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DETERMINATION OF IL-2 SERUM LEVELS WITH SUSCEPTIBILITY TO HBV, HCV IN SOME IRAQI PATIENTS

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A**BSTRACT**
Hepatitis is an inflammation of the liver that is most commonly caused by one of the five types of hepatitis viruses; A, B, C, D and E. These types are of a greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread worldwide; in particular, types B (HBV) and C (HCV). Therefore, the present study aimed to investigate serum level of cytokins (IL-2). Interleukin-2 (IL-2) is an immunoregulatory cytokine produced by T cells and plays an important role in antitumor immunity. For cytokine serum level 100 hepatitis Iraqi patients and controls enrolled in this study. Cytokine serum level was determined by using ELISA method. Serum level of IL-2 showed no significant difference in HBV patients (35.65 ±10.84 pg/ml) compared to HCV patients (36.08 ±14.54 pg/ml) or controls (35.35 ±14.50 pg/ml) respectively.

KEYWORDS: B (HBV), C (HCV), hepatitis, cytokins (IL-2), Interleukin-2 (IL-2).

INTRODUCTION

Hepatitis is an inflammation of the liver that is most commonly caused by one of the five types of hepatitis viruses; A, B, C, D and E. These types are of a greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread worldwide; in particular, types B (HBV) and C (HCV) (WHO, 2013). The balance between virus and host defense defines the course of viral infection and pathogenesis, and persistent viruses such as HBV and HCV are generally not directly cytopathic and have developed immune evasion mechanisms to survive without destroying the host (Bertoletti and Gehring, 2006). For the host, the goal is to prevent, eliminate, or at least control viral infection while limiting undue collateral damage. These interactions are influenced by various host genetic, immunological and viral factors (Saxena *et al*., 2013). IL-2 was discovered in 1975 as a growth-promoting activity for bone marrow-derived T lymphocytes, and it is coded by a gene located on human chromosome 4 (Fallahzadeh *et al*., 2011; Oo *et al*., 2012). Structurally, IL-2 is a protein consisting of 133 amino acids and has a molecular weight of 15400 Daltons. It is synthesized and released from activated T cells and has a key role in cell mediated immune response, and it was found that IL-2 increases lymphokine release from T, B and NK cells and has many immunological effects (Wrenshall *et al*., 2014). Additionally, it is a potent T cell growth factor that is assumed to amplify lymphocyte responses in vivo (Fallahzadeh *et al*., 2011). It is synthesized and released from activated CD4 + T cells, although expression by native CD8+ T cells, dendritic cells, and thymic cells can also occur (Waldmann, 2006). Also, it promotes production of NK-derived cytokines such as tumor necrosis factor alpha (TNF-α), IFN-γ and granulocyte Monocyte-colony stimulating factor (GM-CSF), and can act synergistically with IL-12 to enhance NK cytotoxic activity (Oo *et al*., 2012). There are several forms of IL-2 receptors that differ in their affinities for IL-2: a low-affinity receptor consisting of an α chain, an intermediate affinity receptor consisting of β and γ chains, and a high affinity receptor consisting of all three chains α, β, and γ (Thornton *et al*., 2004).

MATERIALS & METHODS

The duration of this study was from March 2016 to December 2016. After ethical clearance the study was carried out in the Gastroenterology and Hepatology Teaching Hospital / Baghdad. Those patients have been of different age groups, and different geographic residencies. The diagnosis was made by the consultant medical staff at the hospital, which was based on clinical examination and serology test by ELISA, and confirmed by molecular test real time PCR, all of them didn't receive any treatment. The study was carried out on 100 subjects, 76 of them were suffering from viral hepatitis, and were divided into two groups:

The group I: consists of 38 patients with hepatitis B (HBV), mean ± SD: (43.36 ± 10.68) years.
The group II: consists of 38 patients with hepatitis C (HCV), mean ± SD: (33.63 ± 15.35) years.
The group III: consists of 24 controls mean ± SD: (39.20 ± 11.32) years.

