Serological Study on HBV Infected Patients and Individuals Vaccinated with Recombinant HB Vaccine

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ABSTRACT

Background and Objectives: The specific cellular immune responses play a main role in the hepatic necrosis that occurs with hepatitis B virus (HBV) infection. Interferon-g (IFN-g) and interleukin-2 (IL-2) are considered examples on T helper 1(Th1) cytokines which required for host antiviral immune response and involved in cell-mediate immunity against HBV infection. This study was designed to estimation T-helper 1 cytokines (IFN-g and IL-2) in HBV infected patients and individuals vaccinated with recombinant HB vaccine.

Methods: The study groups were classified into patient group 35 (15 acute hepatitis (AH) and 20 chronic hepatitis(CH)) and 35 vaccinated group (20 responder (RD) and 15 Non-responder (NRD)) and 18 control group, during May to November 2007. Blood samples were taken from patients and hospitals staffs in Nanakaly, Erbil and Rizgary Teaching Hospital to detect hepatitis B surface antigen (HBsAg), anti hepatitis core antibody IgM (Anti-HBc Ab(IgM)), Anti-HBs Ab, IFN-g level and IL-2 level in serum by enzyme linked immunosorbent assay (ELISA).

Results: The concentration of IFN-g and IL-2 levels in the AH group differed significantly compared with healthy control and CH patients ($p<0.01$) by F-test. LSD-analysis for IFN-g also revealed same result while IL-2 level significantly increased in healthy control only. F-test for IFN-g revealed ($p<0.05$) among RD group, NRD group and healthy non vaccinated (HN) control in ≥30 and <30 years old respectively but inverse result was observed in IL-2 levels ($p>0.05$).

Key words: IFN-g, IL-2, HBV, Recombinant HB vaccine.

INTRODUCTION:

Hepatitis B virus is the prototype agent for a virus family called hepadnaviridae. Secreted Th1 cytokines considered as an appropriate response of the immune system to inhibit viral replication and HBV eradication [1]. The level of IL-2 and its receptors increased during the acute phase HBV infection also stimulation occurs to increase the activities of natural killer(NK) cells and $CD8^+$ lymphocytes which participate in the development of immunity to HBV. On the other hand IL-2 decreases in the level of its production in patients with chronic HBV infections [2, 3]. IFN-□, which is known as immune interferon has functions, enhance the ability of macrophages to destroy tumor cells, viruses, and bacteria [4]. T cells in adaptive immunity produce IFN-□ in response to antigen recognition [5]. IL-2 is a glycoprotein synthesized by CD4+ T helper lymphocytes which was formerly called T-cell growth factor [6]. The single most important tool for the prevention and control of hepatitis B infections is the hepatitis B vaccine [7]. Vaccination with HBsAg induces protective immunity through Th cell type dependent production of anti-HBs antibody [8]. Different patterns of cytokine production have been observed in T-cell clones isolated from responder individuals, with either predominant Th2 or

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of both types of cytokines in healthy nonresponder individuals has been demonstrated, therefore defective Th cell function, either Th1 or Th2, lead to failure of immune response to HBsAg [9, 10]. This study was designed to estimation T-helper 1 cytokines (IFN-g and IL-2) in HBV infected patients and individuals vaccinated with recombin

MATERIALS AND METHODS:

ant HB vaccine.

The study groups were classified into patients group with total number of 35 (25 males and 10 females) (15 acute and 20 chronic) patients and Vaccinated group (35 healthy individuals) who were previously vaccinated against HBV. This group classified into responder group (20 individuals), Non-responder (15 individuals) and control group (18 apparently healthy non vaccinated individuals). They were chosen to match the age and sex of the study groups to serve as negative control, during the period between May to November 2007. Blood samples were taken from patients and hospitals staffs and employees in Erbil Teaching Hospital, Nanakaly Hospital for Blood Diseases and Rizgary Teaching Hospital or from blood donors, who voluntarily came to Blood Bank Units. The study protocol includes viral assay which involve detection of HBsAg (Biokit, 3000-1130, Spain), and Anti-HBc Ab(IgM) in serum (Murex anti-HBc IgM, Murex Biotech Limited, C08GE18GB, UK). Hepatitis B vaccine assay for detection of Anti-HBs antibodies in serum (WB-2396,China) and immunological assay for Estimation of serum IFN-g level (human IFN-g BMS228, Austria) and serum IL-2 level (Bender MedSystems) By ELISA test intended for the qualitative detection. Analysis of data was performed by using Statistical Package for Social Science (SPSS) Version 11.5. Results are expressed as mean ± S.E. Statistical differences were determined by LSD test for multiple comparisons after ANOVA. p value < 0.05

