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Research article

T-Lymphocytes Subsets in inactive carrier state of HBV

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ABSTRACT

This study included nine patients with inactive carrier state of HBV and 14 healthy control groups. The number and the percentage of T- Lymphocyte (CD3+ Cells) in the peripheral blood of these groups showed no significant difference. Similar trend was observed when number and percentages of T helper cells (CD4+ cells) and T cytotoxic lymphocytes (CD8+ cells). Moreover, no significant difference in CD4+ /CD8+ cells ratio ($P > 0.05$) in peripheral blood of patients with inactive carrier state of HBV as compared with healthy control group. The levels of total serum bilirubin (TSB) concentration and alanine aminotransferase (ALT) activity were similar to control group. The levels of immunoglobulin concentration (IgG and IgM) in patients group were similar to control group. No remarks of autoimmune phenomena were observed in patients group.

Keywords: CD4+ cells, CD8+ cells, inactive carrier, HBV, T-Lymphocytes.

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INTRODUCTION

The routine diagnosis of hepatitis B virus (HBV) infection remains serologic [1]. The presence of HBsAg indicates the patient is infected with HBV. The absence of IgM anti-core antibody implies that this patient has chronic rather than acute HBV infection. The patient is HBeAg negative with presence of antibody to e antigen (anti-HBe). The presence of HBe Ag and normal level of alanine aminotransferase indicates there is no viral replication and low lever damage [2, 3]. Two billion people have been exposed to the hepatitis B virus (HBV), 5 million cases of acute hepatitis B occur annually and over 350 million people have a chronic infection [4]. In total, hepatitis B results in 500,000-1.2 million deaths annually [4]. The risk of developing chronic HBV infection after acute exposure ranges from 90% in newborns of HBs Ag-positive mothers to 25% to 30% in infants and children under 5 and to less than 5% in adults [5]. In addition, immunosuppressed persons are more likely to develop chronic HBV infection after acute infection [6]. The infection with HBV for long time will

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give an evidence of autoimmune hepatitis type 1 like cases and promotes hepatocarcinoma [3,7]. HBV infect intestinal tract of human. Many studies focused on other intestinal tract versus such as enterovirus also infect intestinal tract but the diagnosis of these virus dependent on different ways that used in diagnosis of HBV [8]. In present study, many biochemicals, serological and cytological testes were evaluated in peripheral blood of patients with inactive carrier state of HBV and 14 healthy control groups

MATERIALS AND METHODS

Patients

Nine patients with inactive carrier state of HBV and 14 healthy control groups were subjected in present study. This study was carried out with the approval of the local ethics committee of ministry of health, Baghdad, Iraq. All samples were collected from central public health laboratory (CPHL) Baghdad, Iraq. The status of patients was detected by specialist physician. The patients who had the liver diseases or infected with hepatitis C virus (HCV), hepatitis delta virus (HDV), and human immunodeficiency virus (HIV) were excluded. Heparinized blood and serum was collected from all these patients and control. Nobody had received antiviral drug treatment during the time of experiment.

Detection of virus markers by ELISA

Hepatitis B surface antigen (HBsAg), IgM anti-hepatitis B core antigen (HBc), IgG anti-HBc, anti-HCV, IgM anti-HDV and anti-HIV were detected in sera of patients and healthy control groups by ELISA technique. HBsAg (Biotest), IgM anti-HBc (Hepanostica organon), IgG anti-HBc (Hepanostica organon), anti-HCV (UBI, USA), IgM anti-HDV (Biokit) and anti-HIV (Murex Diagnosis) kits were used according to manufactures' instructions.

Total serum bilirubin (TSB) and Aminotransferase (ALT)

The standard method of Pyrkov et al. (1986) was followed to check the levels of total serum bilirubin (TSB) in sera of patients and control groups [6]. The standard method of Henry et al. (1960) was followed to estimate the activity of ALT in patients and control sera [9].

Measurement of essential Immunoglobulins (Igs)

Single radial immunodiffusion method was used to measure the concentrations of essential immunoglobulins (IgM and IgG) in sera of patients and control groups. The manufacture's instruction of Sanofi Diagnostic Pasteur was followed to check the concentrations of immunoglobulins.

Detection of autoantibodies

The autoantibodies, anti-nuclear antibodies (ANAs) and anti-smooth muscle antibodies (ASMAs) were checked in sera of patients and healthy control by indirect immunofluorescence technique. The manufacture's instruction of Sanofi Diagnostic Pasteur was followed.

Cells isolation

Lymphocytes were isolated from heparinized peripheral blood by density gradient centrifugation by used lymphoprep [5] and washed three times with HBSS (flow laboratory). The number of viable mononuclear cells was estimated using trypan blue exclusion assay [10].

Preparation of cell smear

10 µl from cells suspension (10^6 cells/ml) was smeared on clean glass slide and the last was air dried and fixed with buffer formal acetone (prepared immediately before use by mixing 8 ml of phosphate buffer, 38 ml distilled water, 33.2 ml of 40% formalin and 60 ml of acetone) for 30 second and the slide rinsed with distilled water and transferred to tris buffer saline (TBS, Fluka) and then dried.