Assessment of IL-2 Serum Levels

Sera of hepatitis patients and controls were assessed for level of IL-2 using commercially available kits (PeproTech; UK) by means of ELISA that was based on similar principles Fig.1.

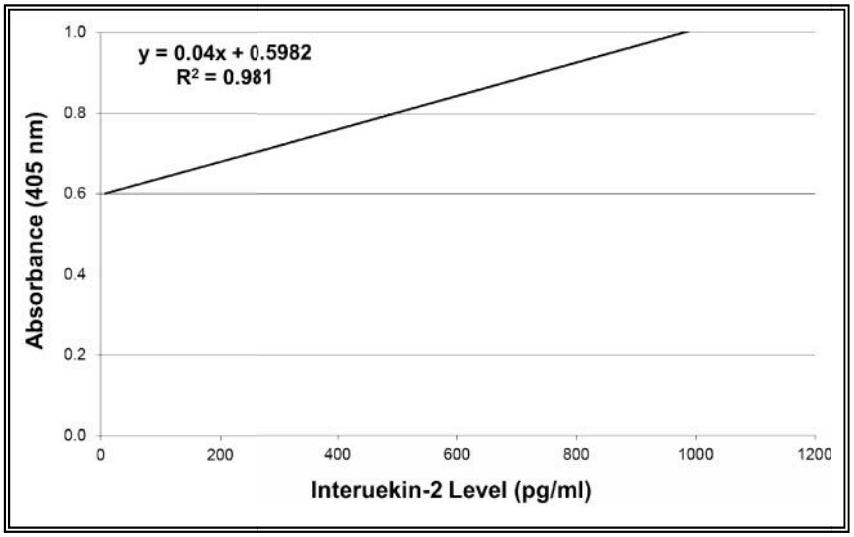

FIGURE 1: Standard curve of LI-2 serum level

Statistical Analysis

Serum level of IL-2 was statistically analyzed using the computer program SPSS (Statistical Package for Social Sciences) version 13.

RESULTS & DISCUSSION

Serum Level of IL-2

Total Patients

The serum levels of IL-2 in total hepatitis B and C patients and control was detected. Serum level of IL-2 showed no significant difference in HBV patients (35.65 ±10.84 pg/ml) compared to HCV patients (36.08 ±14.54 pg/ml) or controls (35.35 ± 14.50 pg/ml) respectively.

Patients Distributed by Age

No significant differences in their serum levels of IL-2 between the two age groups (< 40 and ≥ 40 years) in HBV and HCV patients or controls.

Patients Distributed by Gender

Distribution patients and controls into males and females revealed some significant differences in the serum level of the IL-2. Females HCV showed a significant increase level of IL-2 compared to male patients (39.56 ±16.13 vs. 30.75 ±10.00 pg/mL) (table 1).

TABLE 1: Serum level of IL-2 and in hepatitis B and C patients and controls distributed by gender and age group

Cytokine	Groups	No.	Cytokine Serum Mean Level ± S.D. (pg/ml)		
			Patients		Controls
			Hepatitis B	Hepatitis C	
IL-2	< 40	49	36.30 ± 10.69^{A}	37.48 ± 12.49^{A}	34.36 ± 13.68^{A}
	≥ 40	51	34.53 ± 11.38^{A}	35.34 ± 15.70^{A}	36.33 ± 15.82^{A}
P ≤			N.S.	N.S.	N.S.
IL-2	Males	41	38.27 ± 11.59^{A}	30.75 ± 10.00^{A}	32.73 ± 14.25^{A}
	Females	59	32.73 ± 14.25^{A}	39.54 ± 16.13^{A}	36.22 ± 14.88^{A}
P ≤			N.S.	N.S.	N.S.

