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ORIGINAL ARTICLE

Immune profile of peripheral blood T-lymphocyte subpopulations in chronic hepatitis B

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ABSTRACT

Background: *The role of immune response to chronic hepatitis B virus (HBV) infection is complex; and the specific T-cell response to this infection can determine the duration and the extent of liver disease.* ***Objective:*** *This study aimed at assessing the profile of T-lymphocyte subpopulations in chronic hepatitis B (CHB) patients and its association with HBV replication.* ***Methodology:*** *The case group included 50 CHB patients with normal liver function tests (LFTs); and the control group included 50 age and sex-matched healthy individuals. The HBV markers, LFTs and serum viral load were measured in cases. Blood CD4 and CD8 T-lymphocyte subpopulations and the CD4/CD8 ratio were assessed in both groups by flow cytometry.* ***Results:*** *Our results showed significantly higher CD8 T-cells; significantly lower CD3 and CD4 T-cells; markedly reduced CD4/CD8 ratio in the cases as compared to the controls ($P<0.001$, for all). This T-cell impairment was also significantly linked to HBeAg positivity and elevated level of viraemia. The increased level of CD8 T-cells was significantly linked to both the HBeAg positivity ($P<0.001$) and the elevated level of viraemia ($P=0.005$), whereas the decreased levels of CD3, CD4 T-cells and CD4/CD8 ratio were significantly linked to both HBeAg positivity ($P<0.001$, in all) and the elevated level of viraemia ($P<0.001$, $P=0.001$& $P=0.007$, respectively).* ***Conclusion:*** *T-lymphocyte subpopulations imbalance could be expected by measuring the serum HBVeAg and the viraemia level in CHB patients exhibiting normal LFTs. These parameters are recommended to be measured regularly for the cellular immune function assessment.*

INTRODUCTION

Egypt is loaded with about two to three million CHB infected individuals, with increased risk of development of liver cirrhosis and liver cancer being the most important health problems, yet, may be associated with histologically normal liver and normal LFTs[1]. The role of immune response to chronic HBV infection is complex; and the specific T-cell response to this infection can determine the duration of liver disease and the initiation of liver damage[2]. The cellular immunity is considered the main immune arm that controls the pathogenesis of HBV. The T-cell responses to acute HBV infection are powerful and specific thus, can effectively eliminate the virus, whereas in chronic HBV infection these response are fairly weak[3,4]. This ensures the crucial role of the T-cell responses in controlling HBV infection[5].

The HBeAg, an indicator of active viral replication, is essential in the virus-immune system interaction. It has an immune-modulatory role in recognition and antigen presentation via CD4 T-cells. Moreover, in transgenic murine model systems, secreted HBeAg can change the immune response by triggering non-inflammatory Th2 cells and deleting inflammatory Th1 cells[6].

The CD4/CD8 T-lymphocytes ratio is considered an important marker for the immune system function. A healthy individual exhibits a CD4/CD8 ratio of 1.5 to 2.5, but a reversed ratio is a sign of severe chronic immune response[7]. It was found that high viral load in CHB patients is associated with T-cells functional impairment, resulting in persistent viral infection[8].

This study aimed at assessing the profile of T-lymphocyte subpopulations in chronic hepatitis B (CHB) patients and its association with HBV replication.

METHODOLOGY

Study design

This case control study was conducted over a period of ten months. The study was approved by the Mansoura Faculty of Medicine Institutional Research

Board (code number: R.21.03.1238). The case group included 50 CHB patients (positive HBsAg for >6 months)[9] who attended the Tropical Medicine Outpatient Clinic in Mansoura University Hospitals, Egypt. The control group included 50 age and sex-matched healthy blood donors. The T-lymphocyte subpopulations profile was compared in both groups. All participants gave informed consent for the study.

Inclusion criteria

The inclusion criteria for the cases were:

- CHB (positive HBsAg for >6 months).
- Normal LFTs.

