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The Frequency of Hepatitis C Viral Infections and its Correlation with IL-12 and IL-18 among Major Thalassemic Patients in Baghdad

Rana Akram Hadi [1], Zainab A. Hamid [2]

[1] *Department of Microbiology, Baghdad Medical College, University of Baghdad, Baghdad-Iraq*

[2] *Ph.D. Medical Microbiology, Department of Microbiology, Baghdad Medical College, University of Baghdad, Baghdad-Iraq*

Abstract

Background: hepatitis C virus (HCV) is responsible for nearly 80 - 90% of post-transfusion hepatitis in beta-thalassemia patients. This study aimed to evaluate the appearance of HCV infection in a set of Beta-Thalassemia patients in Baghdad and to assess the IL-12, IL-18, and serum liver enzymes levels among β-thalassemic patients infected with HCV. **Methodology:** A total of 150 sample included in this study, (70 male; 70 female) in Baghdad, 140 beta-thalassemia patients, and 10 healthy control group, from January to September, 2020. Sera of study populations were tested for anti-HCV Ab by ELISA and confirmed by western blot technique;the concentrations of IL-12, IL-18, AST, ALT, and APL were also measured. **Results:** 17.9% (n=25/140) of the major thalassemic patients were positive for HCV Abs.The median of serum concentrations for IL-12 in HCV positive thalassemic patients was 0.41 pg/ml which is lower than the corresponding medians of control group while the median concentration of IL-18 in sera of HCV positive thalassemic patients was 17.07 pg/ml, which is significantly higher ($p<0.001$) than the corresponding median in control group. The median concentration of AST in sera of HCV positive thalassemic patients was 71.0 U/L which was significantly higher than the equivalent medians in HCV negative thalassemic group and control group while. The correlation coefficient for anti-HCV Abs concentrations and their corresponding IL-12 concentrations is 0.084; while for the same antibodies with IL-18 was 0.0979. **Conclusion:** The prevalence of HCV infections is high among β-thalassemia major patients in Baghdad, especially among young adult patients. ELISA technique is a perfect choice for detecting anti-HCV Abs with very high sensitivity. Generally, the infected patients have decreased IL-12 concentrations, elevated IL-18 concentrations, and elevated serum AST concentrations.

Key words: β -Thalassemia, HCV, IL-12, IL-18, liver enzymes, Baghdad

1. Introduction

Beta-thalassemia is an inherited genetic disorder due to mutation in beta subunit gene of hemoglobin, which leads to production of abnormal hemoglobin unable to normally carrying oxygen leading to anemia and tissue hypoxia. In beta-thalassemia there is decrease in synthesis of beta chains of hemoglobin (Hb). The patient has anemia because of enhancing red blood cell damage. This is attributed to existing red blood cells defective and cannot transfer enough oxygen [as in 1]. The main reason for morbidity and mortality in Beta-Thalassemia patients is infections. This caused by the results of functional modification in the immune system because of the different type of blood.

In the same context, regular blood transfusions for patients of Beta-thalassemia that have successfully enhanced to give the chance survival though these transfusions hold a specific hazard of transmission of certain viruses. Because of recurrent blood transfusions hepatitis B, C, G as well as human immunodeficiency virus (HIV) infection can appear. Where both of blood or blood products is considered as about 10% cases [as explained by 2] that convey for the whole of these.

Additionally, hepatitis C virus (HCV) can be considered as an covered positive-sense, singular-stranded virus that belonging to the family Flaviviridae [3]. It transmitted by both of blood or blood products [4].

*Corresponding author e-mail: zainaba.hamid@comed.Uobaghdad.edu.iq; (Zainab A. Hamid).
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Hepatitis C virus is causative agent of post-transfusion non-A-non-B hepatitis in patient with Beta-thalassemia major [as in 5]. Furthermore, HCV is responsible for (80 - 90%) of post-transfusion hepatitis in beta-Thalassemia patients. Unavoidable liver iron overload in patients on regular blood transfusion, as well as HCV infection is displayed to have a potentiating impact on hepatic fibro genesis in Beta-Thalassemia patients [as in 6].

Many immunological disorders may be found in Beta-thalassemia patients [7], both the innate and adaptive immune replies are changed among that the damage of neutrophils and macrophage phagocytic and killing functions that effect on the production of some cytokines [as described by 8].