RESULT:

The results of serum concentration of IFN-g in 14 AH, 18 CH and 15 healthy controls were presented in Table (1) and Figure (1). The concentration of IFN-g level in the AH group differed significantly (31.797 pg/ml) in comparison to that of CH (14.752 pg/ml) and healthy control (17.593 pg/ml) ($p<0.001$) when analyzed statistically by F-test. LSD- test analysis revealed significant elevation in serum IFN-g level of AH patients compared with healthy control ($p<0.05$), but highly significant elevation when compared with CH patients ($p<0.001$). Moreover, the present study estimated a non significant decrease of IFN-g in the CH patients ($P>0.05$) when compared to healthy control. Decrease individuals ≥30 and <30 years old respectively when compared to both group 13 NRD vaccinees (14.217 pg/ml) and (14.604 pg/ml) with 15 HN control (12.417 pg/ml) and (21.618 pg/ml) ($p<0.05$) when analyzed statistically by F-test, Table (2). LSD-test analysis revealed significant elevation in serum IFN-g level of RD group compared to HN in age ≥30 years ($p<0.01$) , while it was not significant in age <30 years ($p>0.05$). On the other hand, it was significant when compared with NRD group ($p<0.05$) in both age groups, but there was non significant differences in NRD group and HN control ($p>0.05$). By comparing the three groups (AH, CH and healthy control) regarding their IL-2 levels, it has been found that patients with AH have the highest mean values (58.225 pg/ml), compared with other study groups, (43.373 pg/ml) and (31.013 pg/ml) in CH and healthy control respectively with significant differences ($p<0.01$) using F-test analysis. However, the comparison between healthy control and AH using LSD -test also revealed significant differences ($p<0.01$) in IL-2, Table (3). Table (4) revealed non significant levels of IL-2 in sera of RD compared to NRD and HN control groups by F-test analysis in age <30 years ($p>0.05$), while RD group

significant differences ($p<0.05$) compared with NRD in age ≥30 years by using LSD-test, Figure (2).

Table (1): Difference in mean serum level of IFN-g (pg/ml) between study groups.

Study groups		No.	IFN-g Mean ±SE	P value (F-test)
Acute HBV		14	31.797±4.463	P< 0.001
Chronic HBV		18	14.752±1.222	
Healthy control		15	17.593±2.098	
HC versus AH	**LSD**	P< 0.04		
HC versus CH		NS		
AH versus CH		P< 0.001		
HC: Healthy control, CH: Chronic hepatitis, AH: Acute hepatitis.				
P<0.001: Highly significant , p<0.05: Significant, NS: Non significant				

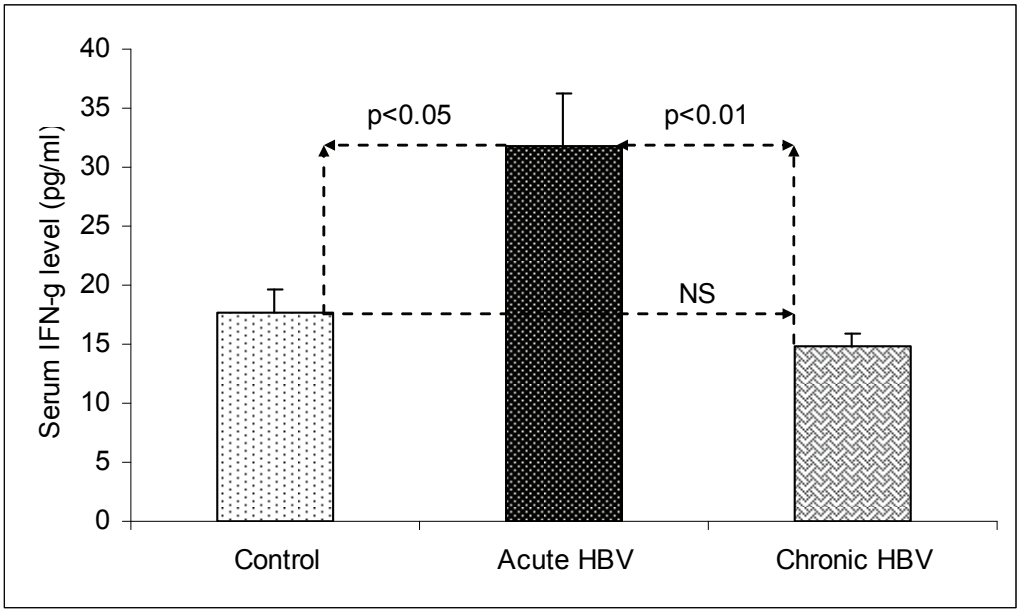

Figure (1) : Serum level of IFN-g in study groups

Table (2): Serum IFN-g in pg/ml between vaccinated study groups and healthy control according to age distribution