Exclusion criteria

The exclusion criteria for all participants were:

- Individuals suffering from hepatitis C, hepatitis D, liver cirrhosis, drug hepatitis, human immunodeficiency virus (HIV), metabolic hepatic disease, autoimmune hepatitis, lupus and rheumatoid arthritis.
- Intravenous drug users and patients under immunosuppressive therapy.

Estimation of LFTs and HBV markers

Total bilirubin (TBil), aspartate transaminase (AST) and Serum alanine amino-transferase (ALT) were investigated using routine automated procedures (upper normal limit: 17.1 µmol/mL, 40 U/L and 40 U/L, respectively). The HBV markers (HBsAg, HBeAg, HBsAb, HBeAb, HBcAb IgM and HBcAb) were evaluated for the cases by ELISA (Dialab, Austria).

Serum HBV DNA quantitation

The HBV DNA load in the cases was evaluated by fluorescent quantitative real-time PCR. This was performed according to the reagent kit that also included the primer (Shenzhen PG Biotech, China). The final reaction volume was 40 µL, and the program used for amplification involved five minutes at 37°C, one minute at 94°C, then forty cycles of five seconds at 95°C, thirty seconds at 60°C. A HBV DNA load of $>10^3$ copies/mL indicated viraemia.

Assessing profile of T lymphocyte subpopulations in peripheral blood

Blood samples were collected from the cases and controls into 2-mL EDTA vacutainer tubes. Antihuman monoclonal antibodies CD4-FITC, CD8-PE and CD3-PE-CY5 were used (Abcam, UK). The used isotype negative control was mouse IgG1-FITC isotype control Ab (Abcam, UK). Samples were analyzed using a flow cytometer, and the result was expressed as percentage of CD4, CD8 and CD3 cells that was positive for antigen marker among total T- cell population. This was performed according to the manufacturers' instructions.

Statistical analysis

The collected data were analysed using Statistical package for Social Science (SPSS) version 25, Armonk, NY: IBM Corp. For assessing the statistical significance of difference between two study groups' means, "Student T Test" was used. To compare more than two study groups' means, one way analysis of variance "ANOVA" was used. For assessing the strength of association between two quantitative variables, "Correlation analysis" was used. For all performed statistical tests, the threshold of significance is fixed at 5% level (*p*-value). The results were considered: significant when $p < 0.05$, and highly significant when $p < 0.001$.

RESULTS

The study was conducted on 50 patients with CHB, with mean age of 48.6 years, and 50 healthy controls with mean age of 47.4 years (P=0.645). The cases (37 males and 13 females) were gender-matched with the controls (40 males and 10 females) (P=0.524). The serum level of HBV DNA of $>10^3$ copies/mL was detected in 80% of CHB infected patients, and the HBeAg was positive in 80% of them. (table 1). The CHB cases showed significantly higher CD8 T-cells; significantly lower CD3 and CD4 T-cells; and markedly reduced CD4/CD8 ratio when compared to controls (P<0.001, for all). This T-cell impairment was also significantly associated with HBeAg positivity and elevated level of viraemia. Positive correlation was present between the level of CD8 T-cells and both the elevated level of viraemia (P=0.005) and the HBeAg positivity (P<0.001), while a negative correlation was present between the CD3 and CD4 T-cell levels and CD4/CD8 ratio and both the elevated level of viraemia (P<0.001, P=0.001& P=0.007, respectively) and the HBeAg positivity (P<0.001, for all), (table 2 and figures 1- 3).

