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Transmitted Water Disease, Assessment of Immunopathogenesis of Chronic Hepatitis B and The Carrier State of Disease

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Abstract: The transmission of viral hepatitis type B (HBV) is of significant public health concern. The infection result depends on how well the virus interacts with the host and in particular, on the ability to respond inherently and adaptively to the humoral and cellular immunity. The purpose of this study is to evaluate clinical, immunology and tracer status (viral). This study showed the relationship between the immune and chronic conditions of Iraqi patients who are chronic hepatitis virus B or HBV carriers. The study included (111) chronically-viral hepatitis type (b) and (112) hepatitis virus surface antigen type (b) healthy carriers from out of patients. The result of this study proved that a non-significant correlation was observed between cellular immune response (CD4 and CD8) among chronic hepatitis B patients. For CD8+ lymphocytes: there was a highly significant decrease ($P<0.001$) in the percentage means of the CD8+ cells in CHB patients, as compared with the carrier groups. For CD4+ lymphocytes: there was a slight decrease in the percentage of these lymphocytes in the peripheral blood of the patients, as compared with the carrier groups, a non-significant importance was recorded between them. The percentage of cytotoxic T-lymphocyte CD8+ was significantly decreased in CHB patients as compared to the carrier group. One of the deciding factors for the form of infection, and the immune response, which developed in accordance with a number of other biochemical factors and genes is chronic hepatitis B immunopathogénesis and carrier condition with the level of cellular immunity.

Key words: Hepatitis B virus (HBV), cell-mediated immunity, CD4+/CD8+ lymphocytes.

Introduction

Hepatitis is an infection that fundamentally spreads through the faecal-oral course, which happens when an uninfected individual is exposed to food or water polluted with the virus. Despite the fact that uncommon, waterborne flare-ups are essentially connected to sewage-sullied or inappropriately cleaned water. Close actual contact (like oral-butt-centric sex) with an infected individual can likewise communicate the infection, however, casual touch between people doesn't spread the contamination. Hepatitis refers to a group of clinical and pathologic disorders that are characterised by hepatocellular necrosis and inflammatory cell invasion of the liver on histopathological evaluation (Kleiner et al., 2014; Liu et al., 2019). Hepatitis results from hepatocyte damage produced by the action of varying forms of chemical agents including drugs, toxins, alcohol or some pathogens such as viruses and others (Choi, 2012; Sitia et al., 2007). Hepatitis B virus causes chronic infection for less than 5% of adults, while infection during infancy or childhood is associated with

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a high risk of persistent infection (Guha et al., 2004; Ramalingam, 2015; Trépo et al., 2014). Complications include cirrhosis, as a result of chronic active hepatitis, hepatocellular carcinoma (HCC), hepatitis B carriers are 200 times more in respect to get liver cancer than non-carriers (Puotiet al., 2006; Rehermann, 2013)

The strength of the innate and adaptive, humoral and cellular immune responses, as well as the kinetics of the virus-host interaction, determine the fate of HBV infection (Ponte et al., 2018; Wang et al., 2005).

Although it recognises HBV-infected cells and generates and retains protective HBV-specific memory, the adaptive cellular immune response plays a crucial role in the host's defense against viruses like HBV (Isogawaet al., 2013; Trépo et al., 2005). Specific antibody patterns have been linked to various stages and consequences of HBV infection, and they are commonly employed as diagnostic tools. Antibodies to HBs Ag are the initial markers of acute HBV infection. Antibodies towards HB Ag are a symptom that a person is recovering from acute self-limited HB. Anti-HBe seroconversion is linked to less severe liver disease (Boonstra et al., 2008; EASL, 2017).

