Study of the serum concentrations of TNF-α and Interleukin-2 in chronically patients with Hepatitis B and C

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Abstract

Viral hepatitis is an important endemic disease in Iraq , this work was curried in the period between January and June 2007 . And was aimed to evaluate the serum levels of cytokines in the chronic patients. Obtained results showed that their is a clear elevation in the levels TNF- in HBsAg positive group (211.27±28 pg/ ml), and(189.77±23.5 pg/ ml) in patients with HCV in comparison to control group (59.45±22 pg/ ml). also levels of IL-2 recorded a remarkable increment , In HBsAg positive group (101.47± 29 pg/ml), and in HCV patients (88.78±19.5 pg/ml) in comparison to control group (62.22±14 pg/ ml) **.** These results indicate the predominant role of Th-2 than Th-1 which lead to chronicity of the disease in those patients.

دراسة تراكيز الحركيات المناعية IL-2 و TNF-α نمط المزمن الفيروسي بالتهاب الكبد المصابين المرضى في B و C

الخلاصة

إن التهاب الكبد الفيروسي احد الأمراض المهمة المتوطنة في العراق. اجري هذا البحث في الفترة بين كانون الثاني وتموز من عام **2007**، وكان هدف البحث تقييم مستويات الحركيات المناعية في أمصال المصابين بالشكل المزمن من المرض. بينت النتائج التي تم الحصول عليها وجود زيادة معنوية واضحة في تركيز TNF-α ، حيث كانت قيمته في المرضى وعلى التوالي C و B في الأشخاص الذين أعطوا فحصاً موجباً للنمطين بكغم/مل). وقد سجلت الدراسة 22±59.45(بكغم/مل) مقارنة بمجموعة السيطرة الأصحاء 23.5±189.77 بكغم/مل) و (28±211.27(، حيث كانت القيم وعلى التوالي C و B في الأشخاص الذين أعطوا فحصاً موجباً للنمطين IL-2أيضا وجود زيادة معنوية في قيم تراكيز بكغم/مل). هذه النتائج 14±62.22التي سجلت (بكغم/مل) مقارنة بمجموعة السيطرة الأصحاء 19.5±88.78 بكغم/مل) و (29 ±101.47(تشير إلى وجود تفوق واضح لدور الخلايا اللمفاوية المساعدة نوع 2 على النوع 1 الأمر الذي يفسر وجود الحالة المزمنة من المرض.

Introduction

In HBV infection, initially the virus replicates in hepatocytes with few or no cytopathic effects, correlating with the lack of the liver damage. The HBV genome is integrated into the host DNA, and the infection may remain latent , at later stages , accumulation of the filamentous HBV surface antigen HBsAg gives rise to cell-mediated immunity and inflammation, rather than cytopathic effects caused by viral replication (1).

Once the hepatic damage observed during acute or chronic hepatitis appears to be caused mainly by the intrahepatic inflammatory process evoked by the immune response to

HBV, much effort has been made to determine the cytokines profile as well as the cellular sources of the cytokines produced. It was reported that HBV infection is associated with the production of a broad range of proinflammatory cytokines and chemokines such as IL-1β, IL-6, IL-8, IL-12, TNF-α, and IFN-γ (2,3). As well as the anti-inflammatory cytokine IL-10 (4).

In HCV infection, the disease sings and liver injury occur as a result of both cytopathic effects of the viral particles and replication and the immune response to the process of viral multiplication and viral proteins in the liver. As a result of the infection and liver injury, there is a clear changes in the concentrations of some cytokines, mainly TNF-α, IL-1α, and IL-6, IFN-γ, IL-8 (5,6).

In both types of hepatitis, the levels of these cytokines and other chemokines depending on the clinical stage of the disease and many other factors including age and the heath state (7,8).

Previous studies demonstrated various serum Th1/Th2 cytokine profiles in chronic HCV infection (9,10,11, 12, 8, 13) .

It has been suggested that the differentiation of activated T cells toward Th1 cells results in an improvement of hepatitis (1)

Although serum levels of Th1 cytokines, including IFN-γ and IL-2 have been reported to be elevated in HCV infections (9), some others have shown low levels of IFN-γ in patients with HCV infection (14) .

Napoli *et.al.,* (1995) found that IFN –γ and IL02 mRNA were increased in the livers of patients with chronic hepatitis C, suggesting that these cytokines are locally produced by hepatic CD+4 cells and Th1 cytokines mediating the hepatocellular damage(15) .

Osna *et.al.,* (1997) measured serum IFN-gamma, IL-4, IL-10 and IL-12 levels and they found lower IFN- γ and higher IL-10 levels in chronic HCV patients than in healthy controls . They also reported an increase in the serum levels of IFN –γ when IL-10 production had been inhibited and suggested that IL-10 served as a downregulative factor for IFN-γ in chronic HCV infection (16) .

Other studies reports, both Th1 and Th2 cytokines involved in the pathogenesis of HCV and recorded a conflicted results (17).

