Original Article

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Correlation between serum alanine aminotransferase activity and immunologic response and body mass index in obese patients with chronic hepatitis B virus infection

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ABSTRACT

Background & Objective: Chronic B viral hepatitis is a major health problem worldwide. Several studies have reported that obesity is important risk factors altered immune system response in individuals with no underlying causes of liver disease. This study was to examine the correlation between body mass index, serum alanine aminotransferase activity and immunologic response in obese hepatitis B Saudi patients.

Subjects and Methods: One hundred fifty Saudi male patients with hepatitis B viral infection (HBV); their age ranged from 30 to 45 (38.64 ± 7.12) years. Patients were divided in to two equal groups according to their body mass index: Group (A): Included patients with HBV, their body mass index (BMI) was greater than 30 kg/m^2 (the obese group). Group (B): Included patients with HBV, their BMI between 20 and 24 kg/m^2 (the normal-weight group).

Results: An elevation of serum alanine aminotransferase (ALT) activity was found to be associated with increased BMI, also we observed an elevation with regard to the normal weight group in the parameters of white blood cells, neutrophils, monocytes, CD3, CD4 and CD8 for group A. CD3, CD4 and CD8 correlated with BMI only as a total amount, as well as with all measured parameters of blood count.

Conclusion: Obesity adversely affects the immunological response and rate of disease progression in HBeAg-negative chronic hepatitis B viral infection. Body weight control is important in the management of patients with chronic hepatitis B viral infection.

Keywords: immune system, obesity, body mass index, chronic hepatitis B virus infection

INTRODUCTION

Hepatitis B virus (HBV) causes inflammation of the liver in humans and is a major public health problem worldwide. About a quarter of the world population (>2 billion) has been infected with HBV, including 350 million with chronic or lifelong HBV infection (1). HBV is 50–100 times more infectious than human immuno-deficiency virus (HIV) (2, 3).

Clinically, abnormal liver function test results have often been found in overweight and obese people (4-6). Obesity may present histological liver alternations, mainly steatosis (7). Many pathological reports revealed fat droplets or steatosis occurred frequently in the liver biopsy of overweight patients with increased ALT (8, 9). Only in men was the hepatitis B virus (HBV) infection significantly associated with elevated ALT (10).

HBV carriers are at risk of developing life threatening cirrhosis and later on hepatic carcinoma. The long-term aim in the treatment of these patients is to prevent the development of cirrhosis and hepatocellular carcinoma (11). The immune responses to HBV antigens are responsible both for viral clearance during acute infection and for disease pathogenesis. Immune responses involved in viral clearance comprise both humoral and cellular immunity where CD4+ helper T cells contribute to generation of antibodies against viral envelope antigens that clear circulating virus particles and CD8+ cytotoxic T lymphocytes eliminate infected cells (12).

Obesity is a major health problem since excessive body weight constitutes a risk factor in a number of chronic diseases. The prevalence of obesity is increasing worldwide and as per the latest WHO estimates, approximately 500 million adults and nearly forty three million children under the age of 5 years appear to be obese, showing a BMI ≥ 30[13]. Immune-competence is dependent on nutritional status and can be easily dysregulated in states of imbalanced nutrition such as under nutrition (malnutrition) or over nutrition (obesity). However, immune suppression in undernourished state is well known (14). Many authors reported dysregulation and alteration in number of immune cells in obese subjects. Obese subjects showed either increased or decreased total lymphocytes in peripheral blood populations (15) and had decreased CD8+ T cell population along with increased or decreased CD4+T cells (16).

Cohort studies indicate that obesity increases risk of hepatic steatosis and fibrosis in non-diabetic patients with chronic hepatitis infection as overweight diminished response to antiviral therapy and affects the progression of chronic liver disease (17, 18). Weber et al. conducted the first study to describe the relationship between vaccine response and obesity. His group found that, high BMI was associated with a failure to develop detectable antibody response to Hepatitis B vaccine in health-care workers (19). Further, Simo Minnana et al. reported lower antibody response in adolescents with high BMI when administered a three-dose regimen of recombinant hepatitis B vaccine (20). A randomized controlled trial compares triple-antigen vaccine vs standard single antigen vaccine administered in three doses over six months resulted in 71% and 95% protection rates in obese subjects when compared with lean subjects (91% & 99% respectively) (21). Therefore, the purpose of this study was to examine the correlation between body mass index, serum alanine aminotransferase activity and immunologic response in obese hepatitis B virus patients.

SUBJECTS AND METHODS

One hundred fifty non-hypertensive, non-cirrhotic Saudi male patients with chronic HBV infection; their age ranged from

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30 to 45 (38.64 ± 7.12) years, were randomly selected and studied on referral to Gastroenterology and Hepatology Department, King Abdulaziz University Teaching Hospital, Saudi Arabia between the period of March 2014 and December 2015. Only patients diagnosed with chronic HBV mono-infection and undergo Real-Time polymerase chain reaction. All these patients were negative for HBeAg. The inclusion criteria were as follows: positive test for serum hepatitis B surface antigen (HBsAg) and negative test for HBeAg for at least six months, elevated serum ALT level recorded at least 2 distinct instances at an interval of one month, and HBV DNA level > 2000 IU/mL (tested using CobasAmplicor HBV monitor, which has a detection limit of 12 IU/mL). Anti-viral treatment for all enrolled patients followed the American Association for the Study of Liver Disease (AASLD) treatment guidelines (22).