Antibodies work against the glycoproteins of the surface (S), pre-surface 1 (S1) and pre-surface 2 (S2) region are neutralising and appear when HBs Ag is cleared during recovery from HBV infection. Their production is a T-cells dependent process (Durantel and Zoulim, 2016; Puro and Schneider, 2007). CD4+ T helper (TH) and CD8+ cytotoxic T-cells mediate unique cellular immune responses to the hepatitis B virus (CTL). In the context of MHC class II molecules on antigen-presenting cells, CD4 TH cells identify viral peptides (Spickett, 2019; Zhu et al., 2018). HBV-specific CD8+ T cells, on the other hand, detect peptides in the context of MHC class I molecules produced only from endogenously produced proteins. HBV-specific T cells perform a range of immunoregulatory roles after being activated by their particular T cell receptors (Salpini et al., 2015; Sierra-Davidson, 2016). CD4 TH cells, in particular, contribute to B cell activation and differentiation for the generation and maintenance of HBV-specific CD8 T cells and permit dendritic cells to activate CD8 effector T cells (Chisariet al., 2010; Fahey, 2014; Mizukoshi et al., 2004). CD4 T cells, like CD8 T cells, may release IFN- and TNF-, which limit HBV multiplication and gene expression. Finally, CD4+TH cells, as well as CD8+ T cells, the primary effector cells, are capable of directly lysing HBV-infected cells (Humby, 2017; Puro and Schneider, 2007; Thursz et al., 1995).

Khan et al. (2018) and Khongand Overwijk, (2016) have reported that in chronic hepatitis infection the majority of liver infiltration lymphocytes are Th1 type and capable in the secretion of IFN-γ and IL-6, whereas in acute hepatitis cells appear to be predominantly of Th2 type.

Finally, the pathogen interplay during HBV infection may be described as follows: (a) via lyses of infected cells and cytokine-mediated downregulation of HBV replication, the innate immune response regulates the first phase of HBV infection. (b) The adaptive immune response is required for viral clearance and protection against reinfection by HBV-specific immunologic memory. (c) Innate and adaptive immune cells produce cytokines and chemokines, which cause antigen-nonspecific amplification of the intrahepatic cellular infiltration, the formation of necroinflammatory foci, and enhanced serum alanine aminotransferase activity (Dongpinget al., 2006; Knolle and Thimme, 2014; Pennaet al., 2007; Rehermann, 2013).

Methods

Patients

Patients groups include the following:

Chronic hepatitis B patient's groups: A total of 112 patients with CHB were admitted to Hepatology and Gastroenterology Al-Hussain Teaching Hospital in Al-Nasiriya City, Southern of Iraq, aged from 20 to 65 years. The patients were suffering from different clinical symptoms with previous risk factors for transmission of HBV infection.

Control groups: A- A total of 112 healthy HBs Ag carriers were discovered accidentally through attending blood bank for the donation of blood, aged from 18 to 52 years.

Patients and carriers blood samples were submitted for the following:

Sample Collection

The study was done in Central Public Health Laboratories/Viral Hepatitis Reference, Hepatology and Gastroenterology Al-Hussain Teaching Hospital in Al-Nasiriya City. A cross sectional study was conducted in the period between the beginning of February, 2021 to the end of April, 2021.

From each individual enrolled in this study, 10-12 ml of blood was drawn by venipuncture using disposable syringes. The blood was placed in plastic disposable tubes and left to stand at room temperature (20-25°C)

to allow it to clot, then the sera were separated by centrifugation for 5 minutes at 10,000 r.p.m and divided into aliquots (250 µl) and stored at -20°C till examination. Each aliquot of the serum was used at once to avoid freezing and thawing. All sera and reagents were allowed to stand at room temperature before being used in the test.

