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The Impaired Balance of $CD4^+/CD8^+$ Ratio in Patients with Chronic Hepatitis B

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Abstract

Background: The role of immune system in natural course of viral hepatitis has been drawn some attention. One of the main diagnostic markers of the immune system function in different diseases might be the ratio of $CD4^+$ to $CD8^+$ T lymphocytes ($CD4^+/CD8^+$ ratio).
Objectives: This research aimed to measure and compare $CD4^+/CD8^+$ ratio in the patients with chronic hepatitis B (CHB) and control group.
Methods: In this cross-sectional study, thirty-three CHB patients and thirty age and sex-matched healthy controls were included. Immunophenotyping of isolated T cells was performed using specific anti-CD4 and anti-CD8 antibodies by flow cytometry. Consequently, $CD4^+$, $CD8^+$ and the ratio of $CD4^+/CD8^+$were counted and compared between the two groups.
Results: $CD4^+$ counts (%) were considerably reduced in patients with CHB compared to the healthy controls (51.22 ± 10.5 vs. 63.14 ± 9.9, P = 0.00), whereas $CD8^+$ counts (%) were higher in the patients with CHB than healthy controls (48.8 ± 10.5 vs. 36.85 ± 9.86, P = 0.00). Moreover, $CD4^+/CD8^+$ ratio remarkably decreased in the patients with CHB (1.15 ± 0.5) than healthy controls (1.93 ± 0.9) (P = 0.00). Area under curve (AUC) of 0.79 (SE = 0.06, CI = 0.68 - 0.90, P value = 0.05) was reported for $CD4^+/CD8^+$ ratio with a sensitivity of 72.73% and specificity of 73.33% in 1.35 cut-off (likelihood ratio = 2.72).
Conclusions: The research indicated an impaired balance between T cell subsets associated with a higher proportion of $CD8^+$ T cells and a lower proportion of $CD4^+$ T cells and $CD4^+/CD8^+$ ratio in patients with CHB.

Keywords: CD4-CD8 Ratio, Hepatitis B, Immune System

1. Background

Chronic infection with hepatitis B virus (HBV) has infected 257 million people throughout the world of which 75% live in the Asia Pacific Region. Nearly 2 billion people have been infected, and almost 1,000,000 persons die per year (1). The highest prevalence of HBV infection in Iran was reported from Northeast of Iran (Golestan Province) (2, 3).

One of the major determinants affecting disease duration and the beginning of the liver disease is the virus-specific T-cell response. A significant diagnostic marker of immune system function is the ratio of $CD4^+$ to $CD8^+$ T lymphocytes ($CD4^+/CD8^+$ ratio). Many healthy people possess a $CD4^+/CD8^+$ ratio ranging between 1.5 and 2.5 to 1, whereas an inverted ratio characterizes intense chronic immune responses (4).

2. Objectives

The present research aimed to measure $CD4^+/CD8^+$ ratio in the patients with CHB and healthy controls and then compare them to possibly introduce this ratio as one of the newly developed diagnostic markers.

3. Methods

3.1. Subjects

In this cross-sectional study, thirty-three patients with chronic hepatitis B (positive HBsAg for more than 6

months) were selected randomly to be included in the study from those referred to our research center in Sayyad-e-Shirazi Hospital, Gorgan, Northeast of Iran (no.: IR.GOUMS.REC. 1397.091).

Thirty age- and sex-matched persons were recruited as healthy control group (HBsAg negative) from gastroenterology clinic. The exclusion criteria (in both groups) were as follows: autoimmune diseases (such as autoimmune hepatitis, metabolic liver disease, lupus, rheumatoid arthritis, drug hepatitis, and cirrhosis of the liver), The human immunodeficiency viruses (HIV), hepatitis C virus (HCV) and hepatitis D virus (HDV) positive test results, intravenous drug use (IVDU) and under treatment with Immunosuppression drugs for any reason.

A questionnaire was completed for demographic variables, including gender, age, height, weight, waist circumference, body mass index (BMI), address of residency, ethnicity, job, background of the underlying illness, history of alcohol usage, cigarettes and (IVDU). Following data were obtained from the patients' records: HBV-DNA level, hepatitis B surface antigen (HBsAg) seropositivity, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (alk.p), prothrombin time (PT), partial thromboplastin time (PTT), platelet count, hepatitis B surface antibody (HBsAb) and hepatitis B core antibody (HBcAb or anti-HBc). All cases were HBeAg negative.