Study groups	Serum IFN-g (Mean ±SE)				
	No.	Individuals age ≥30 years	P value (F-test)	Individuals age <30 years	P value (F-test)
Responder	20	22.509±3.776	P<0.05	29.223±3.595	P<0.01
Non-responder	13	14.217±1.436		14.608±1.763	
Healthy non-vaccinated control	15	12.417 1.346±		21.618±2.997	
HN versus RD	**LSD**	P<0.01		NS	
HN versus NR		NS		NS	
RD versus NR		P<0.02		P<0.04	
RD: Responder , ND: Non-responder, HN: Healthy non-vaccinated control					
P<0.05:Significant, NS: Non significant					

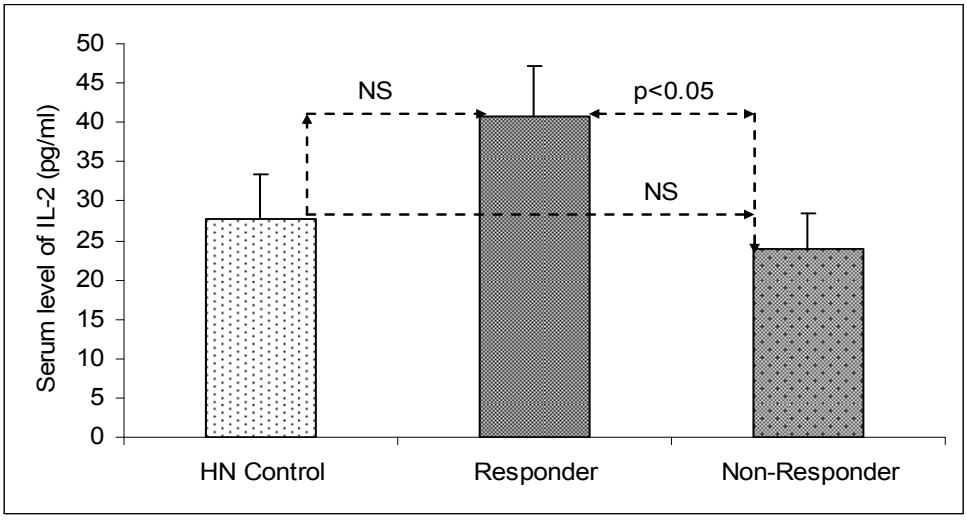

Figure (2): Serum level of IL-2 in age ≥30 years vaccinees and control groups

Table (3): Difference in mean serum level of IL-2 (pg/ml) between study groups

Study groups		No.	IL-2 Mean ±SE	P value (F-test)
Acute HBV		9	58.225±8.261	P< 0.01
Chronic HBV		18	43.373±4.697	
Healthy control		15	31.013±4.467	
HC versus AH	**LSD**	P< 0.01		
HC versus CH		NS		
AH versus CH		NS		
HC: Healthy control, CH: Chronic hepatitis, AH: Acute hepatitis.				
P<0.01: Significant , NS: Non significant				

Table (4): Serum IL-2 in pg/ml between vaccinated study groups and healthy control according to age distribution

Study groups	Serum IL-2 (Mean ± SE)				
	No.	Individuals age ≥30 years	P value (F-test)	Individuals age <30 years	P value (F-test)
Responder	18	40.827±6.285	P<0.05	47.379±7.052	P>0.05
Non-responder	14	23.941±4.459		33.403±7.937	
Healthy non-vaccinated control	15	27.841±5.494		33.128±6.651	
HN versus RD	**LSD**	NS		NS	
HN versus NR		NS		NS	
RD versus NR		P<0.03		NS	
RD: Responder , ND: Non-responder, HN: Healthy non-vaccinated control					
P<0.05: Significant, NS: Non significant					

DISCUSSION:

