Estimation of some T-helper 2 cytokines (IL-4 and IL-10) in HBV Infected Patients and Individuals Vaccinated with Recombinant HB Vaccine

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Abstract:

The specific cellular immune responses play a main role in the hepatic necrosis that occurs with hepatitis B virus (HBV) infection, interleukin-4 (IL-4) and IL-10 are considered examples on T-helper 2 (Th2) cytokines which required for host antiviral immune response and involved in humeral immunity against HBV infection. This study was designed to estimation Th2 cytokines (IL-4 and IL-10) in HBV infected patients and individuals vaccinated with recombinant HB vaccine. Study groups were classified into patient group 35(15 acute (AH) and 20 chronic (CH)), 35 vaccinated group (20 responder (RD) and 15 Non-responder (NRD)) and 18 healthy control (HC) during May to November 2007. Blood samples were taken from patients and hospitals staffs to detection HBsAg, Anti-HBc Ab(IgM), Anti-HBs Ab, IL-4 level and IL-10 level in serum by enzyme linked immunosorbent assay (ELISA) test.

The concentration of IL-4 and IL-10 levels in the AH group differed significantly compared with HC and CH patients ($p<0.01$) by F-test. LSD-analysis for IL-4 revealed same result only between HC and CH ($P<0.008$). Comparison between CH and both HC and AH, respectively revealed significant differences in IL-10 level ($P<0.008$) and ($P<0.03$) by LSD-test. F-test for IL-4 revealed non significant result ($P>0.05$) among RD, NRD and HN control in ≥30 and <30 years old, respectively, but inverse result was observed in IL-10 levels ($p<0.05$).

LSD-test revealed ($P>0.05$) between all study group regarding IL-4, however the level of IL-10 were ($P<0.014$) and ($P<0.01$) in RD and NR groups among ≥30 and <30 years old, respectively. Indeed ($P<0.03$) between RD and HN groups in age <30 years.

Conclusion:

In this study significant elevation of IL-4 and IL-10 levels was observed in AH patients compared with CH patients and healthy control. Same result was seen in IL-10 among RD and NRD in ≥30 and <30 years old, as well as between RD and HN groups in age <30 years.

Keywords: IL-4, IL-10, HBV, recombinant HB vaccine.

Introduction:

Hepatitis is an inflammation of the liver, characterized histologically by hepatocellular necrosis and infiltration of the liver by inflammatory cells (1). The term of viral hepatitis is reserved to the infection of the liver caused by a small group of viruses having a particular affinity to the liver (2). Control of HBV infection requires the combined action of both the innate immune response and the humoral and cellular arms of the adaptive immune response. Following HBV infection, there is an initial hepatitis that may or may not be symptomatic. Successful clearance and resolution of infection depends on the age and immune status of the individual (3).

Interleukin-10 is referred to B cell-derived T cell growth factor and cytokine synthesis inhibitory factor (CSIF) because it inhibits IFN-□□production by activated T cells. It is 18 kDa protein, produced by a variety of cell types, including $CD4^+$ T cells, activated $CD8^+$ T cells, activated B cells, monocytes, macrophages and keratinocytes (4). The effects of IL-10 include reduction of antigen-specific T cell proliferation, inhibition of IL-2 and IFN-□□induced MHC class II expression on monocytes (5).Since IL-10 can be produced by Th2 cells and inhibits Th1 function by preventing Th1 cytokine production (such as IFN-□), also it is considered a T cell cross-regulatory factor and thus it has been referred to as "anticytokine" , so that IL-10 suppresses cell-mediated immunity and stimulates humoral immunity (6).

Interleukin-4 is a 20-kDa cytokine produced by $CD4^+$ Th cells, mast cells, and basophiles. It induces $CD4^+$ T cells to differentiate into Th2 cells while suppressing the development of Th1 cells (7). Most studies of IL-4 have been in mice, where it serves as a growth and differentiation factor for B cells and as a switch factor for synthesis of IgE, it enhances class II MHC expression on B cells, and suppresses IFN-γ dependent macrophage functions, thus inhibit cell mediated immunity (8).

Production of Th2 type cytokines has been associated with progressive virus infections. Activation of Th2 cells can negatively regulate immune responses and may be associated with the immune tolerant state of chronic HBV infection (9). IL-4 and IL-10 cytokines secretion promote suppression of HBeAg/HBcAg specific $CD8^+$ T-cell responses, also the Th2 cytokine response to HBsAg in chronic hepatitis B patients lead to more severe liver damage (10), and may be associated with the persistence of HBV (11). The aim of this study is estimation of some T-helper 2 cytokines (IL-4 and IL-10) in HBV infected patients and individuals vaccinated with recombinant HB vaccine.

