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Dynamics of Hepatitis B Virus Surface Antigen Level with Peripheral Blood Lymphocytes in Chronic Hepatitis B Infection

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Authors' contributions

This work was carried out in collaboration among all authors. Author SAH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SHS and SU managed the analyses of the study. Author BM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Peripheral blood mononuclear cells containing an aggregate of immune competent cells, such as T lymphocytes, B cells and natural killer cells, play an important role in control or persistence of the hepatitis B virus (HBV) infection. Similarly, the expression of hepatitis B viral antigens on the surface of infected hepatocytes can invoke a cytotoxic T–cell response.
Objective: To investigate the dynamic changes in hepatitis B surface antigen (HBsAg) and peripheral lymphocyte subsets of healthy donors and chronic hepatitis B patients.
Methodology: Serum HBsAg was quantified by enzyme-linked immunosorbent assay according to the manufacturer's guidelines. Peripheral blood lymphocyte cell phenotyping was carried out by flow cytometry for all chronic hepatitis B patients and healthy blood donors.

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Results: The results of this study showed a significant correlation between HBsAg level and percentage of T and NK cells *(r=0.366; P=0.01, r=-0.462; P=0.01,* respectively). On the other hand, significance variation in peripheral blood lymphocyte percentage of T lymphocyte subsets in patients were found to be directly proportional to T cell subsets CD4+and CD8+ (*P=0.001)* compared with healthy blood donor controls.
Conclusion: In conclusion this study highlighted the role of the HBsAg level in supressing the immune cells of the innate and adaptive immune system. Understanding the interactions between HBsAg and peripheral blood cells serves as a basis for development of HBV therapeutic vaccines and a prognostic biomarker in persistent HBV infection.

Keywords: Therapeutic vaccine; prognostic biomarker; chronic HBV infection; HBsAg.

1. INTRODUCTION

Hepatitis caused by hepatitis B virus (HBV) is a life threatening liver infection that causes cirrhosis and hepatocellular carcinoma [1]. Globally, more than 2 billion people are infected with HBV [2]. About 350 million people are chronic HBV carriers [3], with over 8% in sub-Saharan Africa [4] where a high rate of asymptomatic HBV infection exists, which poses a significant health risk for close family contacts and public health workers. HBV has been reported in different populations in various parts of Nigeria [2-4]. It is a serious public health problem affecting all ages. The likelihood of becoming a chronic carrier is higher for those infected at infancy and in early childhood [5]. About 10% of infected adults, 90% of infants infected at birth and 50% of children infected between the age of 1-5 years are at risk of developing chronic hepatitis [6].

When the human body is infected by HBV, complex immune responses, such as innate and adaptive immune responses, could be happened to overcome the virus and ultimately terminate the infection. It is generally acknowledged that the humoral antibody response helps the clearance of circulating virus particles and the prevention of viral spread within the host, while the cellular immune response is the most important factor to determine or eliminate infected cells, and is thought to be responsible for viral clearance and disease pathogenesis during HBV infection [7]. Part of the antiviral immune response, the T cell arm, represents a key factor in determining the outcome of infection, and it has been shown that chronic HBV is correlated with the presence of dysfunctional immune responses [8].

Peripheral blood mononuclear cells (PBMCs) containing an aggregate of immune-competent cells, such as T lymphocytes, B cells, and natural killer cells, play an important role in the control or persistence of the HBV infection [9,10]. It is believed that the expression of viral antigens on the surface of infected hepatocytes can invoke a cytotoxic T–cell (CTL) response [11] and different subsets of T cell lymphocytes, which have different responses to viral antigens and effects on the clinical course and prognosis of the infection [12]. Recently, studies on HBV pathogenesis in animal models demonstrated that activated virus-specific T lymphocytes in the liver cells are critical for the pathogenesis of both HBV infection and hepatocellular carcinoma [13].