In the hepatitis patients, some Th1 phenotype cytokines are positively correlated with hepatic inflammatory activities, especially those underlined by CD4+ T cells (Jiang *et al*., 2002). Activated CD4+ T cells can be distributed into two subsets based on their cytokine secretion profiles, which are Th1 subset that produces IL-2, IFN-γ and TNF-α, these are participates in cell-mediated immune responses. In contrast, Th2 subset produces IL-4 and IL-13, and mediates humoral immune responses, in which IL-10 is an up-regulator produced by Treg cells (Bailey-Bucktrout and Bluestone, 2011). Therefore, an increased level of IL-10 may encounter cell-mediated immunity, which is effective in virus clearing.

Several studies have focused on the relationship between viral hepatitis and cytokines, and the broad range of cytokines and chemokines, which has been required for viral clearance, is produced in response to viral infection (Mogensen and Pauldan, 2001). Kitaoka *et al*. (2003) suggested that the Th1/Th2 type cytokines were changed in association with progression of chronic liver disease and in response to therapy. Reiser *et al*. (1997) showed that serum Th2 cytokines are overhead (but at a low level) in a proportion of patients with chronic HCV infection. However, the elevated Treg cytokine levels may represent a systemic response and not a result of increased local production within the liver.

However, a survey by Fan *et al.* (1998) showed that IL-2, IL-4 and IL-10 levels were significantly increased in HCV infected hosts when compared to normal controls, but the production of Th2 cytokines was more predominant.

Immediately following the viral infection, a strong host response is initiated, which includes activation of pre-existing anti-viral defense machinery commitment to apoptosis, and production of specific cytokines. These events lead to the reduction of the viral replication and to the limitation of viral spread (Maini and Schurich, 2010).

Besides that, the interaction of viral proteins with cellular proteins can initiate a cellular reaction that may lead to release the first wave of cytokines; virus induced expression of cytokines is primarily due to stimulation of one or more of signal transduction cascades, leading to activation of specific transcription factors, then release of cytokines, which regulate and modulate the host immune response (Maini and Schurich, 2010).

The results of cytokine serum levels distributed according gender demonstrated that IL-2 revealed significant variations among the investigated groups. Previous studies have demonstrated a correlation between cytokine serum levels and gender, and this may have been influenced by various factors, and among them is the hormonal status (Franceschi *et al*., 2007). In this regard, immune defense capacity has shown differences between human males and females. In addition, males are found to be more prone to infections, while females are at greater risk to develop autoimmune diseases (Zhu *et al*., 2009). These findings were correlated with humoral responses to the foreign antigenic challenge, and the suggestion was that sex hormones may influence immune functions (Goetzl *et al*., 2010). With respect to cytokines, Elahe *et al.* (2006) reported that inflammatory cytokines were differentially regulated in response to hepatic infection in male and female mice. In addition, Klingstrom *et al.* (2008) revealed that the cytokine responses in females and males are not similar during an acute viral infection; implying that the subsequent activation and function of immune responses might differ between female and male patients upon infection. These findings may explain part of the observed sex differences in susceptibility to infectious diseases and in mortality following viral infections.

Such results may suggest that the age is not critical factor that affect the serum level of the investigated cytokine; however, caution must be considered in interpreting these results because the sample size in patients and controls may not permit a firm conclusion. In addition, age can be considered an effected factor when we have subjects at age more than 60 years, because it is often that a dysregulation in the immune functions, and a decline in health and increased sensitivity to various diseases are associated with advanced ages (Poveshchenko *et al*., 2014), and since cytokines are central to immune cell communications, age-associated changes in cytokine production may contribute to these alterations (Poveshchenko *et al*., 2014).

However, in agreement with present findings, Kim *et al*. (2011) demonstrated that serum levels of IFN-γ, IL-10, IL-2 and TNF-α showed no significant differences between two age groups of healthy subjects (< 40 and ≤ 65 years). However, Goetzl *et al*. (2010) reported that the generation of IFN-γ and IL-17 by stimulated T cells was much higher or minimally different for healthy older women as compared with young women. In addition, Kleiner *et al.* (2013) found that IL-4, IL-6, TNF-α, IFN-γ and IL-13 did not show differences between young children and adults, but these cytokines were up-regulated in children between 7 and 17 years, except IL-13, that was instead down-regulated.

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