatitis B infection is about 12% in Nigeria (4).

Hepatitis B virus has the potential to affect the functional integrity of the liver of an infected host. Liver as a homeostatic organ plays a pivotal role in lipid metabolism. Thus, the circulating levels of lipids in plasma depend greatly on the functionality of the liver. In the setting of acute or chronic hepatic dysfunction circulating lipids and lipoproteins are altered with respect to quantity as well as pattern of their electrophoretic mobility (5).

Previous studies documented diverse reports about the alterations of serum lipids in patients suffering from acute hepatitis due to the actions of hepatotropic viruses (6-8). Additionally, it was reported that chronic HBV infection is associated with elevated levels of low density lipoprotein (LDL), which is known to be a predictor of atherosclerotic cardiovascular disease risk (9-13).

Low-density lipoprotein consists of several subclasses of particles with different sizes and densities and they include the large buoyant, intermediate and small dense (sd) LDL particles. It is well documented that sdLDL cholesterol (sdLDL-C) proportion is a better marker for prediction of cardiovascular disease than total LDL-C (14, 15).

Furthermore, activation of the immune response during viral hepatitis leads to the production of many pro-inflammatory cytokines that act as mediators of disease activity (16). These pro-inflammatory cytokines particularly interleukin-6 (IL-6), interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α) appear to accentuate lipogenesis (17).

Even though previous studies have documented that viral hepatitis might interfere with lipid metabolism, however, the link between sdLDL, TNF-α and liver enzymes in individuals with hepatitis B patients has not been fully elucidated. The present study therefore aimed to determine the association between sdLDL, TNF-α, AST, ALT and ALP in chronic hepatitis B patients visiting a tertiary hospital in the south-western part of Nigeria.

2. MATERIALS AND METHODS

2.1 Subject selection

A total of sixty (60) subjects were recruited for this cross-sectional study. The test group comprise thirty (30) clinically diagnosed chronic hepatitis B virus (HBV) infected patients attending clinic at the Department of Gastroenterology, Ladoke Akintola University of Technology Teaching, Osogbo, Osun state,country name. These patients continuously tested positive for HBsAg for more than one year during their periodic visit to the clinic and they had one or more of these features; pallor, jaundice and liver enlargement. The control group comprise thirty (30) age matched apparently healthy HBV seronegative individuals.

A short structured questionnaire was administered to each study participant to obtain information on demography, alcohol use, drug use, smoking habits, medications and established diseases. Persons diagnosed with dyslipidemia and other metabolic conditions, record of alcoholism, smoking, usage of medications that affect lipid status and pregnant women were excluded from this study.

2.2 Ethical consideration

All participants were recruited for this study after ethical clearance was obtained from the ethics committee of Ladoke Akintola University of Teaching, Osogbo, Osun state. Written informed consent was obtained from each participants.

2.3 Blood pressure and anthropometric measurement

The blood pressure was measured using mercury sphygmomanometer with appropriate cuff size. Korotkoff phases 1 and 5 were used. Body weight in kilogram (kg) was measured using a standard weighing scale and height (m) was measured using standiometer. Body mass index (BMI) was calculated as the ratio of body weight (kg) to the square of height (m^2).

2.4 Sample collection and assay methodology

About 6 milliliters (mL) of venous blood was collected from each participant and dispensed into plain bottles to obtain serum which was aliquoted into a small vial and stored at -80^{o}C until time of analysis for the determination of small dense low density lipoprotein (sdLDL), tumour necrosis factor-alpha (TNF-α), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

2.4.1 Detection of Hepatitis B surface antigen (HBsAg).

The serum samples of subjects were screened to detect the presence of hepatitis B surface antigen. Fifty microliter (50μL) of serum was added vertically into the hole on the cassette and the result was read after 15 minutes (Melsin Medical Co., Limited, China). Appearance of two distinct red lines; one line at the control region (C) and the other at the test region (T) indicated positive test. Whereas appearance of only one red line, at the control region (C) indicated negative test. The result was invalid when the line at the test region appeared but the control region failed to appear.

2.4.2 Determination of liver enzymes

The serum activities of ALT and AST were determined colorimetrically using Randox reagents (Randox Laboratories, UK) as described by Reitman and Frankel (18). The serum activities of ALP were determined colorimetrically using Randox reagents (Randox Laboratories, UK) as described by Gesellschaft (19).