However, many previous studies have reported a strong correlation between T lymphocyte response, liver damage cells and HBV clearance [14,15] and, as a result, most investigations have concentrated on lymphocyte subset alteration in the peripheral blood of chronic HBV infection, which has demonstrated that there was imbalance, or impaired balance, of the T cell subsets such as a decrease in CD4/CD8 ratio [16], while other approaches showed an increase or no significant change in these cells [17,18] and ultimately, the dysfunction of the immune responses. These dysfunctions are thought to be a consequence of a prolonged display to large amounts of viral antigens, as demonstrated for hepatitis B e antigen (HBeAg) [19,20], while the effects of hepatitis B surface antigen (HBsAg) in this dysfunction is more controversial.

Detection of HBsAg is the first serological marker of the hepatitis B infection and its persistence for more than six months is regarded as a chronic infection. Currently, HBsAg quantification is receiving renewed attention for its diagnostic-clinical role, due to its importance as a facilitating factor to differentiate disease status in chronic HBV infection; moreover, it is a good predictor of early virological response to antiviral therapy [21,22]. Overall, HBsAg is produced by several pathways, including translation of transcri-

ptionally active cccDNA molecules, the intrahepatic virus reservoir acting as a template for replication and from the translation of viral genes transcribed from integrated HBV sequences in the host genome [16]. Moreover, soluble HBsAg is present in the serum of HBV patients as sub viral, not infectious particles, exceeding the number of virions by a factor of 10^2–10^5 [23]. Some reports have described that HBsAg could be involved in the synchronization of the immune response, partially in disturbances of the appropriate immune response and the amount of HBsAg in peripheral blood might influence the HBV-specific CTL response [24,25].

Because the impact of HBsAg serum levels on HBV-specific cellular and humoral immune responses has not yet been explored, in this paper we aimed, firstly, to assess the differences of lymphocyte subsets among chronic hepatitis B (CHB) patients and normal controls at baseline, and in different stages of HBeAg positive and negative subjects. Secondly, we aimed to define the relationship between HBV-specific T cell response and HBsAg serum levels.

2. MATERIALS AND METHODS

The study is a cohort study approved by the Ethics Committee of the Specialist hospital Sokoto. Blood samples were collected between January 2018 and December 2019, from consecutive CHB patients, recruited as the study group from the general outpatient department of the Specialist hospital Sokoto, and from fifty healthy individuals who were used as a control group. Informed consent of individuals in both groups was obtained prior to their enrolment in the study. Ten millilitre (10 mL) venous blood samples were collected from the antecubital fossa of each participant. Serum was separated, divided into aliquots and maintained frozen at −80°C until testing. All patients and controls were sero-negative for hepatitis C virus, delta virus, human immunodeficiency viruses and tuberculosis, and did not consume alcohol. The patients had been positive for HBsAg for more than six months and had clinical features of chronic HBV infection according to the hospital records and clinician reports.

2.1 Serological Assays

The serum HBeAg, anti-HBe, HBsAg and anti-HBs status of the subjects and controls were checked by a commercial third generation enzyme-linked immunosorbent assay (ELISA) (MONOLISA® Bio-Rad) according to the manufacturer's guidelines.

2.2 Quantitation Serum HBsAg Assay

Serum HBsAg was quantified using an Elecsys assay (Roche Diagnostics, Germany). The serum samples were diluted 1:400 in a 2-step procedure (twice 1:20) using the diluent solution provided by the manufacturer. Then, the diluted serum samples were measured following the manufacturer's protocol for HBsAg assay. If the result of the cut-off index (c.o.i) was between 1 and 1000, the final result was the c.o.i X400, whereas if the c.o.i > 1000, the sample was retested at a 1:8000 dilution (3 steps of 1:20 dilution) and the final result was c.o.i X8000. However, if the c.o.i was < 1, the sample was retested undiluted and the result obtained was used as the final result. This method was validated by others, and a very strong correlation was found between this method and the Architect quantitative assay (Abbot) which is used for the measurement of the HBsAg level [26].