Interleukin-12 latterly distinguished cytokines that play a vital role in the induction for both of T-helper cell type 1 and T-helper cell type 2 [as in 9]. Moreover, Interleukin-12 is considered as an significant immunoregulatory cytokine, which has been essentially produced by antigen-giving cells. This is conveyed through infection regulates innate replies and identifies the kind of adaptive immune replies [10]. In Beta-Thalassemia patients [11], IL-12 generation has been expressively repressed when associated to regular individual, representing the contribution of this cytokine in the defeat of erythropoiesis in Beta-Thalassemia patients [as in 12]. Reduced IL-12 level and improved IL-18 level were recognized in patients with Beta-Thalassemia main [as described by 13].

Interleukin (IL)-18 has been initially exposed as a feature, which improved IFN-γ generation from anti-CD3-stimulated Th1 cells, particularly in the existence of IL-12 [as in 14]. Consequently, there is a dearth of information on the environment of the contribution of IL-18 in HCV pathogenesis and mass defense, which is studied. The current study is given to identify IL-18 levels in patients of HCV infection as well as is to evaluate the character of IL-18 in the determination of HCV. Furthermore, it is focused on the alters in the serum level of Interleukins 12&18 in Beta-Thalassemia patients as well as the associated to HCV infections, then Beta-Thalassemia is considered as one of the chief health challenges in the whole country, trouble in immune function that did not attract more consideration.

This study aimed to evaluate the appearance of HCV infection in a set of Beta-Thalassemia patients in Baghdad/Iraq and to assess the levels of IL-12, IL-18, and liver enzymes in these patients.

Methodology

A total of 150 sample included in this study, (٧٠male; ٧٠female) in Baghdad, 140 beta-thalassemia patients, and 10 healthy control group (9 male;1 female) who were attended Al Karama Teaching Hospital and Ibn AL-baladi Maternity Hospital &Children's Teaching Hospitalfrom January to September 2020; aged 4-46 years, they received regular blood transfusions and treatment for their disease, thalassemia. All the laboratory testing was done in National Blood Transfusion Center and Central Public Health Laboratory (CPHL).Five ml of venous blood sample was collected by medical staff from each patient just before transfusion. The sera of the patients were separated and frozen at -20 °C till tested.HCV Abs were screened by ELISA Kit (Fortress HCV kit, Fortress Company/ UK), patients with positive anti-HCV Abs were confirmed in CPHLby using HCV Blot 3.0 Western Blot Assay kit. Age and gender of patients were recorded. The sera were also tested for liver function tests (ALT, AST and ALP) using automated clinical chemistry analyzer (ARSHITECT c400, USA), and estimation of IL-12 & IL-18 levels were estimated by ELISA kits (Melsin Medical Company, Limited/ China).

Results

The current study revealed that 17.9% (n=25/140) of the major thalassemic patients were positive for HCV Abs by ELISA technique, while all others are negative as described in figure 4.1. Then, positive cases were confirmed by the confirmatory assay, Western Blot Assay, when all the positive cases by ELISA were also positive by the confirmatory assay, this result gives a sensitivity of 100% for the ELISA technique.

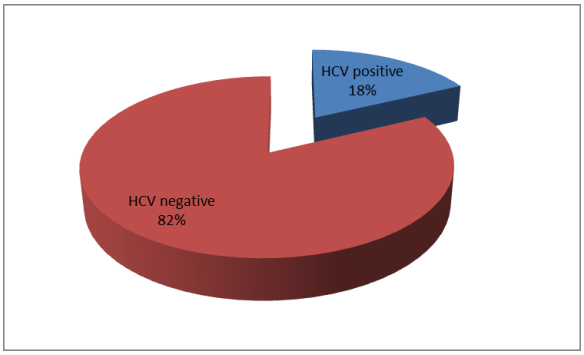

Figure 1: HCV prevalence among major thalassemic patients

More than half of HCV positive thalassemic patients are in age group 21-30 yrs old (56%, n=14/25); while the majority of HCV negative patients are in age group 11-20 yrs old; the distribution of thalassemic patients according to age groups in relation to HCV positivity is statistically highly significant (p<0.001), the control group included 10 persons enrolled in 3 age groups as demonstrated in table (1). The median age of the control group (31.7 yrs old) is higher than that of HCV positive patients (26.36 yrs old) which in turn higher than the corresponding in HCV negative patients (16.8 yrs old) as shown in Table 1.