Materials and Methods:

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 IL-4 level (human IL-4 BMS225/2, Austria) and serum IL-10 level (human IL-10 BMS215/2 Austria) 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. P value < 0.05 was considered statistically significant.

Results:

The serum concentration of IL-4 in 14 AH, 18 CH and 15 healthy controls were presented in Table (1). The concentration of IL-4 level in the AH group differed significantly (30.219±8.688 pg/ml) in comparison to that of CH (37.803±5.46 pg/ml) and healthy control (14.151±4.039 pg/ml) ($p<0.01$) when analyzed statistically by F-test. LSD- test analysis revealed highly significant elevation in serum IL-4 level of CH patients compared with healthy control ($P<0.008$), but no significant relation was reported when compared with AH patients ($p>0.05$).

Moreover, Table (2) revealed non significant differences in IL-4 levels in sera of RD compared to NRD and HN control groups by F-test and LSD-test analysis in age ≥30 and <30 years old respectively ($p>0.05$).

Table 1: Difference in mean serum level of IL-4 (pg/ml) between study groups

Study groups		**No.**	**IL-4 Mean ±SE**	**P value (F-test)**
Acute HBV patients		13	30.219±8.688	P< 0.01
Chronic HBV		14	37.803±5.46	
Healthy control		14	14.151±4.039	
HC versus AH	**LSD**	NS		
HC versus CH		P<0.008		
AH versus CH		NS		
HC: Healthy control, CH: Chronic hepatitis, AH: Acute hepatitis.				
P< 0.05: Significant , NS: Non significant				

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

Study groups	**Serum IL-4 (Mean±SE)**				
	No.	Individuals age ≥30 years	P value (F-test)	Individuals age <30 years	P value (F-test)
Responder	19	17.192±4.936	P>0.05	35.724±8.479	P>0.05
Non-responder	12	10.777±3.59		14.081±6.301	
Healthy non-vaccinated control	14	8.851±2.402		18.274±6.795	
HN versus RD	**LSD**	NS		NS	
HN versus NRD		NS		NS	
RD versus NRD		NS		NS	
RD: Responder, NRD: Non-responder, HN: Healthy non-vaccinated control					
NS: Non significant					

By comparing the three groups (AH, CH and healthy control) regarding their IL-10 levels, it has been found that patients with CH have the highest mean values (19.424±3.358 pg/ml), compared with other study groups, (9.924±1.94 pg/ml) and (9.0197±0.845 pg/ml) in both AH and HC, respectively, with significant differences ($p < 0.01$) using F-test analysis. However, the comparison between CH and both HC and AH, respectively, revealed significant differences in IL-10 level ($P < 0.008$) ($P < 0.03$) by LSD-test, Figure (1).

Table (3) revealed significant elevation of IL-10 level in sera of RD compared to NRD and HN control groups by F-test analysis in age ≥ 30 and < 30 years old ,respectively, ($p <0.05$). Indeed, RD group showed significant differences ($p<0.03$) compared with HN in age < 30 years by using LSD-test, the same result also reported between RD and NRD in age ≥30 and < 30 years old respectively ($P<0.01$) and ($P < 0.014$) using LSD-test. However no significant elevation was reported when compared between NRD and HN in age ≥ 30 and < 30 years old respectively ($p > 0.05$), as well as between RD and HN in age ≥ 30 using LSD-test Figure (2 and 3).