CD4 and CD8 T-Cell Assay

A. Principle
   Is an immune enzymatic assay based on the specific capture of the TCD4 or TCD8 lymphocytes, with paramagnetic micro particles coated with capture antibodies (Carinelli et al., 2015).
B. Procedure
   1. Mixing the blood sample in a suspension of paramagnetic microparticles (coated with anti-pan T antibodies for the capture of T lymphocytes). Dispense the mixture in the wells of a microtitration plate associated with a magnetic frame.
   2. After aspiration of the residual blood, a peroxidase-labeled monoclonal antibody with anti-CD4 (or anti-CD8) specificity is added to the sample in the well (separate measurement for TCD4 and TCD8 cells for the same sample).
   3. On completion of the labeling step, the peroxidase immobilised in the antigen-antibody complexes are revealed by its incubation in the presence of a substrate, after the unbound fractions have been removed (washing).
   4. After the reaction stopped, the measurement of the absorbance values of the wells is performed using a spectrophotometer at a wavelength of 450 nm.
   5. The absorbance values are proportional to the number of TCD4 and CD8 cells in the blood samples.

Results

Cellular Immunity Among the Studied Group

The CD8 and CD4 T-cell assay is a major test in the immunological follow up of diseases with immune dysfunction. The percentage of CD8+ and CD4+ in CHB patients and carrier group have been estimated as shown in Table 1 and Figure 1, and the results revealed that for CD8+ lymphocytes there was a highly significant decrease ($P<0.001$) in the percentage means of the CD8+ cells in CHB patients, as compared with the carrier groups. For CD4+ lymphocytes, there was a slight decrease in the percentage of these lymphocytes in the peripheral blood of the patients, as compared with the carrier groups and a non-significant importance was recorded between them.

Normal value:

CD4 : 1400 -2600 TCD4 / µL

CD8 : 1050 -2000 TCD8 / µL

Additionally, the result of this study proved that a non-significant correlation was observed between cellular immune response (CD4 and CD8) among chronic hepatitis B patients. Table 2 and Figure 2 show the level of CD8 comparison among treatment and control groups while indicating high significance in treatment groups more than in controlled levels; on other hand, Figure 3 demonstrated the levels of CD4.

The results in Table 2 demonstrated the positive correlation and highly significant difference between control and treatment group indicating strong correlations showing more obvious trends in the data.

Discussion

The mean ± SD of the CD8 concentration in a sample of treatment individuals with hepatitis B patient's males and females were found to be 1138.288±91.93µL and

Table 1: The variation in the percentage of CD4 and CD8 in the blood of both groups

Type of group		*N*	*Mean*	*SD*	*F*	*Sig*
CD8	Treatment	111	1138.288	±91.927	1.68	HS
	control	111	818.351	±109.76		
	total	222	978.319	±189.49		
CD4	Treatment	112	1422.04	±77.956	0.55	HS
	control	111	1319.41	±82.890		
	total	223	1370.95	±95.331		

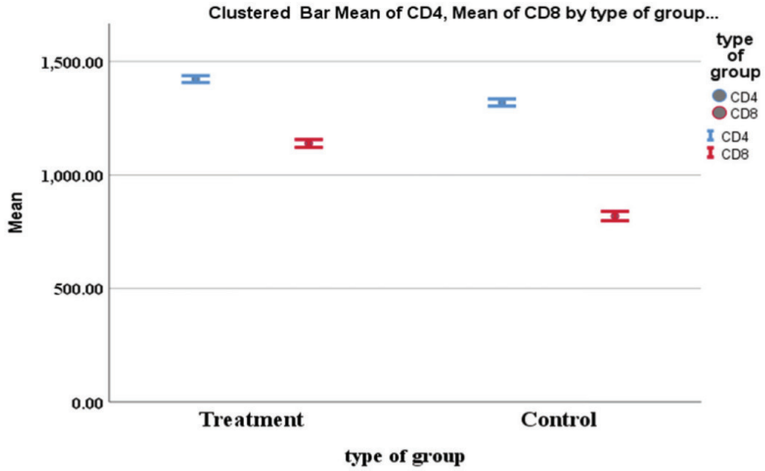

Figure 1: The difference in the percentage of CD4 and CD8 in the blood of chronic patients and carrier group.