Table 1: Descriptive analysis of age of thalassemia patients (HCV positive and HCV negative) and control group

		Study groups			P-value
		Thalassemia patients		**Control (N=10)**	
		HCV positive (N=25)	**HCV Negative (N=115)**		
Age groups	≤10 years	2 (8%)	29 (25.2%)	0 (0%)	<0.001**
	11-20 years	3 (12%)	55 (47.8%)	0 (0%)	
	21-30 years	14 (56%)	25 (21.7%)	5 (50%)	
	31-40 years	6 (24%)	3 (2.6%)	4 (40%)	
	> 40 years	0 (0%)	3 (2.6%)	1 (10%)	
Total		25	115	10	
Mean		26.36	16.80	31.7	<0.001**
Standard Deviation		8.97	8.73	5.66	

***: High statistical significance (p<0.001)*

The results of this study indicated that females are more frequently infected with HCV (56%, 14/25) than males (44%, 11/25), while the inverse is reported in HCV negative thalassemic patients, however, these results were statistically non-significant when compared with each other and with the control group (p>0.05) as mentioned in table (2).

The optical density was measured to reflect the concentration of HCV antibodies using ELISA technique; the median optical density of positive cases were 1.6 which was significantly differ from the median of the HCV Abs negative thalassemic patients and the normal control group as illustrated in table (3). The serum concentration of the IL-12 was measured in different groups and the results revealed that the median of serum concentrations for IL-12 in HCV positive thalassemic patients were 0.41 pg/ml which was lower than the corresponding medians of control group and HCV negative thalassemic patients (table 4), the results were statistically highly significant (p<0.001).

Table 2: Gender distribution of study groups

		Study groups			P value
		Thalassemia patients		**Control (N=10)**	
		HCV positive (N=25)	**HCV Negative (N=115)**		
Sex	Female	14 (56%)	56 (48.7%)	1 (10%)	0.157^{NS}
	Male	11 (44%)	59 (51.3%)	9 (90%)	
Total		25 (100%)	115 (100%)	10 (100%)	

NS: None statistical significance (p>0.05)

Table 3: Descriptive analysis of HCV ELISA result in thalassemia patients and control group

HCV (OD)	Study groups		
	Thalassemia patients		**Control (N=10)**
	HCV positive (N=25)	**HCV Negative (N=115)**	
Median	1.60	0.05	0.05
Percentile 25	1.20	0.05	0.05
Percentile 75	2.00	0.06	0.05
P value	<0.001**		
HCV positive vs HCV Negative	<0.001**		
HCV positive vs control	<0.001**		
HCV Negative vs Control	0.988^{NS}		

*NS: None statistical significance (p>0.05), **: High statistical significance (p<0.001)*

The median concentration of IL-18 in sera of HCV positive thalassemic patients was 17.07 pg/ml, which is significantly higher (p<0.001) than the corresponding median IL-18 concentration in HCV negative thalassemic patients (1.21 pg/ml) or control group (1.35 pg/ml). Moreover, the differences in medians of HCV negative thalassemic patients and control group is statistically significant (p<0.025), these results are illustrated in table (5).

The median concentration of AST in sera of HCV positive thalassemic patients was 71.0 U/L which was significantly higher (<0.001) than the equivalent medians in HCV negative thalassemic group and control group; the differences in medians of last two group were not significant as demonstrated in table (6).

The results of this study showed that medians of serum concentrations of ALT for HCV positive thalassemic group, HCV negative thalassemic group, and control group were 32.0 U/L, 24.0 U/L, and 30.0 U/L respectively. The overall results of the three groups is statistically significant; however, when compared separately, the differences in results for HCV positive thalassemic patients and control group were statistically non-significant (p= 0.337), while the median of ALT for HCV positive thalassemic patients is significantly differ (p=0.002) from the results of the HCV negative thalassemic patients,(table 7)

Table (4): Descriptive analysis of IL-12 concentrations in thalassemia patients and control group

		Study groups		
		Thalassemia patients		**Control (N=10)**
		HCV positive (N=25)	**HCV Negative (N=45)**	
Serum IL-12 (pg/ml)	Median	0.41	0.75	0.62
	Percentile 25	0.41	0.62	0.56
	Percentile 75	0.46	2.21	2.21
P value		<0.001**		
HCV positive vs HCV Negative		<0.001**		
HCV positive vs control		<0.001**		
HCV Negative vs Control		0.224 NS		

*NS: None statistical significance (p>0.05)**: High statistical significance (p<0.001)*

Table 5: descriptive analysis of IL-18 concentration result in thalassemia patients (HCV or without HCV) and control group

