The use of Interleukin -10 as a biomarker for diagnosis of viral hepatitis type C Infections and related liver function in beta-thalassemic major patients.

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Abstract

Introduction; IL-10 is an important cytokine in the pathogenesis of both infectious and inflammatory processes. The central and necessary role of IL- 10 in protecting against severe or inflammatory pathology has been clearly shown in models of experimental infection with intracellular pathogens using IL-10 genetically deficient mice. IL-10 is a multifunctional cytokine with potent immunoregulatory and anti-inflammatory properties. It prevents the release and function of a number of proinflammatory cytokines. It has been postulated that inadequate levels of IL-10 can determine long-term escape of pathogens from immune control and give rise to persistent infections. The **aim** of the study is to use of Interleukin -10 as a biomarker for diagnosis of viral hepatitis type C Infections and its relation to liver enzymes in thalassemia patients with viral hepatitis. **Patients and methods**: A cross sectional study was done on thalassemia patients in gastrointestinal center – Medical city directory in Baghdad from the beginning of March to the end of August 2017. A total of 80 subjects were participated in the present study, (45 thalassemia patients without hepatitis and 35 patients with Thalassemia who receive regular blood transfusion and diagnosed having viral hepatitis type C and were participated in the study. liver enzymes, and IL-10. Alanine transaminase (ALT), and aspartate transaminase (AST) were measured according to standard procedures. **Result;** there is significant increase in the concentration of IL-10 in the serum of HCV positive patients as compare with control patients. Also, there were significant increase in serum liver enzymes (ALT and AST and ALP), in patients with positive HCV, as compare to control with those with non-viral thalassemia hepatitis.

Key words; HCV, Liver enzymes, IL-10, thalassemia patients.

استعمال الإنترلوكين -10 كعلامة بايولوجية لتشخيص التهاب الكبد الفايروسي و ما يتعلق بها من نشاط انزيمات الكبد لدى مرضى الثلاسيميا نوع بيتا الكبرى المصابين بالتهاب الكبد الفيروسي.

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الخلاصة:

الانترلوكين -10 هو سيتوكين متعدد الوظائف منضم للمناعة قوي وله خصائص مضادة للالتهابات. ويمنع تحرر و الإفراج عن وظيفة عدد من السيتوكينات المضادة للالتهابات. وقد افترض أن مستويات غير كافية من إيل-10 يمكن أن تحدد الهروب على المدى الطويل من مسببات الأمراض من السيطرة المناعية وتؤدي إلى الإصابات المستمرة. **الهدف** من الدراسة هو تحديد تركيز الانترلوكين -10 وعلاقته بإنزيمات الكبد في مرضى الثلاسيمياالمصابين بالتهاب الكبد الفايروسي الوبائي. **المرضى وطرق العمل** : دراسة مقطعية اجريت لمرضى الثلاسيميا المحالين الى مركز الجهاز الهضمي و امراض الكبد- دائرة مدينة الطب في بغداد. بدات الدراسة من بداية مارس وحتى نهاية أغسطس 2017. و شارك في الدراسة 80 شخصا، (45 مريضا بالثلاسيما بدون التهاب الكبد و 35 مريضا مصابا بالثلاسيميا مع التهاب الكبد الفيوسي نوع سي. وقد تم قياس انزيمات الكبد و الانترلوكين -10 . **النتائج:** هناك زيادة كبيرة في تركيز الانترلوكين -10 في مصل مرضى التهاب الكبد الفايروسي نوع سي مقارنة مع مرضى السيطرة. كذلك هنالك زيادة معنوية لمستوى انزيمات الكبد في المرضى الذين يعانون مرضى التهاب الكبد الفايروسي نوع سي مقارنة مع السيطرة الذين لا يعانون من التهاب الكبد الفيروسية.

الكلمات المفتاحية: الانترلوكين -10 ،إنزيمات الكبد، مرضى الثلاسيميا

Introduction

In the United States, viral hepatitis is most commonly caused by hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV). These three viruses can all result in acute disease with symptoms of nausea, abdominal pain, fatigue, malaise, and jaundice. Furthermore, chronic hepatitis carriers remain infectious and may transmit the disease for many years. [1, 2].

