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Research article

T-Lymphocytes Subsets in Patients with chronic hepatitis that showed autoimmune phenomenon

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ABSTRACT

This study included 7 patients with autoimmune chronic hepatitis that infected previously with HBV (AIH-HBV), and 16 healthy human as a control group. No significant difference in number and percentage of T- Lymphocyte (CD3+ Cells) in the peripheral blood of patients with AICH as compared with control group. In addition, no significant increase in the percentage and number of CD4+ cells ($P < 0.001$) was reported and no significant decrease in the number and percentage of CD8+ cells ($P < 0.01$) was observed. These finding was concomitant with increase in CD4+/CD8+ ratio as compared with healthy control. In present study, it can conclude that the collaboration activity of both cells (CD4+ and CD8+) plays the crucial role in severity of AIH-HBV.

Keywords: T lymphocytes, autoimmunity, CD4+ Cells, CD8+ cells

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INTRODUCTION

Autoimmune hepatitis (AIH) is an inflammatory liver disease, affecting mainly females, characterized by elevated serum transaminase activity, positive organ and non-organ specific autoantibodies, elevated IgG, and a histological picture of interface hepatitis. There are two types of AIH according to their serology: type 1 is characterized by anti-nuclear (ANA) and/or antismooth muscle (SMA) antibodies; type 2 by anti-liver kidney microsomal type 1 (anti-LKM-1) antibody [1]. AIH a chronic necroinflammatory disorder of unknown etiology, is characterized by immunologic and autoimmunologic features [2]. It is a disorder in which immune reactions against antigens are believed to be the pathogenic mechanism [3]. It is now evident that the recognition of self is essential to the normal functioning of the immune system [4] that AIH points to a regulatory disorder within the immune system.

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In recent study by Zgair (2013) the high percentage of CD4+ and low percentage of CD8+ was observed in patients with AIH type 1 [5]. In current study, we try to find the level of number and percentages of T lymphocytes (CD3+ cells), T helper (CD4+ cells) and T cytotoxic (CD8+ cells) in peripheral blood of patients that previously infected with HBV and showed autoimmune phenomena.

MATERIALS AND METHODS

Patients

This study was carried out with the approval of the local ethics committee of ministry of health, Baghdad, Iraq. In the present study, 23 subjects, including 7 patients with autoimmune hepatitis phenomenon and infected previously with HBV (AIH-HBV) and 16 healthy control group were collected from central public health laboratory (CPHL) Baghdad, Iraq. The status of patients was detected by specialist physician. No patient has taken any immune suppressive drugs for 1 month prior to the date of this study. The patients who had the liver diseases or infected with hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis delta virus (HDV), and human immunodeficiency virus (HIV) were excluded Heparinized blood and serum was collected. Nobody was received immunosuppressive or antiviral drug treatment.

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 [7].

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 volunteers (control) by indirect immunofluorescence technique. The manu-facture's instruction of Sanofi Diagnostic Pasteur was followed to check the ANAs and ASMAs in patients and healthy volunteers' sera.

Cells isolation

Mononuclear cells were isolated from heparinized peripheral blood by density gradient centrifugation by used lymphoprep [5] and washed three times with HBSS (flow laboratory).

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 of Galun et al [8] with little modification was followed. The slides were submerged in methanol supplemented with 0.6 % of H2O2 (Fluka) for 15 min and they were rinsed in distilled water and put in PBS. The slides was incubated 37 ^{o}C for 60 min 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 min 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 min 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

Clinical, biochemical and serological features of studied group

Number of female was higher than mail (6:1). Only one case tested positive for HBs Ag test, while six cases gaive positive for anti-HBs. All cases were carried autoantibodies (ANA and ASMA) in their sera. The level of TSB and ALT was higher than control significantly. In present study, the levels of serum immunoglobulins (IgG, IgM and IgM) were higher than levels of immunoglobulins in sera of healthy control (**Table 1**).

Table 1. Clinical, biochemical and serological features of patients with AIH-HBV and healthy volunteers groups.

Information	AIH-HBV		Control	
No. patients	7		16	
Six	F	M	F	M
	6	1	7	9
Rang of age in years	22-45	50	24-35	20-40
Mean of age in years	38	50	28	33
HBs Ag	1		0	
Anti-HBs	6		0	
Anti-HBc IgM	0		0	
Auto-antibodies	7		0	
ANA	7		0	
ASMA	6		0	
TSB mg/dl (0.2-1 mg/dl)	1.6 ± 0.9 *(P<0.01)		0.90 ± 0.12	
ALT (2-15 Iu/L)	22 ∓ 6.3 (P<0.01)		9.3 ± 5.3	
IgG mg/dl	2830 ± 510 *(P<0.01)		1360 ± 513	
IgM mg/dl	330 ± 98 *(P<0.01)		25 ± 75	
IgA mg/dl	440 ± 2189 *		300 ± 27	

*Significant difference from control group (P<0.05).

