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Evaluation ofserum levels of IL-10and IL-6 in patients with HCV at Diwaniyah Teaching Hospitals, Iraq

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Syoof khoman Alramahy[b],Najwan abdul karim[a]

[b] College of science, University of Al-Qadisiyah, Email:syoofalwan@yahoo.com

[*]College of science, University of Al-Qadisiyah, Email:haideralsudani466@gmail.com

ABSTRACT:
Hepatitis C virus (HCV) infection constitute serious global health problems due to their high morbidity and mortality.This study evaluated the serum cytokine levels (IL-10 , IL-6) of hepatitis B, as well as the differences in such levels between patients and healthy controls.30 blood samples from patients infected with HCV and 30 blood samples from healthy individuals as a control group were collected from the unit of Viruses at Diwaniyah Teaching Hospital, Women's Hospital and Educational Children in Diwaniyah Governorate, duringthe period from July 2018to February2019. In this study ELISA technique indicated that serum levels of IL-10 and IL-6 were irregular in patients with HCV , the level of IL-10 was decreased significantly in patients with HCV (115.64±24.05) compared to control group (234.71±147.62),whereas IL-6 level was increased non significantly in patients with HCV (385.11±244.96) pg/ml compared with control group (287.15±210.45) pg/ml. Conclusionfrom the current study,low level of IL-10 in patients with HCV compared to control group, high level of IL-6 in patients with HCV compared to control group.

KEYWORDS: Interleukin 6, Interleukin 10 , Hepatitis ,HCV , Serum Level.

1. INTRODUCTION

Hepatitis is one of the most common health problems caused by HCV (Shrivastava*et al*,2013), which are one of the most important hepatitis virus leading to acute and chronic infection and is one of the main factors related to liver cirrhosis and liver cancer (Heidari*et al*,2016) .The remainder and stability of the virus and divrsity in clinical outcomes of HCV infection are associated with environmental factors, the virus itself, immunological differences, hereditary factors affecting the evolution of liver disease and the gene polymorphism of cytokines that affect the development of HCV infection (Conde*et al*,2014). The host response to hepatitis viruses includes several components of the immune system , from these components cytokines which include , chemokines, interleukins, interferons , and TNF, all of which act significant role in distinguishing the infected cell and regulate the immune response and then eliminate the virus and destroy the infected cell (Dondeti*et al*,2016). From these cytokines, IL-6, which is located on the human chromosome 7 and contains (5 exons and 4 introns) and IL-10, which is located on the first human chromosome (Zhai*et al*,2011). These interleukins play an important role in immune preservation against HCV infection. They are produced by expanse of cells like T helper 1 and T helper 2.Th1 produces pro-inflammatory cytokines like IL-6, which plays an important role in the removal of intracellular injury. Th2 produces anti-inflammatory cytokines like IL-10, which plays a role in controlling humoral immune response. The production of these cytokines is very complicated. The imponderables in the production of pro-inflammatory and anti-inflammatory interleukins significantly affects the evolution and persistence of HCV infection. It also affects the immune response of the host and the evolution of liver disease from chronic infection to hepatocellular carcinoma (AL-sharif,2011).There was a relationship between gene polymorphism and the level of IL-6, where did he find GG genotype at site 174 to IL-6 was higher in patients with hepatitis C virus and that the level of IL-6 was higher in patients who carry this genotype (Mourtzikuo*et al*,2014). The aim of the study was to estimate the levels of IL-6 and IL-10 and study their relationship with HCV.

2-Materials and methods:

2.1 Samples Collection:

Collected60 blood samples from patients auditors to unit of viruses in Diwaniyah teaching hospital, Women's Hospital and Educational Children, which grouped in to 30 patients infected with HCV (14 female and 16 male) and 30 healthy individuals as a control group for the period from July 2018 until February 2019. 5 ml per blood sample was collected by

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venepuncture from which2.5 ml was put in a plantube and standapproximatelyhalfanhourforcoagulation and then

centrifuged at 3000 cycles / minutefor 15 minutes . The serum collected in appendrof tubes and stored at freezing.

2.2ELISA Kit method

Human IL-6 and Human IL-10ELISA Kit was done according to company instruction as following: Elabscience

2.3ELISAKit components:

ELISA components for evaluation of serum levels of IL- 10 and IL-6 in patients with HCV,as in table (1)

Table (1) ELISA kit components.