Indirect immunoperoxidase staining

The standard method [3] was followed. The slides were submerged in methanol supplemented with 0.6 % of H2O2 (Fluka) for 15 minutes and they were rinsed in distilled water and put in PBS. The slides was incubated 37 ^{o}C for 60 minutes with 50 µl of primary monoclonal antibodies diluted to 1:600 (CD 3+ or CD 4+ or CD 8+ markers) (these markers were prepared in mice by Biokit company) in humid chamber and washed three times with tris buffer saline (TBS). Slides were incubated at 37 ^{o}C for 60 minutes with 50 µl of peroxides conjugate (anti-mice Immunoglobulin) (Biokit) diluted with PBS up to 1:400. The slides were washed with TBS three times and after that 50 µl of 3,3 Diaminobenzide tetrahedrochlorid (Sigma) supplemented with 3 µl of H2O2 (Fluka) and incubated for 15 min at 37 ^{o}C. The slides washed with TBS three times and placed in hematoxylin for one second and they washed with PBS and put in the same buffer for 5 minutes to develop the color and it fixed by put the slides in serial dilutions of methanol (70, 80 and 95 %) for 5 seconds in each dilutions after that Canada balsam was added and slides were covered with suitable cover slip. All slides were examined with compound microscope and the percentage of cells was counted.

Statistical analysis

All values have been used to give a mean value and the standard deviation calculated. The difference was analyzed using Student's t-test, and one-way ANOVA test (followed by Tukey test) with Origin version 8.0 software. A value of $P<0.05$ was considered to be statistically significant.

RESULTS

Laboratory and clinical features of patients with inactive carrier state of HBV

Current study investigated nine patients previously infected with HBV and diagnostic as inactive carrier state of HBV. **Table 1** shows no difference was observed between patients and control in terms of six and ages. All examined cases tested positive for HBs Ag and no patients carried anti-HBs in their sera.

Table 1. Clinical and laboratory factories of many groups of patients & control.

Information	HBV-HC		Control	
No. patients	9		16	
Six	Female	Male	Female	Male
	3	6	7	9
Rang of age in years	24-38	23-42	24-35	20-40
Mean of age in years	31	32	28	33
HBs Ag	9		0	
Anti-HBs	0		0	
Auto antibodies	0		0	
ANA	0		0	
ASMA	0		0	
TSB mg/dl	0.9 ± 0.3 NS		0.90 ± 0.12	
ALT	6.6 ± 2.6 NS		9.3 ± 5.3	
IgG mg/dl	1300 ± 350 NS		1360 ± 513	
IgM mg/dl	139 ∓ 47 NS		25 ± 75	
IgA mg/dl	328 ± 143 NS		300 ± 27	

NS: non significant

Autoantibodies (ANA and ASMA) were not found in patients sera. The levels of essential immunoglobulins and liver function tests (TSB and ALT) were similar to control.

T lymphocytes subsets in peripheral blood of patients with inactive carrier state of HBV

The present study checked the number and percentage of T lymphocytes subsets in peripheral blood of patients with inactive carrier state of HBV and healthy control. **Table 2** showed no significant difference in percentage and number of T lymphocyte (CD3+ cells), T helper (CD4+ cells) and T cytotoxic cells (CD8+ cells) as compared with control group ($P>0.05$).

DISCUSSION

About 10% of patients suffering from acute type B hepatitis fail to clearance Hepatitis B Surface antigen (HBs Ag) from the blood and become chronic carriers in adult. But this percentage is 90% in children [8]. The virus is not cytotoxic for hepatocytes and the liver cells damage is related to immunological reactions patterns of host [11]. Previous studies support the hypothesis that two factors are important in pathogenesis of HBV induced hepatocellular injury; viral replication and immune response [12]. In present study many biochemical, serological and cytological studies were included. Most of these tests were similar to control group. Moreover, No difference in number of T lymphocytes subsets was observed. The patients group showed normal level in markers of immunology that related with immune response and viral activity. That means the virus in this group of patients was inactive and that reflect on the activity of immune systems toward immunopathogenesity. That explains the presence of HBs Ag but no remarks of disease severity.

Table 2. Number and percentage of total T lymphocytes (CD3+ cells), T helper (CD4+ cells) and cytotoxic T lymphocytes (CD8+ cells) in peripheral blood of patients with inactive carrier state of HBV and healthy control groups (NS: non significant**)**

		CD_3		CD_4		CD_8		CD_8 / CD_4
		No/ 1100 cell	%	No/ 1100 cell	%	No/ 1100 cell	%	%
Control n=14	mean	814.73	73.43	548.25	49.44	278.7	25.09	1.99
	SD	98.9	6.45	67.8	4.55	46.4	32	0.29
inactive carrier HBV n=9	Mean	780.3	71.37	503.3	46.08	285.7	26.18	1.790
	SD	60.28	3.47	38.9	3.29	39.9	3.05	0.27
	Diff. of sign.	Ns	Ns	Ns	Ns	Ns	Ns	Ns

Conflict of interest

The authors declare that they have no conflict of interests.

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