Table 1: Demographic data of CHB cases

Characteristics	Cases (n=50)	
Mean age ± SD (years)	48.6±12.07	
Sex (male/female)	37/13	
Serum HBV DNA (copies/mL) (n-%)	$<1.0 \times 10^3$	10(20%)
	1.0×10^3 - 1.0×10^5	12(24%)
	1.0×10^5 - 1.0×10^7	15(30%)
	$>1.0 \times 10^7$	13(26%)
HBeAg (n-%)	HBeAg negative	10(20%)
	HBeAg positive	40(80%)

Table 2: T-lymphocyte subpopulations in peripheral blood of controls and cases

Groups	n	CD3		CD4		CD8		CD4/CD8 ratio	
HBV state									
		mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Controls	50	80.5	13.7	41.7	7.4	24.3	5.1	1.8	0.7
Cases	50	59.7	10.1	33.4	5.9	32.4	6.8	1.1	0.4
P		<0.001		<0.001		<0.001		<0.001	
Serum HBV DNA of cases (copies/mL)									
		mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
$<1.0 \times 10^3$	10	68.5	7.5	38.1	6.3	29.6	3.8	1.3	0.4
1.0×10^3-1.0×10^5	12	65.7	4.5	35.5	1.4	29.2	5.7	1.3	0.3
1.0×10^5-1.0×10^7	15	59.7	6.2	31.9	6.4	32.3	7.2	1.1	0.5
$>1.0 \times 10^7$	13	47.3	6.8	29.6	4.6	37.5	6.4	0.8	0.3
P		<0.001		0.001		0.005		0.007	
HBeAg of cases									
		mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
HBeAg negative	10	72.4	3.7	40.6	3.9	24.6	2.7	1.7	0.2
HBeAg positive	40	56.5	8.6	31.6	4.9	34.3	6.1	1.0	0.3
P		<0.001		<0.001		<0.001		<0.001	