2.3 Quantification of Peripheral Blood Lymphocytes

The antibodies used for fluorescence activated cell sorter (FACS) analysis were divided into two panels. One panel used three colour direct immunofluorescence reagent TriTEST CD4 fluorescein isothiocyanate (FITC)/CD8 phycoerythrin (PE)/CD3 peridinin chlorophyll protein (PerCp) to determine the percentage and absolute counts of mature human T lymphocytes (CD3+), Helper/ inducer (CD3+CD+) and (CD3+CD8+) T lymphocytes. The second panel used four colour immunofluorescence reagent MultiTEST CD3 FITC/CD16+CD56 PE/ CD45PerCp/CD19 allophcocyanin to determine the percentage and absolute counts of mature human T lymphocytes (CD3+), NK cells (CD-CD16+CD56+) and B cell lymphocytes (CD3-CD19) in erythrocyte–lysed whole blood samples. All reagents were obtained from Becton Dickinson (San Jose, CA) and were used as per the manufacturer's instructions.

2.4 Statistical Analysis

The data of experiments were analysed using SPSS 21.0 software. Descriptive data were described using "mean± standard deviation". Comparisons between patients and control were analysed using an independent t test. The

correlation between the two indicators (peripheral blood lymphocytes (PBL) and HBsAg) was performed using Spearman rank correlation. The *P* value <.05 was considered statistically significant.

3. RESULTS

All patients (n= 50) enrolled in this study were infected with HBV infection for more than 6 months, and most of them were male (n=31). The peripheral immune cell profiles of control and patients at baseline are shown in Fig. 1. Similarly, the peripheral immune cell profiles of positive and negative HBeAg status CHB patients at baseline are shown in Fig. 2. Some study subjects were positive for HBeAg (n= 10), and the rest were negative (n=40). Table 1 shows the percentage of PBL subsets in patients and healthy control to determine whether any particular immunophenotypic profiles could be related to disease outcomes. There was no significant difference found in the percentage of Total T cells (CD3+), B cells and NK cells as compared with the control, whereas, in contrast, the patients had a significant reduction in the percentage of T helper cells (*P=.05*). The same reduction was seen in CD8+ (cytotoxic T cells) and CD4+/CD8+ cell ratio, as compared with healthy donors (*P*=.01), respectively. A highly significant increase was observed in serum aminotransferase (ALT and AST) (*P*=.01), respectively, in comparison with the healthy control. A correlation between HBsAg level and percentage of PBL subsets was observed in T cells (r=0.366) and NK cells (r=-0.462, *P*=.05), while no significant correlation was found with B cells, T helper, T cytotoxic, CD4+/CD8+ ratio and lymphocyte absolute number (r=0.04, r=0.226, r=0.091, r=0.23 and r=-0.017, respectively).

4. DISCUSSION

When people are infected with HBV, a high percentage of patients are able to clear the virus from their bodies as a result of a combination of cellular and humoral immune responses, and for a low percentage of patients, despite the presence of these immunological mechanisms of HBV elimination, the virus is rarely eradicated from their bodies and they develop chronic hepatitis and hepatocellular carcinoma. The key features of chronic HBV infection are the impairment of immune responses [27] that relate to the cellular composition of the liver, and the presence of excessive viral antigens caused by persistent infection [8].