Reagent	Quantity
Micro ELISA Plate	8 wells ×12 strips
Reference Standard	2 vials
Reference Standard & Sample Diluent	1vial 20mL
Concentrated Biotinylated Detection Ab	1vial 120µL
Biotinylated Detection Ab Diluent	1vial 10mL
Concentrated HRP Conjugate	1vial 120µL
HRP Conjugate Diluent	1vial 10mL
Concentrated Wash Buffer (25×)	1vial 30mL
Substrate Reagent	1vial 10mL
Stop Solution	1vial 10mL

2.4 Assay procedure:

Before begging the assay, all kit reagents and samples were bring at room temperature.

1 - Prepare the washing solution by diluting 50 ml of the washing solution and completed the size to 1000 ml distilled water and preserved at 4 C until use.
2. Prepare a series of dilutions for the solution using the dilution solution supplied by the company, where the range ranged between 5-1000 pg / ml.

3 - Add 100 ml of samples to the drill and then covered the dish after it was roasted and incubated for two hours at room temperature.

4. Add 50 mL of Biotin to each hole.

5-Wash the drilling to remove the antibody and other substances not associated with the use of the washing solution, which was prepared in the first paragraph at a rate of 5 times in each wash by adding 350 ml.

6. Add 100 ml of Avidin Conjugate and cover the dish with the plate cover, then stir and incubate at room temperature for 2 hours.

7. The washing phase was repeated again as in the fifth paragraph to remove the non-binding enzyme Avidin Conjugate.

8. Add 100 ml of Substrate solution in all the holes, then cover the dish and incubate for half an hour at room temperature.

9. Add 100 ml of Stop solution to all the holes and cover the dish and incubate for half an hour at room temperature.

10 - Read at 450 nm wavelength.

11. The karaf paper explained the absorption of the measurements and the measurement curve was drawn from thesepoints as shown infigure (1) and (2).

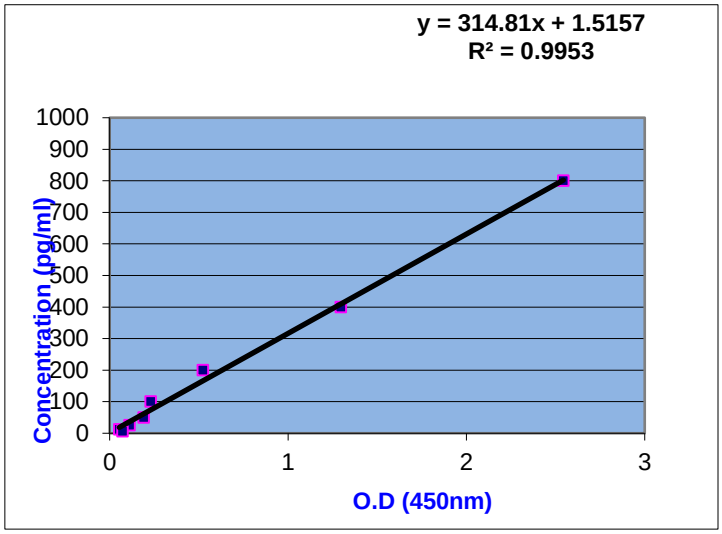

Figure (1) The standard curve for Human IL-6 interleukin

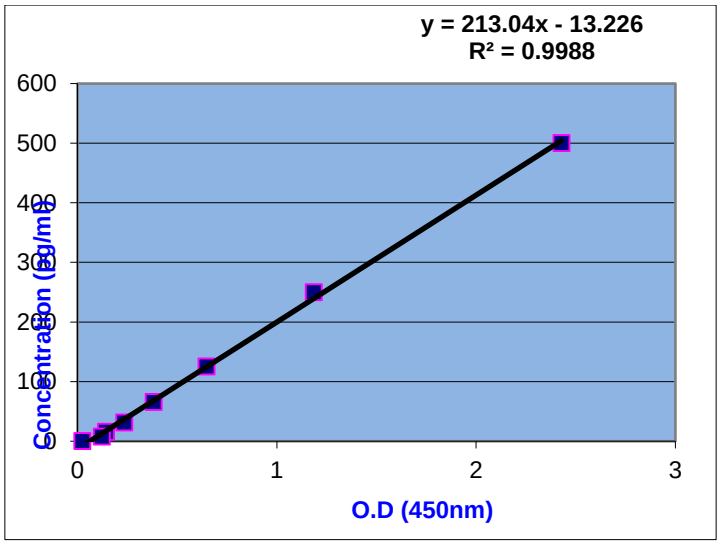

Figure (2) Standard curve forHuman interleukin IL-10

3-RESULT AND DISCUSSION :

3.1 Estimated concentration of(IL-10) in patients with HCV and control group.

The results of the present study showed a significant difference in the level of IL-10 in patients with HCV compared to healthy at a potential level P≤0.05, the concentration of IL-10 in patients with HCV was decreased (115.64±24.05).