3.2. Flow Cytometry Analysis

Blood samples were collected into 2-mL EDTA vacutainer tubes and transported to Stem Cell Laboratory at Gorgan University of Medical Sciences. Staining of the samples was done using the single-staining method. The monoclonal antibodies used to identify these cell subsets were as follows: mouse anti human CD4-FITC (cytomatingene, Iran) and anti human CD8-PE (cytomatingene, Iran). Isotype negative controls were mouse IgG1-FITC isotype control Ab (cytomatingene, Iran) and PE mouse IgG1k isotype control (Biolegend, United States). The samples were prepared by the whole blood lysis technique (lysing solution and Ficoll for red blood cells) and analyzed on a flow cytometer (BD accuri c6) (Appendix 1 in Supplementary File).

3.3. Statistical Analysis

Data were analyzed by SPSS version 16.0, and Graphpad Prism 5.0 was used to depict graphs. The comparative analysis of the cellular population in different groups was performed using parametric (independent samples *t*-test) method. Values of $P < 0.05$ were considered statistically significant.

4. Results

The demographic data of the patients with CHB and control group included the male to female ratio (23/10 vs. 18/12, P value = 0.42) and mean age (SD) (45.70 (13.65) vs. 46.73 (16.3), P value = 0.78) were not significantly different between the two groups. All patients were HBeAg negative and the HBV DNA level was undetectable (< 200 copies/mL). As shown in Table 1, the $CD4^+$ count (%) and $CD4^+/CD8^+$ ratio were significantly lower in patients with CHB than healthy controls (Table 1).

Gender and age group had no significant effect on the $CD4^+/CD8^+$ ratio in the CHB group, however, it was reported higher in men than women. In the healthy control group, this ratio was reported higher in women and younger than 45 years. Comparing the $CD4^+/CD8^+$ ratio between men and women in the two groups showed a statistically significant difference (Table 2).

In order to estimate the diagnostic value of $CD4^+/CD8^+$ ratio, ROC curve analysis was performed. Area under curve (AUC) of 0.79 (SE = 0.06, CI = 0.68 - 0.90, P value < 0.0001) was reported for $CD4^+/CD8^+$ ratio with a sensitivity of 72.73% and specificity of 73.33% in 1.355 cut-off point (likelihood ratio = 2.727) (Figure 1).

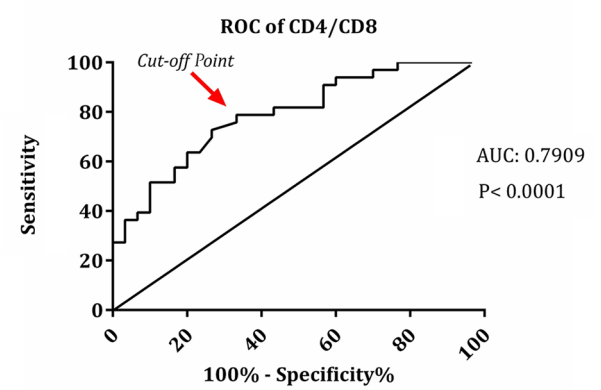

Figure 1. ROC curve of $CD4^+/CD8^+$ ratio in patients with chronic hepatitis B; area under curve (AUC) of 0.79 was reported for $CD4^+/CD8^+$ ratio with a sensitivity of 72.73% and specificity of 73.33% in 1.355 cut-off point.

5. Discussion

Persistent HBV infection is associated with the host's disability in the control of the infection (5). The HBV has non-cytotoxic activity, and resulting hepatotoxicity often goes back to the host immune response (6). In fact, $TCD4^+$ and $CD8^+$ are key cells in cell-mediated immunity and play a leading role in the host immune responses to viral infections (7). A direct association exists between $TCD4^+$ cell response and clinical outcomes of the HBV infection. The potent $TCD4^+$ cell response to acute HBV infection solely is able to clear HBV infection, while this strong immune response is not seen in the chronic phase of the disease.

Table 1. Comparison of $CD4^+$ and $CD8^+$ Counts (%) in Patients with Chronic Hepatitis B and Healthy Controls[a, b]

Groups	$CD4^+$		$CD8^+$		$CD4^+/CD8^+$ Ratio
	Cells/μL	Percentage	Cells/μL	Percentage	
CHB (n = 33)	1098.88 ± 812.96	51.22 ± 10.5	1006.82 ± 725.02	48.8 ± 10.5	1.15 ± 0.5
HC (n = 30)	1469.56 ± 484.22	63.14 ± 9.9	874.73 ± 402.35	36.85 ± 9.86	1.93 ± 0.9
P value	0.034[c]	0.000[c]	0.381	0.000[c]	0.000[c]

Abbreviations: CHB, chronic hepatitis B; HC, healthy control.
[a]Values are expressed as mean ± SD.
[b]Independent sample *t*-test was applied to compare both groups in a column.
[c]P values lower than 0.05 are considered significant.