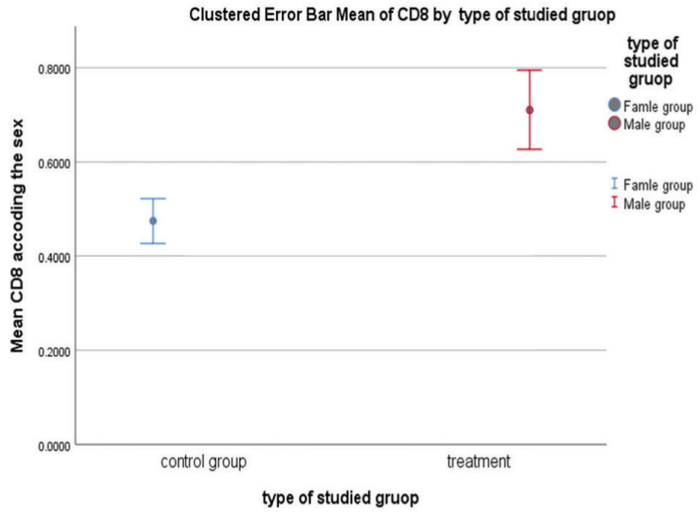

Figure 2: The difference in the percentage of CD8 in the blood of chronic patients and carrier groups according to the sex groups.

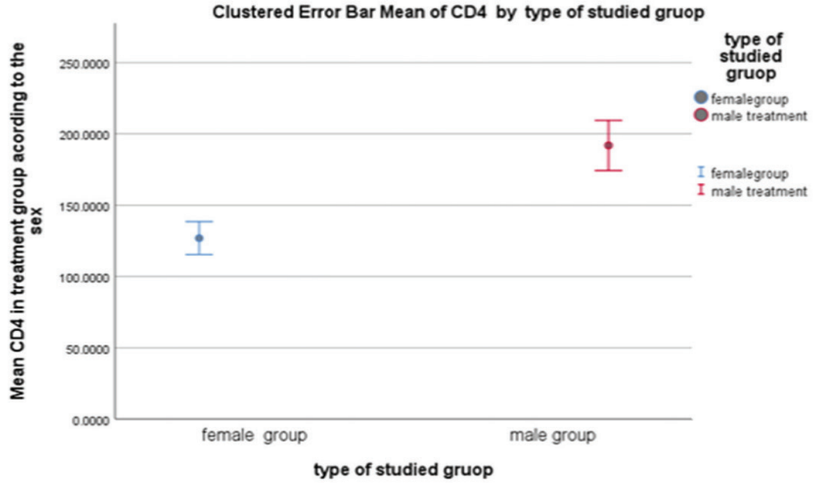

Figure 3: The difference in the percentage of CD4 in the blood of chronic patients and carrier group according to the sex groups.

Table 2: The correlation between cellular immunity (CD4 and CD8) among chronic hepatitis B patients

		CD8	*CD4*	*Mean*	*Std. D*	*N*
CD8	Pearson Correlation	1	.424**	978.32	±189.49	222
	Sig. (2-tailed)		.000			
	N	222	222			
CD4	Pearson Correlation	.424**	1	1370.959	±95.34	223
	Sig. (2-tailed)	.000				
	N	222	223			

**. Correlation is significant at the 0.01 level (2-tailed).

1035.29±91.927µL respectively, while the mean± SD of CD4 concentration in serum of control males and females were 1453.04±77.97µL and 1252.04±77.956µL, respectively. Data in Figure 3 showed that there is a significant elevation (P<0.05) in CD8 concentration in serum of total CHB patients (1138.288±91.927µL) than the concentration of CD8 in serum of healthy controls (818.351±109.76 µL). The results that have been obtained and extracted in the present study are in agreement with previous reports by Durantel and Zoulim (2016) and Janssen et al. (2018), who stated that serum CD8 and CD4 concentrations in patients with CHB were significantly higher than in controls. The high level of chronic hepatitis B patients in the serum of patients found in the present study may be due to the destruction of hepatocytes more than in the carrier group.