The exclusion criteria were as follows: patients with hepatitis C virus, alcoholic liver disease and radiologic evidence of hepatitis C cirrhosis (HCC). The Scientific Research Ethical Committee, Faculty of Applied Medical Sciences at King Abdulaziz University, approved this study. All participants were free to withdraw from the study at any time. Patients were divided in to two equal groups according to their body mass index: Group (A): Included HBV patients with a BMI more than 25 kg/m^2 (the obese group). Group (B): Included HBV patients with a BMI between 18.5 and 23 kg/m^2 (the normal-weight group).

METHODS

Evaluated Parameters

A. Flow cytometry analysis: The human leukocyte differentiation antigens CD3, CD4 and CD8 (Beckman Coulter, Marseille, France). The samples were analyzed by flow cytometry using Cytomics FC500 and CXP software (Beckman Coulter).

B. Analysis of peripheral blood cells: The analysis of peripheral blood cells (e.g., total and differential count) was performed on a Beckman Coulter AcT 5diff hematology analyzer. The values are expressed in percentages and absolute numbers.

C. Serum alanine aminotransferaseand viral serology tests: Blood samples were collected from the antecubital vein. Subjects had blood drawn at the same time in the morning on each occasion (between 8 and 10 AM). Serum alanine aminotransferasewas measured by the colorimetric enzymatic method using an automatic spectrophotometer (Bioclin, Quibasa, Belo Horizonte, MG, Brazil).

D. Body mass index (BMI): Weight and height scale (Metrotype –England) was used to measure weight and height to calculate the body mass index (BMI). Body mass index was calculated by dividing the weight in kilograms by the square of the height in meters (Kg/m^2) (23).

Statistical analysis

Independent t-test was used to compare differences between both groups. Statistical analysis of data was performed using SPSS (Chicago, IL, USA) version 17. The relationship between continuous variables and BMI was assessed by Pearson or Spearman rank correlation. All data were expressed as the mean ± SD. P<0.05 indicated statistical significance.

RESULTS

The two groups were considered homogeneous regarding the demographic variables (table 1). The mean age of the obese group was 39.16 ± 7.33years, and the mean age of the normal weight group was 37.64 ± 6.98years. There was no significant differences in fasting glucose, triglyceride, total cholesterol, high-density lipoprotein-cholesterol, systolic blood pressure and diastolic blood pressure between the obese and normal-weight groups. However, body weight, body mass index (BMI), alanine aminotransferase (ALT) and HBV viral load were significantly different between the obese and normal-weight groups.

Table 1: *Comparison of clinical data between HBV patients in both groups*

	Group (A)	Group (B)
Age (year)	39.16 ± 7.33	37.64 ± 6.98
BMI (kg/m2)	31.15 ± 4.32*	20.36 ± 4.67
ALT activity (IU/L)	80.78 ± 6.84*	23.31 ± 4.92
Fasting glucose (mg/dL)	103.23 ± 15.63	98.43 ± 16.54
Triglyceride (mg/dL)	132.62 ± 17.54	129.37 ± 12.38
Total cholesterol (mg/dL)	195.21 ± 25.42	192.84 ± 17.52
HDL-C (mg/dL)	50.44 ± 12.60	47.11 ± 14.28
Systolic blood pressure (mm Hg)	120.68 ± 17.73	116.62 ± 13.36
Diastolic blood pressure (mm Hg)	80.23 ± 7.41	77.52 ± 6.93
HBV DNA (IU/mL)	3.57 ± 0.85 × 106*	5.14 ± 0.64 × 105

BMI: Body Mass Index; ALT: Alanine aminotransferase; HDL-C: High-density lipoprotein-cholesterol; (*) indicates a significant difference between the two groups, P < 0.05.

The number of white blood cells, total neutrophil count, monocytes, CD3, CD4 and CD8 were significantly elevated in obese individuals when compared with normal controls (Table 2). The Pearson's correlation coefficients test for the relationship between body mass index & ALT activity, white blood cells, total neutrophil count, monocytes, CD3, CD4,and CD8 in both groups showed a strong direct relationship in both groups (Table 3, 4).

Table 2: *Mean value and significance of white blood cells, total neutrophil, monocytes, CD3, CD4 and CD8 count of group (A)*

	Group (A)	Group (B)
White blood cells count (10^9/µL)	9.31 ±3.41*	5.87 ±3.11
Total neutrophil count (10^9/µL)	6.12 ±2.38*	4.15±2.16
Monocytes (10^9/µL)	0.58 ±0.16*	0.37 ±0.11
CD3 count (10^9/L)	1.86±0.93*	1.39 ±0.78
CD4 count (10^9/L)	1.41±0.85*	1.12±0.76
CD8 count (10^9/L)	0.82±0.34*	0.51±0.25

(*) indicates a significant difference between the two groups, P < 0.05.