Table 2. Comparison of $CD4^+/CD8^+$ Ratio in Patients with Chronic Hepatitis B and Healthy Control According to Age Group and Gender[a]

Groups	$CD4^+/CD8^+$ Ratio					
	Age Group, y			Gender		
	≤ 45	> 45	P Value	Male	Female	P Value
CHB (n = 33)	1.11 ± 0.58	1.17 ± 0.40	0.718	1.23 ± 0.52	0.94 ± 0.31	0.109
HC (n = 30)	2.22 ± 0.92	1.67 ± 0.79	0.092	1.64 ± 0.63	2.36 ± 1.05	0.027
P value	0.001	0.024	-	0.031	0.001	-

Abbreviations: CHB, chronic hepatitis B; HC, healthy control.
[a]Values are expressed as mean ± SD.

The $TCD4^+$ cells are indirectly involved in viral overthrow, but trigger the secretion of interleukin-2 (IL-2) cytokine via $TCD8^+$ cell stimulation. The $TCD4^+$ cells reinforce the virus-specific $TCD8^+$ cell responses, and the absence of these cells undermines the activity of $TCD8^+$ cells. There is the possibility of remaining active $CD4^+$ memory T cells and virus-specific CTLs for more than 23 years after the primary viral infection. The $TCD8^+$ cells play a major role in the control and clearance of HBV infection. The $TCD8^+$ cells are able to eliminate a viral infection using cytolytic and non-cytolytic mechanisms. The absence of these cells causes an increase in viral load and liver damage. Chronic infection can lead to dysfunction in HBV-specific $TCD8^+$ cells so that they are incapable of proliferation and cytokine production (8).

Researchers proposed that cytotoxic $CD8^+$ T cells attack to all infected and non-infected hepatocytes that leads to extensive liver injuries in chronic hepatitis B patients (9). Thus it is necessary to conduct a comprehensive study on the immune mechanisms in hepatitis B so that poor immune response to viral control will be more clearly. Cytotoxic T lymphocytes (CTLs) cannot differentiate infected hepatocytes from non-infected cells; so this immune response will damage all liver cells regardless of whether the infection itself is harmful to the host (10).

Several studies have addressed the role of the immune system in diseases such as acquired immune deficiency syndrome (AIDS) and hepatitis C, but few studies are available regarding chronic hepatitis B. Studies in China and Thailand have shown controversial results. In this study, we have found that patients with CHB have significantly lower $CD4^+/CD8^+$ ratio (1.15 ± 0.5) compared to the controls (1.93 ± 0.9) ($P < 0.000$). Our findings were in accordance with You et al. (11) in China and Thailand in 2009 who showed that the $CD4^+/CD8^+$ ratio was significantly lower in the patient group (1.04 ± 0.45) than in the healthy group (1.67 ± 0.33).

But in a study in china (2011), although a similar but higher $CD4^+/CD8^+$ ratio was found in Patients with CHB (1.23 ± 0.11) to our study. This may be explainable by different inclusion and exclusion criteria (12). In the study conducted by You et al. (13) in China and Thailand in 2008, the $CD4^+$ count (%) in the patient group (n = 216) was significantly lower than the healthy group (32% vs. 38%) consistent with our study. In another study by You et al. (14) in China and Thailand in 2008, the $CD8^+$ counts in the patient group (34.39 ± 9.22 cells/μL) were significantly higher than the healthy group (24.02 ± 4.35 cells/μL). Future studies are suggested regarding the treatment and vaccination by helping the immune systems by mechanisms that can discriminate cells, and by preventing inflammation; thus preventing subsequent complications.

5.1. Conclusions

In conclusion, the present research revealed an impaired balance of T cell subsets associated with higher $CD8^+$ and decreased $CD4^+$ T lymphocytes and $CD4^+/CD8^+$ ratio in patients with CHB.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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Footnotes

Authors' Contribution: Nesa Shokoohifar, Salman Ahmady-Asbchin, Saeed Mohammadi, and Sima Besharat had substantial contributions to the conception and design of the work and the acquisition, analysis, and interpretation of data. Fatemeh Roudbari, Saeed Mohammadi, and Sima Besharat contributed to drafting the work, revising it critically for important intellectual content, and final approval of the version published. Taghi Amiriani, Alireza Norouzi, and Behnaz Khodabakhshi contributed to the conception and design of the work, in drafting the work, revising it critically for important intellectual content, and final approval of the version published. Nesa Shokoohifar and Iman Shahabinasab contributed to the acquisition and interpretation of data, also in drafting the work and revising it. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interests: The authors declare that there is no conflict of interest.

Ethical Approval: This research project was approved by the Ethics Committee of Gorgan University of Medical Science with the code of ethics of ir.goums.rec.1396.4.

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