Figure 1 demonstrated the increase in CHB patients concentration could be due to an overproduction of inducible CD4 of T lymphocyte synthase and CD8 in T cell and CHB compared with normal tissue (Stanley, 2018). The study of experimental chronic hepatitis B in animals has confirmed an increased activity of inducible CD4 and CD8 activities (Zhang et al., 2011), with a raised making of CD of T cell (Humby, 2017). Cell-mediation was involved in a number of pathophysiological processes. In mammals, overproduction of CD4-CD8 has been reported in a number of clinical disorders (Froghi et al., 2021). It is important to focus on the fact that the synthesis and production are related to the cartilage response to different types of stimuli. For example, CD of T cell-stimulating in CHB was significantly higher in patients with the chronic comparison with carrier group despite there is no difference in CD combination between them (Zhou et al., 2018). In the field of the cellular immune response, the present study also highlights the role of cellular immune response in the pathogenesis of disease in particular cytotoxic T-lymphocytes (CD8) and helper T- lymphocytes (CD4). The result of this study proves that there is a highly significant decrease (P<0.001) in the percentage of the CD8+ cells in CHB patients as compared with the carrier group.

These results are in concurrence with Peng (2008) who found a highly significant decrease in the mean percentage of T–cytotoxic (CD8+) in CHB patients than the carrier group. This finding was also detected by Lee et al. (2005) and Perralla et al. (2006), they reported that the percentage number of circulating T–lymphocyte (cytotoxic CD8+) were reduced significantly in chronic patients as compared to the carrier group.

Cytotoxic T lymphocytes (CTL) play an important part in disease etiology. CTL activity is always a double-edged sword since it is thought to be involved in both virus-infected cell clearance and liver damage (Guidotti and Chisari, 2006; Wherry and Kurachi, 2015). So the reduced number of CD8 T cells in peripheral blood of chronic patients may indicate that these patients are suffering from hypoimmune responsiveness and have a worse prognosis than the carrier group who manifested a normal immune response (percentage of CD4 and CD8 within normal range), therefore the development of disease to chronicity occurs as a result of dysfunction or dysregulation of these cells (Humby, 2017; Perralla et al., 2006; Puro and Schneider, 2007).

On the contrary, a non-significant decrease in the percentage of the CD4+ cells was noticed between both groups. These results were found to be in accordance with the results of other studies (Koziel, 1998; Vassopoulos et al., 2008; Yang et al., 2007).

The results demonstrated a non-significant decrease of (CD4+) in both the chronic and carrier group, hence in some cases, the CD4+ level is present within the normal range in some patients with CHB but that lead to dysregulation in liver function which might represent a predictive indicator in the prognosis of the disease (Yang et al., 2007).

On the other hand, some researchers reported that there was a decrease in the level of (CD4+) in CHB patients as compared to an asymptomatic carrier (Dongping et al., 2006; Knolle and Thimme, 2014; Penna et al., 2007; Rehermann, 2013). Eventually, a possible defect of the T-helper and cytotoxic negative selection of Th1 cells in the thymus during T-cell maturation resulted in an aberrant state called "holes" in the repertoire for T cell receptor (TCR); it was necessary to react with specific MHC complex to produce mature cells with specificity for P25 peptide encoded by S region of HBsAg (Yang et al., 2007).

Also, cytokines participate in all stages of the immune response, they have an effect on propagation, discrimination and migration of various cells in the immune system and standardize both humoral and cellular immune response.

At last, this study proved that there are significant differences between the experimental study group and the control group or healthy people carrying the type B virus, which reinforced the evidence that goes towards the fact that cellular immunity is considered one of the determining factors for the type of infection and then the immune response that will develop in line with a set of other biochemistry and genetic factors and its main role play as immunopathogenesis of chronic hepatitis B and carrier state of disease.

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