2.4.3 Determination of sdLDL

Small dense LDL (sdLDL) was analyzed based on the principle of solid phase enzyme linked immunosorbent assay (ELISA) using a unique monoclonal antibody directed against distinct antigenic determinant on sdLDL molecule immobilized on microtitre wells with kits supplied by ElabScience, Biotech, Ltd (USA). The standard working solution, biotinylated detection ab working solution and HRP conjugate working solution were prepared according to the manufacturer's instruction.

One hundred microliter (100μL) of sdLDL standards, samples and controls were added to appropriate wells. One hundred microliter (100μL) of biotinylated detection ab, solution was added to each well, they were mixed thoroughly and then incubated at 37^{o}C for 60 minutes, after which the wells were washed 3 times. One hundred microliter (100μL) of HRP conjugate was then added to each well and was incubated at 37^{o}C for 30 minutes after which the wells were washed 5 times. Ninety microliter (90μL) of substrate reagent was added to each well and was incubated at 37^{o}C for 15 minutes after which 50μL of stop solution was added to each well to stop the reaction. Absorbance was read at 450nm with a microtitre well reader. The grades of standard were used to plot a curve of absorbance against concentration for the calculation of sdLDL concentration.

2.4.4 Determination of TNF-alpha

Tumour necrosis factor-alpha(TNF-alpha) was analyzed based on the principle of solid phase enzyme linked immunosorbent assay (ELISA) using a unique monoclonal antibody directed against distinct antigenic determinant on TNF-alpha molecule immobilized on microtitre wells with kits supplied by ElabScience, Biotech, Ltd (USA). The standard working solution, biotinylated detection ab working solution and HRP conjugate working solution were prepared according to the manufacturer's instruction.

One hundred microliter (100μL) of TNF-alpha standards, samples and controls were added to appropriate wells. One hundred microliter (100μL) of biotinylated detection ab. solution was added to each well, they were mixed thoroughly and then incubated at 37^{o}C for 60 minutes, after which the wells were washed 3 times. One hundred microliter (100μL) of HRP conjugate was then added to each well and was incubated

at 37^{o}C for 30 minutes after which the wells were washed 5 times. Ninety microliter (90µL) of substrate reagent was added to each well and was incubated at 37^{o}C for 15 minutes after which 50µL of stop solution was added to each well to stop the reaction. Absorbance was read at 450nm with a microtitre well reader. The grades of standard were used to plot a curve of absorbance against concentration for the calculation of TNF-alpha concentration.

2.5 Statistical analysis

Data analysis was done using SPSS version 21.0. All values were expressed as mean±standard deviation for test and control groups. Comparison of variables was done using Student's t-test and Pearson's correlation was used to determine the relationship between variables. $P < 0.05$ was considered to be statistically significant.

3. RESULTS

The demographic data, anthropometric and biochemical parameters of the study participants are summarized in table 1. The mean age, BMI and blood pressure of the case and control subjects were not statistically significant ($P >0.05$). The mean levels of sdLDL, TNF-α and mean activities of AST, ALT were significantly elevated in hepatitis B patients compared with control. ($P < 0.05$).

Table 2 shows the correlation between sdLDL and other biochemical parameters in HBV patients. Small dense LDL had significant positive correlation with TNF-α (P=0.03), AST (P=0.01) and ALT (P=0.00). There was also positive correlation with ALP but not significant ($P > 0.05$)

Table 3 shows the correlation between TNF-α, AST, ALT and ALP. TNF-α had significant positive correlation with AST (P=0.02) and ALT (P=0.00) but not with ALP ($P > 0.05$)

Table 1. Demographic, anthropometric and biochemical parameters of the study participants

Parameters	HBV	Control	*P*-value
Age (years)	35.6±8.7	32.3±6.4	0.64
BMI (kg/m^2)	23.7±3.9	24.8±4.3	0.14
SBP (mmHg)	128.4±12.5	125.2±8.4	0.34
DBP (mmHg)	78.2±7.4	75.6±5.9	0.22
AST (IU/L)	56.8±33.5	28.2±12.5	0.00*
ALT (IU/L)	46.2±23.2	21.1±14.3	0.00*
ALP (IU/L)	65.3±23.7	58.2±16.8	0.16
SdLDL(nmol/mL)	67.9±23.8	29.8±15.9	0.00*
TNF-α (pg/mL)	29.2±13.5	15.7±10.5	0.01*

*Statistically significant at $P<0.05$. Results are expressed as mean±standard deviation. BMI-Body mass index; SBP-systolic blood pressure; DBP- diastolic blood pressure; AST-aspartate aminotransferase; ALT- alanine aminotransferase; ALP-alkaline phosphatase; sdLDL-small dense low density lipoprotein; TNF-α- tumour necrosis factor-alpha.