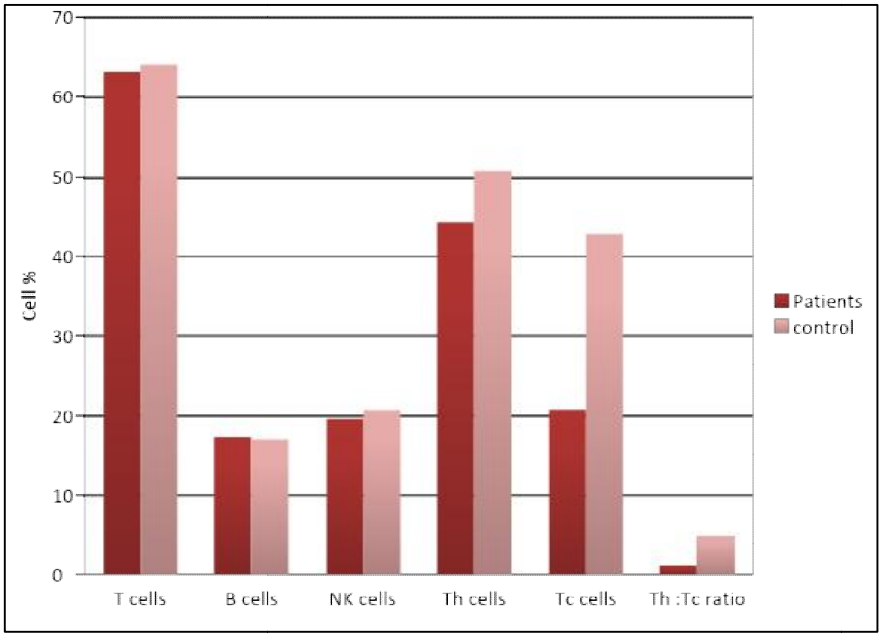

Fig. 1. Peripheral immune cell profiles of control and patients at baseline

T cells: Total T cells, B-cells: Total B cells, NK cells: Total natural killer cells, Th cells: Helper T cells, Tc cells: Cytotoxic T cells, Th:Tc ration: T helper/T cytotoxic cell ratio

Table 1. Comparison of peripheral blood lymphocyte (PBL) percentage at baseline between patients and healthy control

Parameters	Patients (mean±SD) (n=50)	Control (mean±SD) (n=50)	t-statistics (df)	*P* value
Age	41.60±12.194	27.35±7.995	-4.817 (68)	.001*
T cells	63.32±8.103	64.15±7.534	0.407 (68)	.694
B cells	17.42±5.761	17.15±5.019	-0.195 (68)	.855
NK cells	19.64±7.244	20.80±6.678	.641 (68)	.538
Th cells	44.34±14.518	50.80±5.146	1.935	.057*
Tc cells	20.82±20.522	42.85±5.958	4.703 (68)	.000*
Th:Tc ratio	1.2245±.31201	4.9810±3.96725	-4.211 (68)	.00*
LAC (cells/uL)	37.95	34.52	451.000	.524
ALT(IU/mL)	24.38	41.32	209.000	.000*
AST(IU/mL)	20.95	39.95	277.500	.004*

Note: statistically significant (independent t- test). Abbreviations: SD: standard deviation, NK: natural killer cells Th: Helper T cells, Tc: Cytotoxic T cells, LAC: lymphocyte absolute count, ALT: alanine aminotransferase, AST: aspartate aminotransferase