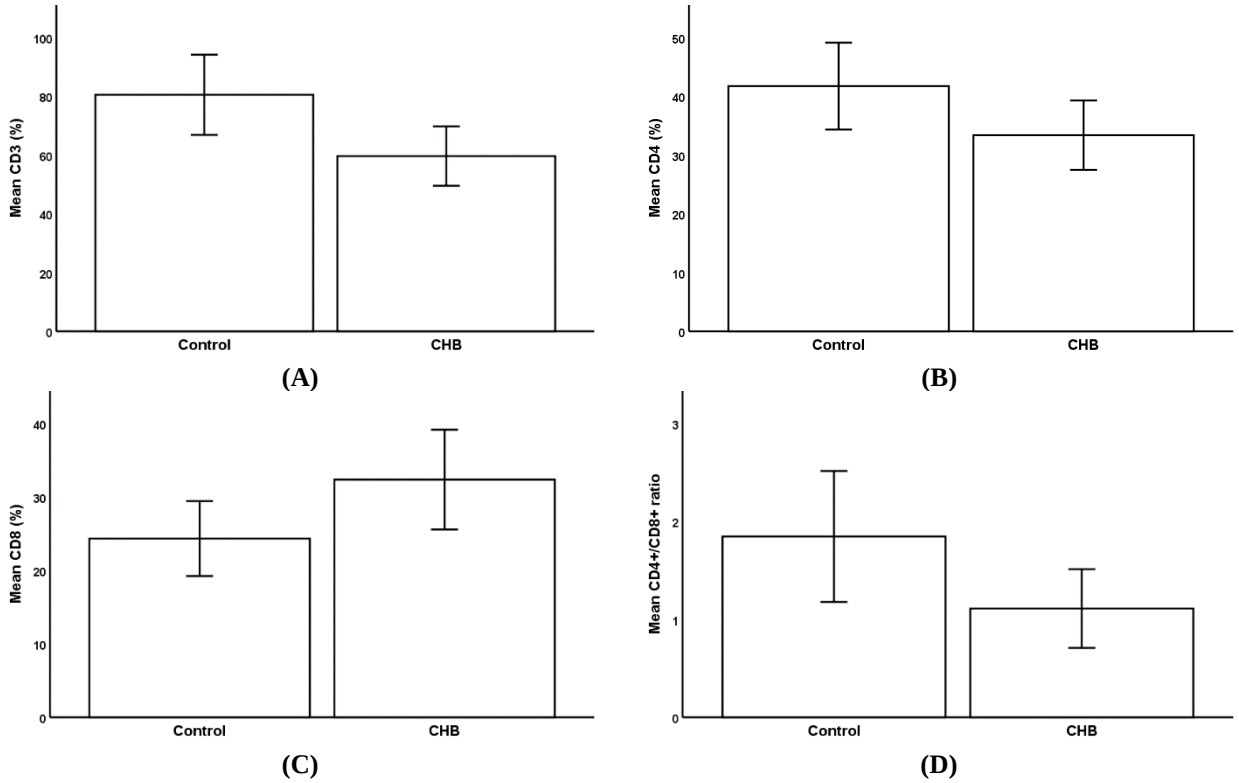

Fig. 1: Column charts for the mean CD3 (A); CD4 (B); CD8 (C) and CD4/CD8 ratio (D) in controls and cases. Error bars represent standard deviation.

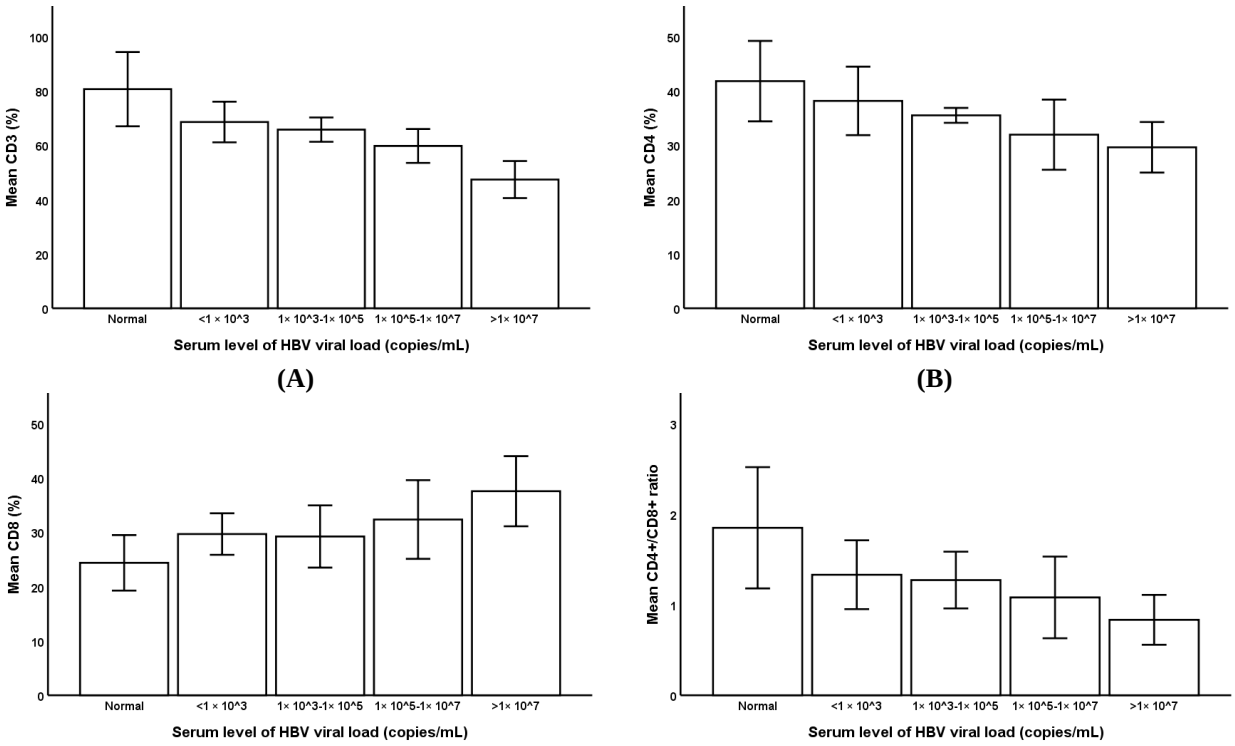

Fig. 2: Column charts for the mean CD3 (A); CD4 (B); CD8 (C) and CD4/CD8 ratio (D) according to the serum viral load. Error bars represent standard deviation.