HCV can be transmitted parenterally, perinatally, and sexually. Transmission occurs by percutaneous exposure to infected blood and plasma. The virus is transmitted most reliably through transfusion of infected blood or blood products, transplantation of organs from infected donors, and sharing of contaminated needles among IV drug users, [3].

Hepatitis is an infection of the liver caused by several viruses, the most common of which are Hepatitis A, B and C. Both Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) spread mainly through contaminated blood, blood products, sexual contact and contaminated needles, [1, 4].

Interleukin (IL)-10 is a member of the IL-10 cytokine family and one of the most crucial suppressors and regulators of the immune system.

IL-10 and other members of the IL-10–like family have been shown to confer hepatoprotection [2,3]. IL-10 family cytokines are categorized into three subgroups based primarily on their biological functions. The first group contains only IL-10 itself which mainly represses excessive inflammatory responses. The second group, namely IL-20 subfamily cytokines, is composed of IL-19, IL-20, IL-22, IL-24, and IL-26 [4,5]. IL-10, protects epithelial cells from invasion by extracellular pathogens such as

bacteria and yeast. Also, they enhance tissue remodeling and wound-healing activities, which help to maintain tissue integrity and restore homeostasis of epithelial layers during infection and inflammatory responses, [6].

Finally, the last group is the type III IFN group (or IFN *λ*s), which contains cytokines IL-28A, IL-28B, and IL-29 *(7)*. IL-10 is a multifunctional cytokine with potent immunoregulatory and anti-inflammatory properties. It prevents the release and function of a number of proinflammatory cytokines [8].

It has been postulated that inadequate levels of IL-10 can determine long-term escape of pathogens from immune control and give rise to persistent infections [9]. Therefore, the physiological role of IL-10 during infectious diseases is likely to reduce tissue damage resulting from the unfavorable and excessive effects of inflammation. However, an inappropriate production of IL-10 during a virulent infection may compromise the effectiveness of the immune system, allowing fulminant or persistent infection [3,4].

Hepatitis is an infection of the liver caused by several viruses. The most common were Hepatitis A, B and C. Both HBV and Hepatitis C Virus (HCV) spread mainly by contaminated blood, blood products, sexual contact and contaminated needles, [10].

Hepatitis C virus (HCV) is one of the most important etiologic agents of postransfusional hepatitis and a common cause of chronic hepatitis, cirrhosis and hepatocarcinoma , [10, 11].

Approximately, two billion people in the world have been infected by Hepatitis B virus (HBV), 350 million of whom are chronic carriers of the virus[1,2]. Worldwide HBV isolates have been classified into eight genotypes: A, B, C, D, E, F, G and H. The eight genotypes have

a characteristic geographical distribution, [12]. Several studies have revealed the association of HBV genotypes with the severity of chronic liver disease, but the results are not consistent, [12-13].

The **aim** of the study is to evaluate the relation between of some biochemical markers in viral hepatitis C patients namely AST, ALT, alkaline phosphatase.

Subjects and methods

A cross sectional study was done in gastrointestinal center – Medical city directory on Thalassemia patients in Baghdad from the beginning of March to the end of August 2017. One hundred and forty patients referred to gastroenterology center (GIT) in medical city in Baghdad for further investigation for detection of viral hepatitis and other liver diseases.

Only eighty patients with Beta Thalassemia patients were completed the whole investigation and participated in the study. 45 neo diagnosed and untreated thalassemia patients affected by HCV-related chronic hepatitis.

A total of 80 subjects were participated in the present study, (35 thalassemia patients without viral hepatitis and 45 thalassemia patients who receive regular blood transfusion and diagnosed having viral hepatitis were participated in the study.

An informed written consent was obtained from every subject before taking blood sample.

Excluded Criteria;

Patients with liver diseases other than HCV, or other medical diseases such as; hypertension, diabetes mellitus, endocrinal disorders or neurological diseases, autoimmune diseases, alcohol abuse, and drug induced

liver injury were excluded. In addition, patients with evidence of other chronic or acute infective processes (altered white blood cells count, temperature, urinary tract infection, airway infections) were excluded. Or patients receiving antiviral drugs were also, excluded from this study.

Diagnosis of patients was based on clinical (medical history, physical examination), instrumental (ultrasonography), and laboratory investigations (serum HCV antibodies, HCV-RNA and liver function tests) data.