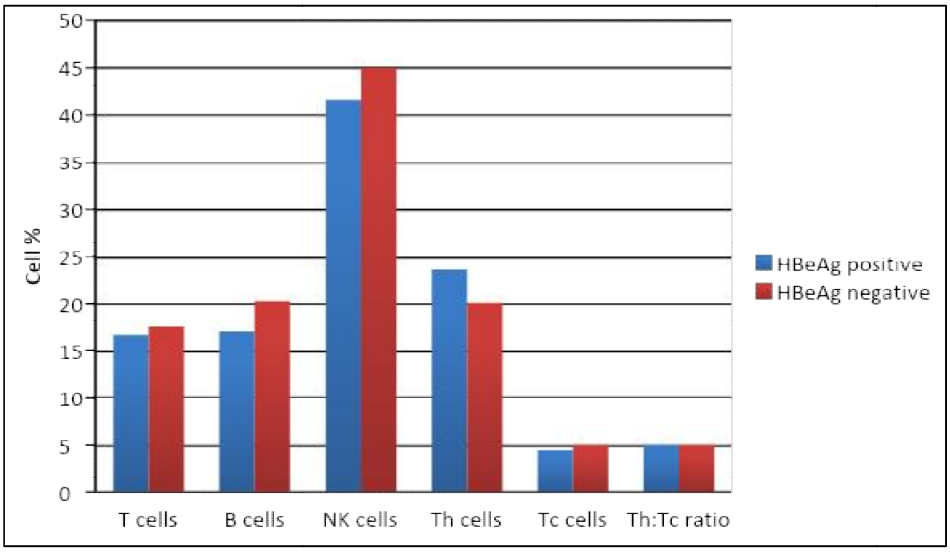

Fig. 2. Peripheral immune cell profiles of positive and negative HBeAg status CHB patients at baseline

T cells: Total T cells, B cells: Total B cells, NK cells: Total natural killer cells, Th cells: Helper T cells, Tc cells: Cytotoxic T cells, Th: Tc: T helper/T cytotoxic cell ratio

Even though many studies have discovered the role and alteration of immune responses during HBV infection, many questions remain to be elucidated, such as the relationship between changing the cellular immune response with HBsAg, HBeAg and HBcAg levels. It is very important to continue attempting to answer these questions so as to develop new strategies of therapeutic aspects. In our study, we first attempted to observe and analyse the dynamic changes of PBL subsets in CHB with normal control, and then to discover the correlation between PBL subsets and HBsAg level in CHB patients. All of our aims lead to exploring the relationship between lymphocyte change and disease progression.

The adaptive immune response during chronic HBV infection depends on Antigen presenting cells (APCs), such as Kupffer cells and, in particular, denderic cells (DC) which are important cells for the presentation and maturation of HBV specific T cells, and the important effectors of HBV clearance. APCs present foreign antigens to (CD4+ and CD8+) T cells and produce cytokines (IL-12 and TNF-α)

which induce IFN-γ production and proliferation of (CD8+) T cells and IL-12, as well as inducing (CD4+) T cell differentiation into the T helper cells Type 1(Th1) subset [21,28].

The outcome of HBV infection is usually influenced by the type of cell-mediated response which is expressed in the early phase of infection, such as in the chronic phase, the HBV–specific T cell responses are weak in peripheral blood cells [29], especially during exposure to the high viral and antigen load [30].

In the present study, our results showed there was no suppression of total peripheral T cell population, B cells and NK cells in chronic HBV patients in comparison with healthy controls, despite the fact that HBV infection causes antibody and cell mediated immune responses [31]. Our data, to some extent, differs from earlier studies that showed a decrease in the percentage of T cell population (CD3+) [12,32], but the same results have been observed with other studies [33] which indicated that there was no significant differences in T, B or NK cells. Considering the importance of the cytotoxic T cell mediated response for elimination and suppression of HBV replication [34], selective reduction of cytotoxic T cells (CD8+), but not the population of helper T cells (CD4+) is clearly evidence that patients having a higher viral load (> 2000 IU/mL) is indicative of persistent chronicity of HBV infection [35]. However, our results identify a slight reduction in percentages of CD4+ and CD8+ cells as compared with healthy controls. These results may indicate the lack of CD4+ T cell role in the impairment of CD8+ T cell activity and antibody production [36,37].

The CD4/CD8 ratio is a reflection of immune system health. Several reports have observed the CD4+/CD8+ ratio in patients with CHB infection. Some investigators showed this ratio to be increasing or decreasing [38] and no significant difference was found in this ratio [29].

The present results showed that the CD4+/CD8+ ratio was lower than in normal controls, and this result is in concordance with You et al. (2008) who confirmed the existence of CD4+/CD8+ to be down in chronic hepatitis B patients [36]. As we know, the CD4+/CD8+ ratio reflects the state of the immune response with a certain range [39] and the up-regulation of this ratio refers to a strong immune response in patients. In contrast, the reduction of even less than one indicates a weak immune function [12] and is attributed to either liver damage or to viral replication [16].