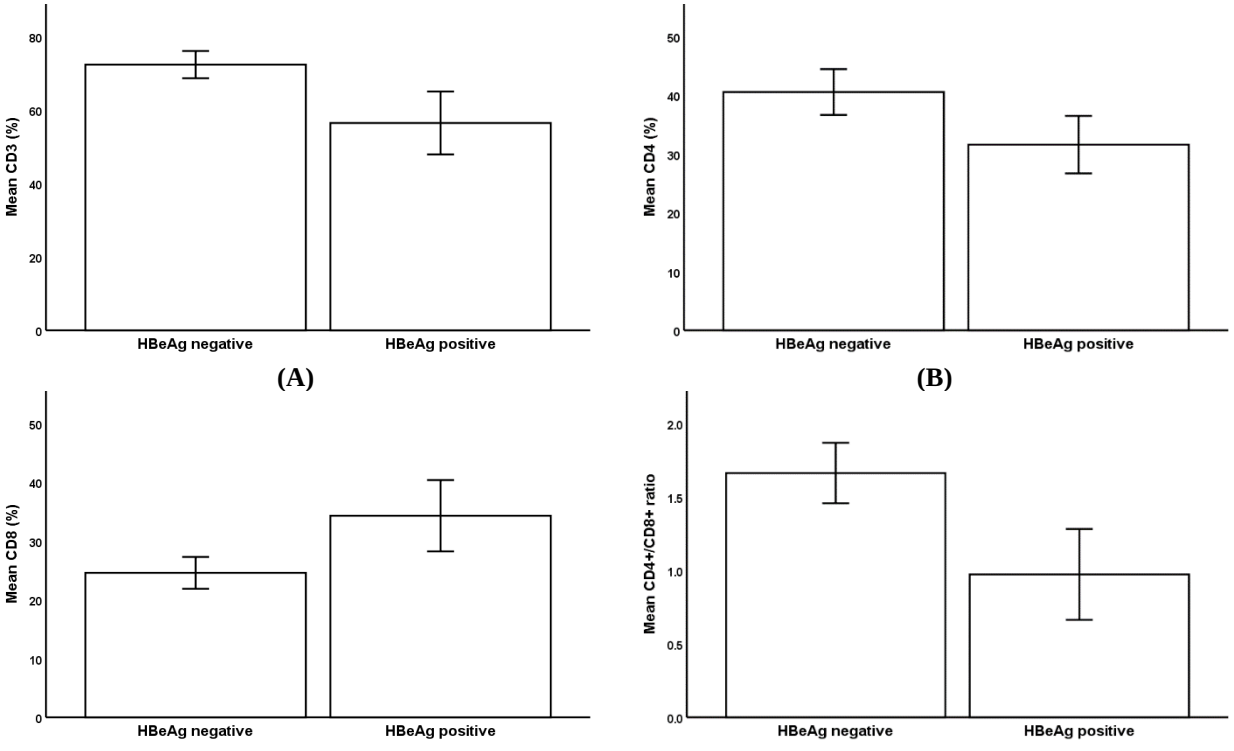

Fig. 3: Column charts for the mean CD3 (A); CD4 (B); CD8 (C) and CD4/CD8 ratio (D) according to HBeAg positivity. Error bars represent standard deviation.