Age and body weight was recorded. Also, about 5 ml of venous blood was drawn from all subjects. The blood lifted to clotted then centrifuged to separate the serum. The separated serum kept in deep freezing, until collection of all samples to be used in measurement of liver enzymes, and IL-10. Alanine transaminase (ALT= SGPT), and aspartate transaminase (AST= SGOT) were measured according to standard procedures, [14].

The circulating IL-10 levels were determined using a quantitative sandwich ELISA according to the manufacturer`s instructions (R &D systems, Germany). ELISA plate wells were coated with 100 µl/well of anti-human IL-10 capture antibody in coating buffer and incubated overnight at 4°C. After incubation, excess coating buffer was discarded and wells blocked with 100 µl/well of blocking buffer (1% BSA in PBS) followed by incubation at 37°C for 1 h. Plates were washed 3 times in washing buffer (0.05% tween-20 in PBS), 100 µl of plasma samples and IL-10 standards diluted in buffer were added to appropriate wells, and

incubation at 37°C for 2 h followed. Plates were washed as before and detector biotinylated antibody (100 µl) added to each well followed by incubation at 37°C for 1 h, then washed as before. Streptavidin-HRP (Horse-Radish Peroxidase) conjugate (100 µl) was added to each well followed by incubation of plates at 37°C for 1 h, and washing as before. Substrate solution, 100 µl was added to each well and plates incubated at room temperature for 15 minutes for color development. Stop solution (50 µl) was added to each well and plates were read at 450 nm, [15].

Detection of viral markers:-

1-ELISA for detection of hepatitis B surface antigen (HBsAg), also ELISA was used for detection of antibodies for HCV (screening test, [16,17].

SPSS was used for **statistical** analysis was used. All data were presented as Mean and Slandered deviation (SD). Unpaired student T test was used to compare between means of variables. P value less than 0.05 or 0.01 was used as significant value.

Results

Thalassemia Patients distributed into two groups;

Group 1 consist of 45 thalassemia patients without liver hepatitis as control group, and 35 thalassemia patients diagnosed as having viral hepatitis type c (patients with positive HCV).

Table 1 The age and body weight of all patients'

Parameters	**Control (45)**	**HCV patients (35 patients)**
Age (years)	30.14 ± 9.2	22.3 ± 6.5
Body weight (KG)	7.8 ± 54.5	46.9 ± 6.6
IL-10 (pg./ml)	8.74 ± 2.5	33.62 ± 7.3

In **table 1** , there is significant increase in the concentration of IL-10 in HCV patients (33.62 ± 7.3) as compare with control patients (8.74 ± 2.5).

In addition, by comparing the mean level of liver enzymes (ALT and AST), ALP, in patients with +ve Anti-HCV as compare to control with those with non-viral hepatitis. (**Table 2)**.

Table 2 showed a significant increase in AST enzyme activity (GOT) in the thalassemia patients suffering viral hepatitis (HCV=47.3 ± 6.54 UI/L) as compare with thalassemia patients without viral hepatitis, (23.6 ± 3.4 UI/L).

Moreover, there is significant increase in ALT enzyme activity (GPT) in the thalassemia patients suffering viral hepatitis (HCV= 70.56 ± 8.2 UI/L) as compare with thalassemia patients without viral hepatitis, (24.6 ± 2.15 UI/L).

Also, there is significant increase in ALP activity in the thalassemia patients suffering viral hepatitis (HCV= 224.6 ± 61.1 UI/L) as compare with thalassemia patients without viral hepatitis, (54.43 ± 4.4 UI/L).

Furthermore, there is significant increase in total bilirubin concentration in the thalassemia patients suffering viral hepatitis (HCV=2.68 ± 0.31mg/dl) as compare with thalassemia patients without viral hepatitis, (0.51 ± 0.09 mg/dl).

Table 2 Show the concentration of GOT, GPT, ALP and total bilirubin in thalassemia patients

Parameters	**Control (45)**	**HCV patients (35 patients)**	**P value**
AST (SGOT) (Ul/l)	23.6 ± 3.4	47.3 ± 6.54	0.05
ALT (SGPT) (Ul/l)	24.6 ± 2.15	70.56 ± 8.2	0.01
ALP (Ul/l)	54.43 ± 4.4	224.6 ± 61.1	0.01
Total bilirubin (mg/dl)	0.51 ± 0.09	2.68 ± 0.31	0.05

Forty percent (40% of thalassemia patients suffered from jaundice and 43 % had thalassemia features. All patients were on regular blood transfusion with a mean frequency of 12.94 ± 4.31 times per year.