Table 3: *Shows the Pearson's correlation coefficients test value and the relationship between the BMI &ALT activity, white blood cells, total neutrophil, monocytes, CD3, CD4 and CD8 count of group (A)*

	Pearson's value (*r*)
ALT activity (IU/L)	0.42*
White blood cells count (10^9/µL)	0.46*
Total neutrophil count (10^9/µL)	0.35*
Monocytes (10^9/µL)	0.32*
CD3 count (10^9/L)	0.41*
CD4 count (10^9/L)	0.33*
CD8 count (10^9/L)	0.34*

ALT: Alanine aminotransferase; Significance was calculated by Spearman or Pearson correlation (2-tailed), *p < 0.05; r, correlation coefficient.

Table 4: *Shows the Pearson's correlation coefficients test value and the relationship between the BMI &ALT activity, white blood cells, total neutrophil, monocytes, CD3, CD4 and CD8 count of group (B)*

	Pearson's value (*r*)
ALT activity (IU/L)	0.41*
White blood cells count (10^9/µL)	0.42*
Total neutrophil count (10^9/µL)	0.41*
Monocytes (10^9/µL)	0.35*
CD3 count (10^9/L)	0.34*
CD4 count (10^9/L)	0.31*
CD8 count (10^9/L)	0.32*

ALT: Alanine aminotransferase; Significance was calculated by Spearman or Pearson correlation (2-tailed), *p < 0.05; r, correlation coefficient.

DISCUSSION

As the impact of obesity on the immune system in the HBV Saudi patients is not well known, our study was conducted to explore the association between the obesity and immune system in obese HBV Saudi patients. In our study, obese HBV Saudi patients showed elevated Serum alanine aminotransferase (ALT) activity than HBV patients with normal body weight; our findings are in line with the results of many previous studies as in HBV patients, obesity is associated with elevated ALT activity (24). Serum alanine aminotransferase (ALT) activity is a commonly used surrogate marker for the evaluation of hepatocellular damage (25). Several epidemiologic studies have reported that obesity is an important risk factor for elevated ALT activity in individuals with no apparent underlying cause of liver disease (26-28). In addition, results of the present study provided evidence for an association between ALT activity and BMI in patients with hepatitis B. A comparable relation between ALT activity and BMI has also been observed in a healthy blood donor population (29, 30). It has been reported that high-normal ALT activity predicts insidious, continued liver damage in patients with chronic hepatitis Band it has been speculated that obesity may carry with it a continuum of risk of subtle hepatic injury even in individuals with mild ALT elevation (31, 32).

In our study, obese HBV Saudi patients showed increased number of white blood cells, total neutrophil count, monocytes, CD3, CD4 and CD8 than HBV patients with normal body weight, also there was a strong direct relationship between body mass index and white blood cells, total neutrophil count, monocytes, CD3, CD4, CD8 in both groups. Our findings are in line with the results of many previous studies as ***Moulin et al.*** who showed in his study that obesity is associated with the modulation of immune parameters (33), elevated numbers of circulating immune cells as neutrophil, monocyte, leukocyte and total WBC (34, 35), as well as elevated activation levels of certain WBC and suppressed immune cell function (15). Also, several authors have reported a chronic inflammation status in individuals with higher BMI (36-38) which was associated with elevated amounts of white blood cells, neutrophils, and monocytes in the blood of all participants with BMI higher than that of the control group (39).

Our findings indicated an association between BMI and cell subpopulation counts in peripheral blood. In the whole tested population we denote an increase of white blood cells, neutrophils, monocytes, CD3, CD4 and CD8 counts. We agree with the suggestion of ***Kintscher et al.*** who observed an increased number of CD3 and CD4lymphocytes in the peripheral blood of obese women correlating with BMI (40). Also, ***Antuna-Puente et al***. found that BMI is positively correlated with the number of macrophages in adipose tissue (41).

Results of the present study add to the growing body of evidence indicating that obesity is involved in the progression of chronic hepatitis B. However, the present study was limited by the fact that we did not measure other biomarkers of hepatic steatosis or inflammation, such as γ-glutamyltransferase (42).

The current study has important strengths and limitations. The major strength is the randomized nature of the study; hence, we can extrapolate adherence to the general population. In the other hand, the major limitations is the small sample size in both groups may limit the possibility of generalization of the findings in the present study. Finally, within the limit of this study, weight reduction is recommended for modulation of abnormalities in liver function. Further researches are needed to explore the impact of weight reduction on serum alanine aminotransferase activity and immunologic response in obese patients with chronic hepatitis B virus infection.

CONCLUSION

Obesity adversely affects the immunological response and rate of disease progression in HBeAg-negative HBV. Body weight control is important in the management of HBV.

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