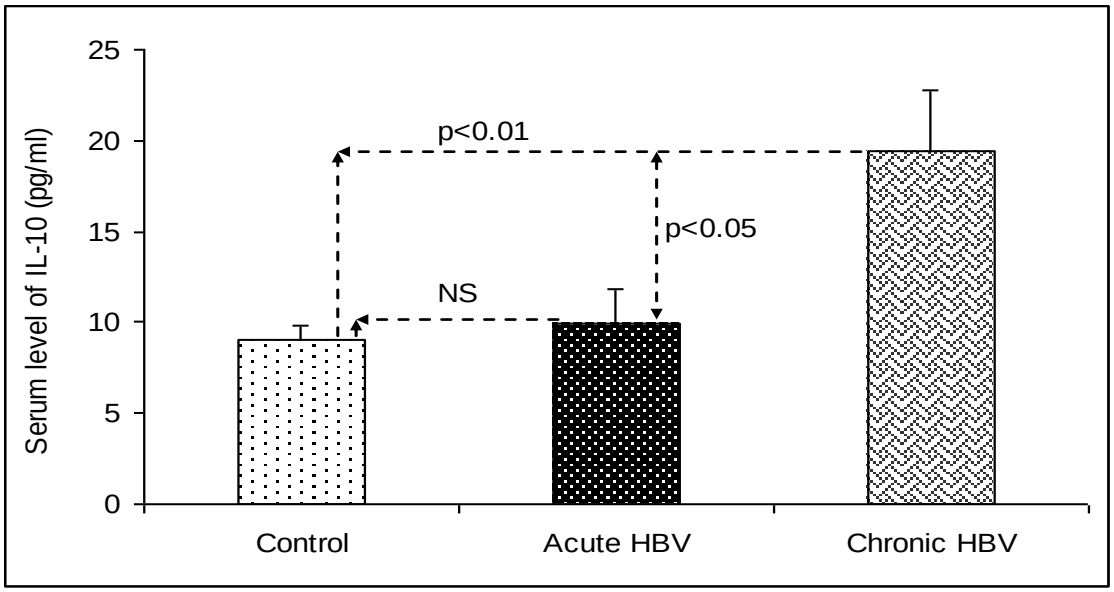

Figure 1: Serum level of IL-10 in pg/ml between study groups

Table 3: Serum IL-10 in pg/ml between vaccinated study groups and healthy control according to age distribution

Study groups	**Serum IL-10 (Mean±SE)**				
	No.	Individuals age ≥30 years	P value (F-test)	Individuals age <30 years	P value (F-test)
Responder	19	12.27±2.771	P<0.05	17.987±2.357	P<0.05
Non-responder	11	7.201±0.4025		9.112±1.024	
Healthy non-vaccinated control	17	8.585±0.647		9.309±1.372	
HN versus RD	**LSD**	NS		P<0.03	
HN versus NRD		NS		NS	
RD versus NRD		P<0.014		P<0.01	
RD: Responder, NRD: Non-responder, HN: Healthy non-vaccinated control					
P<0.05: Significant, NS: Non significant					

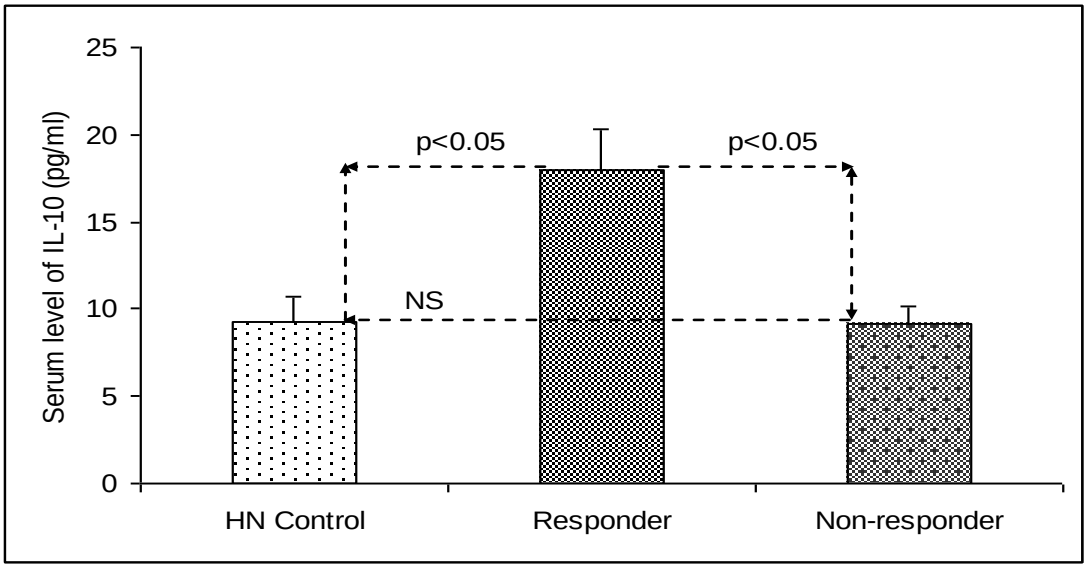

Figure 2: Serum level of IL-10 in pg/ml in age < 30 years vaccinees and control groups