On the other hand, slight differences in the CD4+/CD8+ ratio were found by some investigators [40] and it might be suggested that the impaired immunoregulation may play a big part in the failure of HBV clearance, thus the reduction in CD4+/CD8+ ratio affects the presence of HBV and causes chronic HBV infection [41].

Our study confirmed that there is an elevated ALT and AST level (values reflect clinical definition) in hepatitis B patients, when compared with healthy controls. Our results were in agreement with previous reports that detected an increased ALT level in CHB infection, and there was no distinction between HBeAg negative and positive patients [42]. However, ALT and AST level does not correspond to the proper size of T cell lymphocytes. Cooper et al. [43] observed that there was a high number of lymphocytes in CHB patients with normal or elevated aminotransferase level, while another study indicated that there was no correlation detected between the size of CD4+ and CD8+ on one hand, and the ALT level on the other [30]. Thus, in chronic hepatitis liver diseases, it can be concluded that high levels of AST and ALT do not correspond to the dynamics of inflammatory infiltrate.

Our observation in the present study was that there was no significant difference in T cell lymphocytes between two patients who had positive and negative HBeAg protein. However, this result is only in concordance with some findings [33], while being different to Sun, et al. who found that some T cell subsets are increased in CHB patients, which may play some role in the immune tolerance of chronic HBV infection.

Several recent reports suggest that HBsAg quantification displays a very useful role in the clinical management of chronic HBV, being able to conclude the response to antiviral therapy as well as to help in optimizing the clinical classification of patients. Even though the virological correlates of HBsAg levels remain controversial, variable HBsAg amounts have been predicated to reflect different degrees of immune control [44]. However, the relationship between HBsAg serum levels and the cellular antiviral immune response has not been investigated.

In the current study, we attempted to evaluate the association between the status of peripheral immunocompetent cells in chronic HBV infection and serum HBsAg load to assess the potential relationship between the antiviral immune profile and sero-virological parameters of HBV infection. Overall, the data indicated a significant negative correlation between HBsAg quantity and NK cells, while a positive correlation was seen with the total peripheral T cell population. Our results demonstrated that there was no suppression of total peripheral T cell population or adaptive immunity, but the HBsAg level induced suppression of B cells and NK cells in the peripheral lymphocyte pool. However, our data to some extent differed from an earlier study that showed that the suppression of adaptive immune response was influenced by the amount of HBsAg [34,44]. Many reports have described that the HBsAg level could be involved in the regulation of the immune response [36] and the direct or indirect suppression of the T cell immune response. Interestingly, there are few reports indicating the direct suppression of T, B, NK and NKT cells by increasing the HBsAg load in chronic HBV infection [36]. In 2005, Chen et al. [45] showed that the number of hepatic NK cells was decreased with the expression of HBsAg antigen and their cytotoxicity in transgenic mice, and these findings are in agreement with our results. However, the amount of HBsAg in peripheral blood might influence the HBV-specific CTL response.

In summary, by analysing lymphocyte subsets (T, B and NK cells) of peripheral blood in CHB at baseline, the changes in immune system response between patients and healthy donors was explored. Moreover, we observed the important correlation between HBsAg load and immune cell response. Our results showed that there was no significant alteration in T, B and NK cells, while significant changes were seen in CD4+, CD8+ and CD4+/CD8+ ratio in chronic HBV.

5. CONCLUSION

In conclusion, this study found a significant correlation between HBsAg level and immune cells that are contributing to the innate and adaptive immune system. The understanding of the interactions between HBsAg and peripheral blood cells, and the alteration in percentages of these cells, could help in the development of a HBV therapeutic vaccine and also serve as a good biomarker in HBV persistent infection.

CONSENT

Informed and written consent of individuals in both groups was obtained prior to their enrolment in the study.

ETHICAL APPROVAL

The study is a cohort study approved by the Ethics Committee of the Specialist hospital Sokoto.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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