F: female.

M: male.

Number and percentage of T lymphocytes in peripheral blood of patients with AIH- HBV

Table 1 shows the number of total T lymphocytes (CD3+ cells), T helper cells (CD4+ cells) and cytotoxic / suppressive T lymphocytes (CD8+) per 1100 of leukocytes in peripheral blood of patients with AIH-HBV and healthy volunteers groups. The table also shows the percentages of all these cells in peripheral blood. No significant increase was observed in number of all cells as compared with control group. But the significant increase was observed in ratio of CD4/CD8 of the patients as compared with healthy control ($P<0.05$).

DISCUSSION

Clinical and laboratory characteristics of AIH disease are associated with extrahepatic autoimmune syndromes and prompts response to immunosuppressive treatment, circulating autoantibodies, and hypergammaglobulinemia. The mechanism of this disease remains uncertain [9]. The changes in number of T lymphocytes play a certain role in imbalance of immune system and severity of chronic hepatitis B and AIH1 [5, 10]. Moreover, these changes may affect on the response of host to diseases. That modification in the immune system alarms may responsible for the development of autoimmune phenomena and involve of several of autoimmune diseases. In current study, many clinical, biochemical and serological markers that related with AIH was checked and evaluated in the studied clinical cases (AIH-HBV). It was found that most of clinical, biochemical such as concentration of TSB and activity of ALT was elevated in studied group. Moreover, the number of T lymphocytes subsets was also evaluated. Most of markers of AIH1 was observed in patients group that studied in present study that bring to our mind, the infection with HBV for long time promotes AIH 1 symptoms. However, in current study, we did not find significant deference in number of T helper and T cytotoxic as compared with control. While, Zgair [5] found a significant difference between the number of T helper cells and T cytotoxic cells in patients as compared with control. This finding suggested that the collaboration action of both cells (CD4+ and CD8+) but not each one individually play a central role in severity of AIH-HBV. It can concluded that the HBV may be responsible for generation of AIH symptoms and the combination activity of T helper and T cytotoxic cells play the central role in severity of this disease.

Table 2. Number and percentage of T lymphocytes in peripheral blood of patients with AIH- HBV and healthy control.

		CD_3		CD_4		CD_8		CD_4 / CD_8
		No/ 1100 cell	%	No/ 1100 cell	%	No/ 1100 cell	%	%
Control n=16	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
AICAH n=7	Mean	807.15	72.83	553.57	50.43	238.7	21.4	2.558
	SD	68.36	3.76	48.66	5.1	64.45	4.6	0.66
	Diff. of sign.	Ns	Ns	Ns	Ns	Ns	Ns	P<0.05

Conflict of interest

The author declares that he has no conflict of interests.

REFERENCES

[1] **Vergani D, Mieli-Vergani G.** (2008) Aetiopathogenesis of autoimmune hepatitis. *World J Gastroenterol* **14**: 3306-3312

[2] **Krawitt EL.** (1994) Autoimmune hepatitis: classification, heterogeneity, and treatment. *Am J Med* **96**:S23–S26.

[3] **Meyer zum Buschenfelde KH, Lohse AW, Manns M, Poralla T.** (1990) Autoimmunity and liver disease. *Hepatol* **12**:354–363.

[4] **Cohen IR, Wekerle H**. (1972) Autosensitisation of lymphocytes against thymus reticulum cells. *Science* **176**:1324–1325.

[5] **Zgair AK** (2013) Involvement of (IgG and IgM)-secreting B lymphocytes in severity of autoimmune hepatitis type1. *Med Microbiol Immunol* (2013) DOI 10.1007/s00430-012-0287-5.

[6] **Pyrkov LM, Sokolova TA, Kopyakova LG, Rozen IS** (1986) Microassay of total serum bilirubin on the ABFTs-1 bilirubin analyzer. *Biomed Eng* **20**:156-157.

[7] **Henry RJ, Chiamori N, Golub OJ, Berkman S** (1960) Revised spectrophotometric methods for the determination of glutamic oxalacetic transaminase, glutamic pyruvic transaminase and lactic acid dehydrogenase. *Am J Clin Path* 34:381-398.

[8] **Galun E, Iian Y, Livni N, Ketzinal M.** (1994) Hepatitis B virus infection associated with hepatopoietic tumors. *Am J Pathol* **145**:717-23.

[9] **Roberts EA**. (1995) Autoimmune hepatitis. *Ind J Pediatr* **62**:525–531.

[10] Garella G, Chatenoud L, Degos F, Bach MA. (1982) Regulatory T-cell subsets imbalance in chronic active hepatitis. *J Clin Immunol* **2**: 509-13.

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