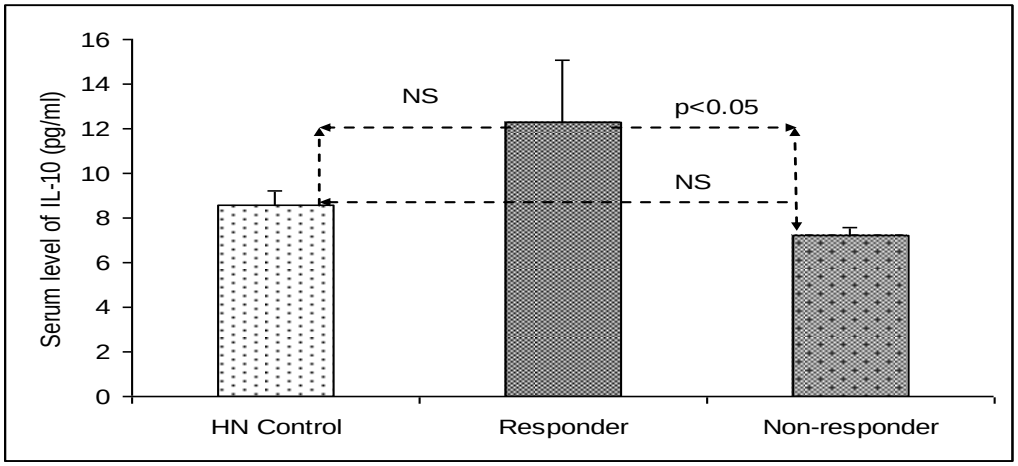

Figure 3: Serum level of IL-10 in in pg/ml in age ≥ 30 years vaccinees and control groups

Discussion:

Secretion of Th2 cytokines, such as IL-4 and IL-10 is thought to be detrimental for B cell differentiation and production of specific antibody (12). In this study, it was determined that the serum IL-4 concentration was elevated during acute phase non significantly when compared with HC ($p>0.05$) Table (1), this finding is in contrast with those of Mansour *et al*., (1994) who reported very low or normal levels of IL-4 in the sera of patients with HBV infection during the acute stage, suggested that type of the stimulus could determine the production profile for each cytokine (13).

In the present study, statistical analysis revealed a significant elevation of serum Th2 cytokines (IL-4 and IL-10) in CH infection as compared to healthy subjects ($p<0.01$), Tables (1) and Figures (1). These results were in agreement with pervious results by Jiang *et al.*,(2002) who found that Th2 type cytokine production elevated in chronic viral infection, suggested that Th2 cytokine has been associated with progressive virus infections, negative regulation of immune responses and may be associated with the immune tolerant state of chronic HBV infection (11). Similarly Atsukawa *et al.,* (2001) showed that Th2 cytokine levels increased significantly during chronic HBV infection, suggested that Th2 (IL-10) suppress secretion of Th1 (IFN-γ) cytokine, and generate macrophage inactivation (14).

However serum IL-10 levels in CH patients significantly increased ($p<0.05$) in comparison with AH patients by using LSD test. IL-10 is one of the key cytokines in the Th2 response. It is a pleiotropic cytokine able to inhibit the synthesis of other cytokines secreted by the Th1 subpopulation, also IL-10 induce B-lymphocyte differentiation into plasmocytes and immunoglobulin synthesis. Therefore, IL-10 has an important role, acting like a general suppressor of the cell-mediated response and increasing the level of humoral immunity. For this reason persistence of high IL-10 levels in the convalescence phase is important in the secretion of surface antibodies against HBV and development of immunity (15).

The levels of IL-4 were not significantly differ ($p>0.05$) among study groups in both age groups, also the results showed non significant elevation of IL-4 in RD compared to NRD studied group, Table (2). In contrast to IL-4, The present study documented that the level of circulating IL-10 was significantly elevated in RD group compared to NRD and HN control groups ($p<0.05$) in both age groups ≥30 and <30 years old as shown in Table (3), although there was significant decrease in NRD compared to RD group, in the same age group ($p<0.05$).