Table 2: Correlation between sdLDL and other biochemical parameters.

SdLDL	R	*P*-value
AST	0.929	0.01*
ALT	0.745	0.00*
ALP	0.294	0.162
TNF-α	0.813	0.03*

*Statistically significant at *P*<0.05 (2-tailed)

Table 3: Correlation between TNF-α and liver enzymes.

TNF-α	R	*P*-value
AST	0.835	0.02*
ALT	0.665	0.00*
ALP	0.440	0.146

*Statistically significant at *P*<0.05 (2-tailed)

4. DISCUSSION

Liver is an important homeostatic organ that is mainly responsible for the synthesis of lipids. Moreover the synthesis of key enzymes for lipid metabolism takes place in the liver (20). Liver also regulates the catabolism of various plasma lipoproteins via hepatic cellular surface receptors which help to maintain the levels of lipids and lipoproteins in humans (21). Thus these processes depend upon the integrity of the cellular function of liver. Hepatocellular damage or injury can interfere with these processes thereby leading to alteration of lipids and lipoprotein patterns.

Small dense LDL is a major component of LDL-cholesterol and it is believed to be a very promising biomarker for the prediction of cardiovascular event because it possesses more atherogenic potential than other fractions of LDL-cholesterol and it has the profound ability to exhibit prolonged residency in the sub endothelial space (22-24). Additionally, sdLDL particles have reduced affinity to the liver LDL receptor, consequently they stay longer in the circulation (25, 26).

Experimental evidence suggests that most proinflammatory cytokines especially TNF-α plays an important role in liver injury induced by hepatitis B virus and TNF-alpha may also be associated with persistent HBV infection and severity (27, 28).

The present study revealed that both sdLDL and TNF- alpha are significantly elevated in chronic HBV patients when compared with control subjects ($P < 0.05$). Additionally, we also observed a significant positive correlation between sdLDL and TNF-α in HBV patients. The underlying mechanism for this association is not entirely clear but one possible explanation is that TNF- alpha has the ability to modify the activities of hepatic lipase thereby causing it to increase the lipolysis of triglyceride-rich LDL with consequent increased formation of sdLDL (29-33).

The present study also demonstrated significantly higher levels of hepatic aminotransferases (AST, ALT) in HBV patients when compared with control subjects. This is consistent with finding of previous studies (8,13,34) and this has been attributed to a localized autoimmune reaction mediated by major

histocompatibility complex-1-hepatitis B surface protein complex which results into the degeneration of hepatic tissue and during this process the cell membranes become more permeable, thereby leading to leakage of the hepatic aminotransferases into the blood stream (6, 35).

Our findings also revealed a significant positive correlation with between sdLDL and hepatic aminotransferases. This agrees with findings of previous studies that reported significant rise in AST and ALT in proportion to raised LDL and triglycerides levels in patients with HBV infection (13, 36-38).

Furthermore, the present study revealed that there is a significant positive correlation between TNF-alpha and hepatic aminotransferases and this is consistent with reports of previous studies (28, 39, 40). The significant positive association that exists between TNF- alpha and hepatic aminotransferases, indicates the progression of inflammation and severity of injury induced by HBV infection (41).

5. CONCLUSION

The present study demonstrated that there is significant relationship between sdLDL, TNF- alpha and hepatic aminotransferases. Taken together, sdLDL and TNF- alpha can serve as potential predictors of liver damage induced by HBV. Also based on our findings, HBV patients need to be closely monitored for signs of cardiovascular disease.

While results from cross sectional study on a larger scale would play significant role in understudying the observations reported in this study, longitudinal studies would also facilitate better understanding of the findings of this study.

Competing interest: Nil

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