DISCUSSION

The T-lymphocyte subpopulations in peripheral blood are considered an important marker for the immune system function. Their role has been investigated in several researches concerning hepatitis C and HIV, but few researches are done concerning CHB. This study confirmed T-cell subsets imbalance in CHB cases with normal hepatic function tests, as there were an increased in CD8 T-cells and decreased a proportion of CD3 T-cells, CD4 T-cells and CD4/CD8 ratio. The HBV is not a cytopathic virus but it can cause liver destruction by stimulating a defensive immune reaction that leads to killing the intracellular virus throughout destruction of viral infected cells. So, immunological injury has been suggested to be the main etiological cause of hepatocyte injury [10,11]. Consequently, immune eradication of infected hepatocytes causes infection termination when it is competent; or persistent necroinflammatory infection when it is not[12].

The CHB patients were found to have T-cell failure in previous studies[13,14]. There is a rising insufficient cellular immune function[15]. The considerable reduction of total CD3 T-cell indicated a deficiency in immune-competent cells against HBV infection[16]. Also, Martinet et al reported that soluble viral antigens can inhibit the stimulation of HBV-specific T cells[17].

The CD8 T-cells are crucial for the clearance and control of HBV infection via cytolytic and non-cytolytic mechanisms; and the CD4 T-cells are concerned with viral eradication by stimulating the CD8 T-cells to secrete interleukin-2 cytokine, and consequently reinforcing virus-specific CD8 T-cell response. An increase in viral load and liver injury could occur in absence of this response. In addition, CHB infection makes viral-specific CD8 T-cells unable to produce cytokines. So, decreased level of CD4 T-cells could inhibit CD8 T-cells response despite of increased level of CD8 T-cells[18]. Other researchers anticipated that cytotoxic CD8 T-cells can not differentiate between infected and non-infected liver cells. So, increased level of CD8 T-cells attacks both infected and non-infected liver cells leading to chronic course with widespread liver injury in HBV patients[19].

Our research revealed a positive correlation between CD8 T-cell levels and viraemia (P=0.005), whereas a negative correlation was present between the CD3 and CD4 T-cells levels and CD4/CD8 ratio and viraemia (P<0.001, P=0.001& P=0.007, respectively). A similar finding was reported by you et al as they detected significant T cell impairment associated with the level of viraemia[20]. A previous research found an increase in the HBV-specific CD4 T-cells throughout the first year of antiviral therapy[21]. Another study reported that CD8 T-cells are present, but suppressed and antiviral therapy can improve the CD8 T-cell response in cases[22].

HBeAg indicates elevated viral replication and plays a vital role in HBV chronicity with high viral load[20]. In this study, T-cell impairment was significantly associated with HBeAg positivity. Positive correlation was present between CD8 T-cells level and HBeAg positivity (P<0.001) while a negative correlation was present between CD3 and CD4 T-cell levels and CD4/CD8 ratio and HBeAg positivity (P<0.001, for all). Similarly, Baumert and coauthors reported that HBeAg has a tolerogenic effect with little specific T-cell response and consequently T-cell impairment [23]. Also, Xu et al observed a strong association between T cell response and HBeAg seroconversion after nucleoside analogs treatment which could inhibit HBV replication and consequently increase the cure rate in CHB cases[24]. On the other hand, another study found independent correlation between HBeAg and T-cell impairment. The possible reason is due to infection with a virus which has a mutation that results in HBeAg loss. So, in these cases, viral replication continues in spite of HBeAg eradication and seroconversion to anti-HBe[25]. This may diminish the correlation between HBeAg expression and T-cell injury[20].

CONCLUSION

Our research reported an imbalance in the T-cell subpopulations with elevated levels of CD8 T-cells and reduced levels of CD4 T-cells and reduced CD4/CD8 ratio in CHB cases. T-cell subpopulations imbalance could be expected by assessing serum HBVeAg and viraemia level in CHB cases exhibiting normal LFTs. These parameters are recommended to be measured regularly for the cellular immune function assessment.

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- Each author listed in the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article has not been published anywhere and is not currently under consideration by another journal or a publisher.

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