While the concentration of IL-10 in healthy was increased (234.71±147.62), as in Table (1).

Table (1): Concentration of IL-10 in patients withHCV and control group.

Interleukin	Statistic	HCV n = 30	Control *n* = 30	*P*
Serum IL-10	Mean ±SD	115.64 ±24.05	234.71 ±147.62	0.001

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	Range	**84.56 ±189.38**	**27.89 ±591.81**	**HS**

n: number of cases; SD: standard deviation.HS: highly significant at $P \leq 0.01$; NS: not significant at $P \leq 0.05$

Table (1) shows that the IL-10 level was low in patients with HCV compared to control group, which is contrary to (Hattori *et al*,2003) (Othman *et al*,2013). They found that the level of interleukin 10 was higher in patients with HCV compared with the control group ,while (Kakumu*et al*,1997) (Inglo*et al*,2008)found no significant difference in the level of IL-10 in patients with HCV and control group. HCV is a major cause of chronic liver disease. HCV is poorly understood, but it is possible that immune and viral factors may play a role in pathogenesis (Constatntini*et al*,2002). In general, there are two distinct types of cytokines production ,the first is the production IL-2, TNF α, which play a role in the defences against the virus (Hohler *et al*,2005) and the second is the production of interleukin IL-10, which plays a role in the humoral immune response (Minton *et al*,2005). IL-10 is produced by monocytes, macrophages, and T helper2 cells. It is an anti-inflammatory cytokine that inhibits the production of pro-inflammatory cytokines (Bidwell *et al*,1999).The production of cytokines in individuals depends on a large genetic contribution, which explains the low level of IL-10 in this study where studies indicate that the timing and relative quantities of the production of IL-10 have a role in the elimination of infection and the increase in the production of IL-10 leads to weakness of the immune system in the elimination of the virus and thus the continuation of injury (Bruno *et al*,2011).

3.2 Estimated concentration of IL-6 in patients with HCV and control group.

The results of the present study showed no significant difference in the level of IL-6 in patients with HCV compared healthy at a potential level P≤0.05, concentration of IL-6 in patients with HCV was increased(385.11±244.96) , while the concentration of IL-6 in healthy was decreased (287.15±210.45). As in Table (2).

Table (2): Concentration of interleukin IL-6 in patients with HCV and control group.

Interleukin	**Statistic**	**HCV** **n = 30**	**Control** ***n* = 30**	***P***
Serum IL-6	**Mean ±SD**	**385.11 ±244.96**	**287.15 ±210.45**	**0.114** **NS**
	Range	**118.31 ±896.21**	**12.22 ±787.6**	

n: number of cases; SD: standard deviation. HS: highly significant at $P \leq 0.01$; NS: not significant at $P \leq 0.05$

Table (2) shows that the level of IL-6 was higher in patients with HCV compared with the control group. This result is similar to study of (Atasevene*t al*,2006) (Porta*et al*,2008) that found the level of IL-6 was higher in patients with HCV compared to control group. HCV infection is a major health problem, so the immune response of the host plays an important role in theoutcome of the virus. The response is the production of pro-inflammatory cytokines that play an important role in the eradication of the virus, from these cytokines, IL-6, which is located on the seven human chromosome and contains 5 Exon and 4-intron and

considered multifunctional interleukin that carry out complex biological activities through different mechanisms (Toumpanakis*et al*,2007).Itis produced by a large number of cells, such as Cooper cells, helper T cells type Th1, lymphocytes and (Dienz*et al*,2009). The level of IL-6 in patients with HCV is controversial (Fallahi*et al*,2012). The level of interleukinIL-6 was high in patients with HCV compared with the control group, and this result was close to what we found in our current result where the rise in the level of IL-6 may be associated with or related to viral load and histological index.

4-Conclusions:

According to results from this research , the following conclusions have been reashed , decreased the level of IL-10 in patients with HCV compared to control group ,increased the level of IL-6 in patients with HCV compared to control group.

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