There was a significant decrease in the frequency of blood transfusion per year in patients who underwent splenectomy, compared to the pre-splenectomy period ($P \leq 0.05$).

Discussion

In the present study, there was a significant increase in the concentration of IL-10 in the serum of HCV patients as compare with control patients.

Previous human studies have investigated this balance using neutralizing antibodies against IL-10 in vitro. HCV-specific T cell responses in chronically infected patients were restored by blocking IL-10, resulting in increased IFN-γ production, [18, 19].

Previous study was found in chronically HCV infected patients significant increase in IL-10 and IL-4, and they suggested that Th2 response during chronic HCV infection and this finding is concordant with the present study, [19, 20].

The significant increase in the concentration of IL-10 in the serum of viral hepatitis patients of the present study (HCV patients), may be these patients have a chronic state of liver damage by the virus. This result agree with previous study which done HCV patients who found there were significant increase I the concentration of IL-10 and IL-18 in the serum of chronic hepatitis C patients, [21].

In study done in Baghdad by Mahmood (2005), who found a significant increase in the mean of levels of serum IL-6, IL-10, IL-8, and TNF-α and in contrast, a significant reduction in the IFN-γ levels in hepatitis patients with maintenance haemodialysis therapy in comparison to healthy control group, [22].

These results indicates the predominance of Th2 cytokine which promote the persistency of virus, also HCV core and NS3 induced production of the anti-

inflammatory cytokines, IL-10, [23, 24].

IL-10 by itself and through cooperation with Th1 cytokines (such as IL-12) also regulates Th2 responses to prevent the overproduction of IL-4, IL-5 and IL-13, cytokines that can lead to severe fibrosis in, for example hepatitis C virus, [25, 26].

In the present study, In Thalassemia patients, there were an increase in serum concentrations of Alanine transaminase (ALT) and Aspartate transaminase (AST), and ALP in HCV positive patients as compare with control patients.

Hepatitis virus C infection is the main risk factor for liver injury in transfusion-dependent thalassemics, [27]. Interrelationship between iron overload, HCV infection and liver injury is still controversial. Multicenter cross-sectional studies have reported that the development and the severity of liver injury are strongly related to the extent of liver iron overload and to the presence of chronic HCV infection [28].

Dimitrios *et al*, (2013), on the other hand suggested that in the late stages of liver disease in BTM patients, iron overload may be the critical determinant, since fibrosis is related to the minimal haemosiderosis, independently of HCV history, [29].

In present study, anti-HCV positive patients had higher mean serum ALT, AST and ALP levels than the negative group, and the same finding was true for total bilirubin in HCV positive patients.

A study done by Ocak *et al*., (2006), found that the HCV positive patients had a significantly higher increase in

the activity of serum ALT level than anti-HCV-negative patients, Ameli *et al*., (2008), found that serum iron was significantly higher in anti-HCV positive patients compared to the negative group, [31].

A previous pilot studies had suggested that IL-10 was well tolerated, normalized serum alanine aminotransferase (ALT), decreased hepatic inflammation and reduced liver fibrosis in patients with chronic hepatitis C who had not responded to previous IFN-based therapy. These data suggested that IL- 10 has an important role in chronic inflammation and fibrogenesis in HCV and that IL-10 might be used in the treatment of chronic hepatitis C and other chronic liver diseases, [26, 32].

So in the present study, data suggest that IL-10 can be used as a non-invasive marker for detection of the chronicity and severity of liver inflammation in chronic hepatitis C. [30].

Conclusions

1-Threre is significant increase in the concentration of IL-10 in the serum of patients of HCV.

2- There is significant increase in liver enzymes activities in patients with viral hepatitis.

3- From the present result, it can be concluded that there is a relation between level of IL-10 and liver function tests and it could be used as a biomarker for the infectivity with HCV .

Recommendations.

1. Measurement of the concentrations of Il-6, IL-12 and TNF In the sera of the positive HBV and HCV patients.

2. Study the effect of splenectomy on outcome on the concentration of interleukin in viral hepatitis.

3.Extend the study on all types of viral hepatitis.

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