The concentration of IFN-g level differed significantly in the AH in comparison to that of CH and HC ($p<0.001$) when analyzed statistically by F-test, but LSD analysis revealed significant elevation of AH patients compared with HC ($p<0.05$), and CH patients ($p<0.001$). This result may reflect the inflammatory action of IFN-g which contributes to the successful clearance of the virus, avoiding progression of the infection and persistence of the virus. Such cytokines has been known as the classical macrophage activator, which acts for supporting Th1 driven immune response [11] therefore it has the main role in the elimination of the intracellular pathogens including viruses [12]. This result is also supported by Ando *et al.,* [1] who reported that there are two factors which have a role in acute self-limited hepatitis B, the first factor is HBcAg-specific cytokine (IFN-g) that produced by Th1 lymphocyte. The second is HBsAg-reactive cytotoxic T cells. In most patients with acute hepatitis, CTL responds to epitopes of HBsAg, while there are no such responses in patients with chronic hepatitis .Thus, Th1 might be insufficient for complete removal of HBV in chronic hepatitis but positively correlated with hepatic inflammatory activity [13]. In contrast to our study, Castillo *et al*., [14] found that the normal levels of IFN-g in the patient's sera with acute HBV infection was explained by that, the DNA viruses are poor inducer of IFN-g. On the other hand Priimangi *et al*. [15]reported that IFN-g increased during chronic viral infection, he suggested that such cytokine involved in the pathogenesis of chronic HBV liver disease. Moreover, non significant decrease of IFN-g in the CH patients ($P>0.05$) when compared to HC. Decrease of IFN-g during chronic phase may be contributed to the chronicity of HBV infection [16]. The concentration of IFN-g level in the RD, NR and HN groups differed significantly ($p<0.05$). Significant elevation of IFN-g level also appear in age ≥30 years while it was not significant in HN compared with NR. In age <30 years ($p<0.04$) in RD versus NR, but ($p>0.05$) in HN versus RD and NR groups by LSD analysis. The high detected level of IFN-g among responder individuals may be clarified by the finding of Jahfarzadeh and Shokri, [17] who reported among vaccinated healthy neonates, an increase in IFN-g level occurs in the responder, while decrease in the level of IFN-g occur among nonresponder groups, and suggested that nonresponsiveness to HBs vaccine could be due to dysfunction of APC in non-responders. Celis *et al.* [18] who first described HBsAg-specific $CD4^+$ T lymphocytes derived from peripheral blood of vaccine recipients. These T cell clones proliferated and produced IFN-γ upon stimulation with HBsAg. By comparing the three groups (AH, CH and healthy control) regarding their IL-2 levels, it has been found ($p<0.01$) present between them using F-test analysis. Using LSD-test revealed ($p<0.01$) in comparison between HC and AH table. The increased levels of IL-2 and its receptors during the acute phase, until the total resolution of the HBV infection may allow for higher levels of T-lymphocyte activation during this period. IL -2 is necessary to stimulate and activate NK cells and $CD8^+$ lymphocytes which participate in the development of immunity. On the other, hand investigations carried out with IL-2, found a decrease in the levels of its production in patients with chronic HBV infections [19]. Priimagi *et al*., [15] demonstrated the role of IL-2 in HBV infection; their results showed that, insufficient IL-2 production promotes the development of chronic HBV infection. In contrast to our study, Rossol *et al*., [20] reported that serum levels of IL-2 were elevated in chronic HBV infection, and demonstrated that patients with necroinflammatory activity have higher serum levels of IL-2 than chronic HBV carrier with minimal histological activity. Table (4) revealed non significant levels of IL-2 in sera of RD compared to NRD and HN groups in age <30 years ($p>0.05$),

age ≥30 years by using LSD-test.These results were in agreement with results reported by Karada et al., 10, who demonstrated that following in vitro activation of PBMC with HBsAg, Th1 (IL-2) cytokine production increased significantly in responders compared to nonresponders, which suggested that unresponsiveness to HBsAg may be owing to defect in either HBsAg specific T cell or antigen presentation. Similar investigation has been carried out in normal individuals vaccinated with rHB vaccine, found the absence of Th1 cytokine production in nonresponder subjects 21. Conclusion: In this study significant elevation of IFN-g and IL-2 levels was observed in AH patients compared with CH patients and HC. Same result was seen in IFN-g among RD, NRD and HN control in ≥30 and <30 years old, while inverse result was observed in IL-2 levels. This result may reflect the inflammatory action of IFN-g and IL-2 which contributes to the successful clearance of the virus.

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