These results were in accordance with Watya *et al*., (1997) who have shown in their studies different patterns of cytokine production in T cell clones isolated from low and high responders, they have reported the predominant Th2 response which observed in high responders (16). In comparison to NRD and HN control groups (in ≥30 and <30 years old), our result shows slightly non significant decrease in IL-10 in the sera of NRD. On the other hand, in <30 years old IL-10 significantly elevated in RD compared to HN control study group Figures (2 and 3). Lack of response to HBsAg had been attributed to variety of mechanisms, including defect in generation of primary HBsAg specific T cell and/ or B cell, and defect in Th cell function (17).

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تقدير بعض سايتوكاينات النوع الاول للخلايا المساعدة التائية (IL-4 and IL-8) في المرضى المصابين بالتهاب الكبد الفايروسي نمط ب والافراد الملقحين بلقاح التهاب الكبد نوع ب المحور

(تاريخ الاستلام: ١٢ / ٧ / ٢٠٠٩ ، تاريخ القبول: ١٤ / ٢ / ٢٠١٠)

الملخص

للاستجابة المناعية الخلوية دور مهم في تنخر الكبد المرافق لمرض التهاب الكبد الفايروسي. يعتبر IL-4 و IL-10 من الامثلة على السايكوتينات المنتجة من خلايا Th- 2 و الذي يحتاجها المضيف كاستجابة مناعية ضد الفايروسات بالاضافة لدورهم في المناعة الخلطية ضد مرض التهاب الكبد الفايروسي. صممت هده التجربة لحساب سايتوكينات خلايا Th-2 خاصة IL-4 و IL-10 للمرضى المصابين بالتهاب الكبد الفايروسي نوع ب والاشخاص الملقحين بلقاح HB.

مجاميع المرضى صنفت الى مجموعة المرضى٣٥ مريض (١٥ حاد(AC) و٢٠ مزمن(Ch)) ٣٥ هم مجموعة الملقحين (٢٠ المستجيبين للقاح(RD)و١٥ غير المستجيبين للقاح (NRD)) بالاضافة الى ١٨ شخص كمجموعة سيطرة خلال الفترة من ايار الى تشرين الثاني ٢٠٠٧.

اخذت نماذج الدم من المرضى والاصحاء من عدة مستشفيات منها مستشفى نانكلي واربيل ورزكاري التعليمي لتشخيص كل مما يلي HbsAg و-Anti HBc Ab(IgM) وAnti-HBs Ab وكذلك تقدير مستويات IL-4 و IL-10 في المصل بواسطة فحص الاليزا.

مستويات كل من IL-4 و IL-10 للمرضى المصابين بالتهاب الكبد الفايروسي الحاد اختلفت معنويا بالمقارنة مع مرضى التهاب الكبد المزمن ومجموعة السيطرة ($p<0.01$) عند التحليل الاحصائي بالاختبار F، بينما الاختبار الاحصائي LSD لل IL-4 ايضا اعطت نفس النتائج لكن بين HC و CH عند مستوى ($P<0.008$). عند المقارنة بين CH و HC و AH على التوالي هناك فروقا معنويا في مستوى IL-10 ($P<0.008$) و ($P>0.05$) وذلك باستخدام التحليل الاحصائي LSD.

اختبار F لل IL-4 اعطت فرقا معنويا ($p<0.05$) عند مقارنة RD و NRD والسيطرة الغير الملقحين في المجموعة العمرية ٣٠ ≥ و > ٣٠ سنة بالتتابع ولكن النتائج كانت مغايرة في مستوى IL-10 }($p<0.05$). اختبار LSD لل IL-4 لم تعطي فرقا معنويا ($p<0.05$) بين كل مجاميع الدراسة لكن كانت الفروق معنوية($P<0.014$) و($P<0.01$) عند مقارنة RD و NRD والسيطرة الغير الملقحين في المجموعة العمرية ٣٠ ≥ و > ٣٠ سنة بالتتابع اضافة لدلك كانت الفروق معنوية($P<0.03$) بين مجاميع RD و HN في المجموعة العمرية٣٠ > سنة.

الاستنتاجات : توصلنا في هذه الدراسة ان هناك ارتفاعا معنويا في مستوى IL-4 و IL-10 في مصول المرضى المصابين بالتهاب الكبد الفايروسي الحاد بامقارنة مع المرضى المصابين بالتهاب الكبد الفايروسي المزمن ومجموعة السيطرة. نفس النتائج سجلت بالنسبة لمستوى مستوى IL-10 في مجاميع RDو NRD في المجموعة العمرية ٣٠ ≥ و > ٣٠ سنة بالتتابع كذلك بين مجاميع RD و HN في المجموعة العمرية٣٠ > سنة.