		Study groups		
		Thalassemia patients		**Control (N=10)**
		HCV positive (N=25)	**HCV Negative (N=45)**	
Serum IL-18 (pg/ml)	Median	17.07	1.21	1.35
	Percentile 25	12.42	0.91	1.21
	Percentile 75	30.72	1.21	1.50
P value		<0.001**		
HCV positive vs HCV Negative		<0.001**		
HCV positive vs control		<0.001**		
HCV Negative vs Control		0.025*		

: statistical significance (p<0.05): High statistical significance (p<0.001)*

Table 6: Descriptive analysis of Aspartate amino transferase results in thalassemia patients and control group

		Study groups		
		Thalassemia patients		**Control (N=10)**
		HCV positive (N=25)	**HCV Negative (N=45)**	
AST (U/L)	Median	71.00	31.00	30.50
	Percentile 25	52.00	27.00	27.00
	Percentile 75	103.00	33.00	33.00
P-value		<0.001**		
HCV positive vs HCV Negative		<0.001**		
HCV positive vs control		<0.001**		
HCV Negative vs Control		0.583 NS		

*NS: None statistical significance (p>0.0) **: High statistical significance (p<0.001)*

The measurement of ALP concentrations in sera of study populations revealed that no significant differences (p=0.397) in medians of concentrations among the three groups, HCV positive thalassemic patients, HCV negative thalassemic patients, and non-thalassemic control group, 84 U/L, 121 U/L, and 85 U/L respectively however, when compared separately only the results of HCV negative thalassemic patients and control group were differing significantly (p=0.025) in in their medians of ALP concentrations; these results are described in table (8).

Results of HCV positive thalassemic patients indicated that there is a non-significant very small positive relationship between the concentrations anti-HCV Abs and their corresponding IL-12 concentrations, R = 0.084; this result was statistically non-significant p = 0.691; figure (9). In the current study, the IL-18 concentration of HCV positive thalassemic patients was technically a negative correlation with the concentration of serum HCV Abs, however, the relationship between these two variables is only weak (R = 0.0979), as the nearer the value is to zero, the weaker the relationship, figure (10).

Table 7: Alanine amino transferase concentrations in thalassemia patients (HCV positive and HCV negative) and control group

		Study groups		
		Thalassemia patients		Control (N=10)
		HCV positive (N=25)	HCV Negative (N=45)	
ALT (U//L)	Median	32.00	24.00	30.00
	Percentile 25	24.00	20.00	28.00
	Percentile 75	65.00	30.00	34.00
P value		<0.001**		
HCV positive vs HCV Negative		0.002*		
HCV positive vs control		0.337 NS		
HCV Negative vs Control		0.036*		

*NS: None statistical significance (p>0.05) *:statistical significance (p<0.05)**: High statistical significance (p<0.001)*

Table 8: Descriptive analysis of Alkaline phosphatase result in thalassemia patients (HCV or without HCV) and control group

		Study groups		
		Thalassemia patients		Control (N=10)
		HCV positive (N=25)	HCV Negative (N=45)	
ALP (U/L)	Median	84.00	121.00	85.00
	Percentile 25	14.00	114.00	80.00
	Percentile 75	214.00	129.00	100.00
P value		0.397NS		
HCV positive vs HCV Negative		0.138NS		
HCV positive vs control		0.706NS		
HCV Negative vs Control		0.026*		

*NS: None statistical significance (p>0.05) *: statistical significance (p<0.05)*

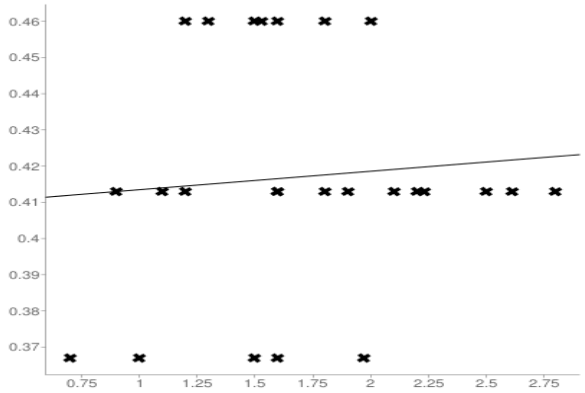

Figure 9: The correlation between HCV Abs concentrations (x) and IL-12 concentrations (y) in HCV positive thalassemic patients

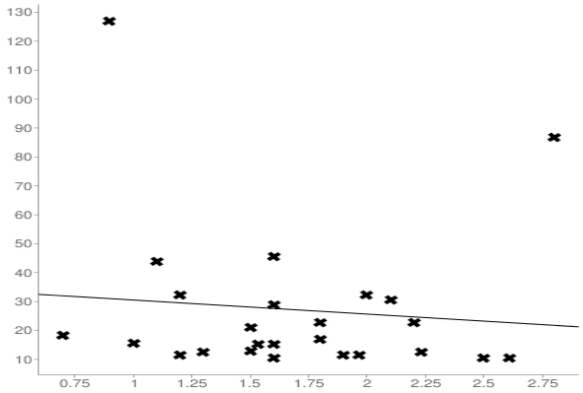

Figure 10: The correlation between HCV Abs concentrations (x) and IL-18 concentrations (y) in HCV positive thalassemic patients

Discussion

The β-thalassemia major patients are continuously subjected to blood transfusion, which make them at risk of blood borne infections like HIV, HBV, and HCV; so it is necessary to screen the transfused blood and the thalassemic patients for these viruses. In this current study, the prevalence of HCV Abs among β-thalassemia major patients was 17.9% which reflect the circulation of this virus in those patients. However, it is important to do quantitative PCR tests on HCV Ab positive cases to detect those who are in need for further management and treatment as the HCV Ab positivity is not necessarily reflect current HCV infection or the need for anti-HCV medications.

The current prevalence of HCV infection is less than that reported in west India [15] and Pakistan [16], but higher than that recorded in Egypt [as in 17]. When the HCV prevalence is compared to equivalent results done in Iraq, the current prevalence is much higher than that recorded in AL-Diwanya province which was 3.8% [18], but less than that mentioned in Duhok Province (35.3%) [19].

More than half of HCV positive thalassemic patients are in age group 21-30 yrs old (56%, n=14/25), when this age group is compared the younger age groups (11-20 and ≤ 10 yrs groups), it is clear that there is a decline in HCV infection in younger ages and the HCV prevalence is decrease as the age is less, two main possible causes behind these findings, first, the possibility of HCV infection is increased as the blood transfusion is more which is in need for longer duration and hence the older the patients the more possible HCV infection, the second reason is that recent improvement in HCV diagnosis and screening made the children less frequently exposed to HCV. However, the older age group (>40 yrs) was surprisingly has zero number of HCV infections which is in part due to the small number of β-thalassemia major patients in this age group (n=3/140) which is equal to 2%, this small number might be due to early death of β-thalassemia major patients due to complications of their disease which impose tissue iron deposition especially in heart [as in 20]

The gender distribution of HCV positivity among β-thalassemia major patients revealed non-significant female predominance, this higher possibility of female HCV infection might be due gender differences in thalassemia prevalence; these results are against results of Hapgood et al who found male predominance of HCV infections among thalassemia patients which might reflect high-risk behaviors of males than females which expose them to HCV virus more than females or due to more frequent need of blood transfusion which is due the gender differences in bone marrow suppression [21]. The results of Jafroodi M, *et al.* [22] were against ours; however, the gender differences were statistically significant which is against our results [22].

In this study, the absorbance of light by the solute was detected using ELISA reader which can measure the optical density of the solution in the wells of microplate reader. The higher the optical density, the higher is the absorbance of light, and as consequence the higher is the concentration of anti-HCV Abs in the sample. All the positive cases have optical densities above the cut off value given by the manufacturer. It is not surprising that the optical densities of HCV Abs positive β-thalassemia major patients is higher than those of HCV Abs negative β-thalassemia major patients and the differences is statistically significant. The use of ELISA technique in this research study was important tool to detect HCV Ab positivity as the rapid cassette method (immunochromatography method) has less sensitivity and specificity than ELISA technique.

IL-12 has an antiviral effect; it is mostly secreted by the cells of innate immunity and induces T helper differentiation to T helper 1 which in turn induces antibody production and humoral immune response [23]. Usually people infected with HCV have an increased level of IL-12, however, in this study; IL-12 concentration in HCV positive β-thalassemia major patients was lower than those with HCV negative, which is against the expected results, this might be due to abnormally lower immune defenses in thalassemic patients which lessen the IL-12 production and this can worsen the complications of HCV infection in thalassemic patients. Similar results were detected by Hashad, et al., 2013 [24].

IL-18 promotes T helper 1 activation and induces cellular immune response with profound inflammatory response especially in the presence of IL-12 [as in 25]. In the current study, the median concentration of IL-18 in sera of HCV positive thalassemic patients was significantly higher than the corresponding median IL-18 concentration in HCV negative thalassemic patients or control group, this result and the previous result of decrease IL-12 might reflect a positive feedback secretion of IL-18 which cannot achieve his function due to decline in IL-12 in HCV positive patient, so more increase in IL-18 concentration is required to activate the immune response in HCV positive patients. It is well documented that increase in IL-18 can cause early resolution from HCV infection [26]; however, this resolution is in need for other cytokines contribution like IL-12 which is decrease in HCV positive β-thalassemic patients. The increase in IL-18 concentration without resolution of HCV infection and cure from this infection might induce ineffective and harmful inflammatory response in liver parenchyma worsening the already present hepatitis.

The hepatocyte injury in HCV infection is associated with the release of AST in the blood causing increase in level of this enzyme; as in normal people infected with HCV, the β-thalassemia patients have an increase

in serum AST level. The liver injury and the subsequent increase in AST in HCV positive β thalassemia patients has two etiologies, first is the iron overload due to blood transfusion in these thalassemic patient, the second cause is the HCV infection, so it is expected that liver injury will be sever in these patient and high titers of AST will be recorded, however, as we did not perform real time PCR and we did not measure the viral load for HCV, we cannot correlate the AST with HCV viral load. The results of Salama KM, *et al*., [27] was in accordance to ours, the mean AST in HCV Ab positive β thalassemic patient was 87 U/L which was significantly higher non-infected thalassemic patients (Salama et al., 2015).The normal median serum AST in HCV negative patient might be due to controlled liver injury due to effective management; similar results were also reported previously by [27].

The median serum ALT levels for all the three study groups (HCV positive thalassemia, HCV negative thalassemia, and healthy control group) were within normal levels; these results are unexpected as ALT is usually elevated in HCV patients and in thalassemia patients. Many studies like those done by Al-Moshary M, *et al*. (Al-Moshary et al., 2020) [28] found results opposite to ours, they detected increase in ALT levels in HCV positive thalassemic patients. Normal median serum ALT level in patients groups of our study might reveal the presence of cofactors that can contribute to this result, these cofactors like adherence to medication, frequency of blood transfusion, and history of splenectomy might decrease the ALT levels but not the AST.

ALP can be elevated in thalassemia patients due to multiple causes starting from live injury and hepatitis and including but not limited to bone complications [29]. Thus it is not uncommon for thalassemia patients to have elevated ALP. Moreover, HCV infections alone can cause abnormally elevated ALP [as explained by 30]. However, and strangely, the medians of serum concentrations of ALP were within normal ranges in the three study groups. According to our results, it seems that ALP is not affected so much by HCV or thalassemia, but serum ALP should also be evaluated in patients with liver fibrosis due to HCV infection.

The anti-HCV Abs reflect humoral immune response activation due to HCV infection, this activation is mediated by cytokines secretion in which IL-12 is one of them. The non-significant very small positive relationship between the concentrations anti-HCV Abs and their corresponding IL-12 concentrations reflect the effect of IL-12 on the activation of T helper 1 cells and B lymphocytes to produce anti-HCV Abs, however, it is not clear if the low production of IL-12 was negatively affecting the production of Anti-HCV Abs which in turn affecting the resolution of HCV from the infected thalassemic patients. To our knowledge, this is the first study that reveals the correlation between IL-12 and anti-HCV Abs.

Although the concentrations of IL-18 were relatively high, this interleukin was unable to effectively activate the humoral immune response which is reflected by its weak negative correlation with the concentration of serum HCV Abs. this negative correlation might in part due to weak stimulation by IL-12 which has relatively low levels and the presence of simultaneously two chronic conditions, the β-thalassemia major and chronic HCV infections, both of them can contribute to defective immune response and hence defective anti-HCV Abs production. The internet-based search for similar correlations in another studies showed absence of such studies.

Conclusion

The prevalence of HCV infections is high among β-thalassemia major patients in Baghdad. ELISA technique is a perfect choice of detection anti-HCV Abs with very high sensitivity. Young adults of β-thalassemia major patients are at higher risk of HCV infection than other age groups. There is non-significant higher female exposure than males among β-thalassemia major patients. β - Thalassemia major patients infected with HCV has decreased IL-12 concentration while IL-18 concentration is increased the serum AST in HCV is usually significantly elevatedwhile ALT is frequently recorded within normal levels. Serum ALP is probably not a good predictor for disease progression in HCV positive thalassemic patients. There is non-significant very small positive relationship between the concentrations anti-HCV Abs and their corresponding serum IL-12 concentrations. IL-18 concentration of HCV positive thalassemic patients was having technically weak negative correlation with the concentration of serum HCV Abs.

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