Mahmood (2005) demonstrated that patients with permanent haemo-dialysis who were seropositve for HCV-antibody showed a significant elevation in serum concentration of (IL-6, IL-10, IL-8, GM-CSF, and TNF-α), in contrast there were decrement in serum value of IFN- γ in comparison to healthy control group (18).

Alkozai (2006) found that levels of IL-2 , IL-10 and TNF-α were increased in patients of hepatitis B and C, while IFN-γ reduced, suggesting activity of Th-2 than Th-1 cells (19).

Materials and Methods

1. Detection of Viral Markers :

1. ELISA for Detection of Hepatitis B surface antigen (HBsAg) :

This is a direct immunoenzymatic method of the sandwich type (20,21, 22) .

2. ELISA for Detection of antibodies to HCV (Screening Test) :

This is an immunoenzymatic method (23)

3. Determination of Cytokines :

Serum levels of IL-2 and TNF-α ,were measured by means of enzyme linked immunosorbent assay using ELISA kits, Measurement done as recommended by the manufacture(Mabtech AB, Sweden).

I. Principle :

Cytokines were measured by using a solid phase sandwich ELISA.

A monoclonal antibody specific for cytokine had been coated onto wells of microtiter plate. The test samples and standards of known human cytokines content are applied, followed by the addition of biotinylated 2nd Ab.

During the 1st incubation, cytokine Ag bound simultaneously to the immobilized (captured) Ab on one site, and to the solution phase biotinylated Ab on a 2nd site. After removal of excess 2nd Ab, streptavidin peroxidase (enzyme) was added. That bound to the biotinylated Ab to complete the four-member sandwich. After a 2nd incubation and washing to remove all the unbound enzyme, a substrate solution was added, which was acting on the bound enzyme to produce colour.The intensity of that colored product was directly proportional to the concentration of h-cytokine present in the original specimen .

Results

In the sera of the positive HBV, the concentrations of TNF-α were significantly elevated ($p<0.01$) the levels were (mean ± SD) 211.27 ± 28 pg./ml in comparison to the control group 59.45±22 (fig.1).

As well as in anti-HCV positive sera the levels of TNF-α were statistically significant elevated ($p<0.01$). The Levels were 189.77±23.5 while in control group 59.45±22 as showed in (fig.1).

In the sera of the positive HBV, the concentrations of IL-2 were significantly reduced ($p<0.01$) the levels were (mean ± SD) 101.47 ± 29 pg./ml in comparison to the control group 126.22 ±14 (fig.2).

As well as in anti-HCV positive sera the levels of TNF-α were statistically significant elevated ($p<0.01$). The Levels were 88.78±19.5 while in control group 126.22 ±14 as showed in (fig.2).

Discussion

Broad range of proinflammatory cytokines and chemokines which required for viral clearance, are produced in response to HBV infection, these are IL-1β, IL-6, IL-8 , IL-12 , TNF-α and IFN-γ (24,25,2, 26, 27, 3, 28,19).

Consistent with observation in human hepatitis, the role of Th1 and Th2 cells responses in the pathogenesis of hepatitis was documented in several experimental liver injury models, it was proposed an important role for IFN-γ-producing antigen specific CD4+ Th1 cells in the pathogenesis of hepatitis and Th2 in chronic infections (29,4).

The results obtained by this work comes in conformity with the results of many previous investigators, which indicating the predominance role of Th-2 on Th1 which indicate the persistency of virus infection.

Wang *et.al.*,(1999) reported a significant increase in the serum concentrations of group of cytokines included TNF- α , IFN-γ, IL-6, and IL-8, in patients with chronic hepatitis B, concluded that the increased cytokines were related to the inflammation of liver cells and multiple factors may play a certain roles in the liver damage(5).

Abayli *et al.*,(2003) studied the serum profile of Th1 and Th2 cytokines in chronically HCV infected patients and found that the Th2 cytokines were increased remarkably include IL-10, IL-4, while the Th1 cytokine IFN-γ revealed no differences and they suggested that Th2 response during chronic HCV infection and this finding is concordant with other recent studies (17).

Recent studies have demonstrated conflicted results on the level of Th1/Th2 cytokines in HCV infection (30 9, 8).

Although serum levels of Th1 cytokines, including IFN-γ, IL-2 have been reported to be elevated in HCV infections (9), some others have shown low levels of IFNγ in patients with HCV infections (14; 19).

Mahmood (2005), documented that the mean serum levels of IL-6,IL-10, IL-8, TNF-α and GM-CSF (granulocyte-moncyte colony stimulating factor) have been showed a significant increase in contrast the IFN-γ levels have been reduced significantly in hepatitis patients with maintenance haemodialysis therapy in comparison to healthy control group in Baghdad, 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. Both TNF-α and IL-10 levels were higher in core and NS3 stimulation in HCV-infected patients than normal (18).

TNF-α system also implicated in the pathogenesis of viral hepatitis, in HCV infections the viral particles induces the expression of the TNF- α in human liver and HCV specific CTL have been showed to secret TNF- α in vitro (2).

Plasma TNF-α level is increased in the patients with chronic HCV infections, However elevated plasma as well as soluble TNF receptor levels were mainly obseved with acute hepatitis and correlate with serum transaminase levels .(31, 19, 32).

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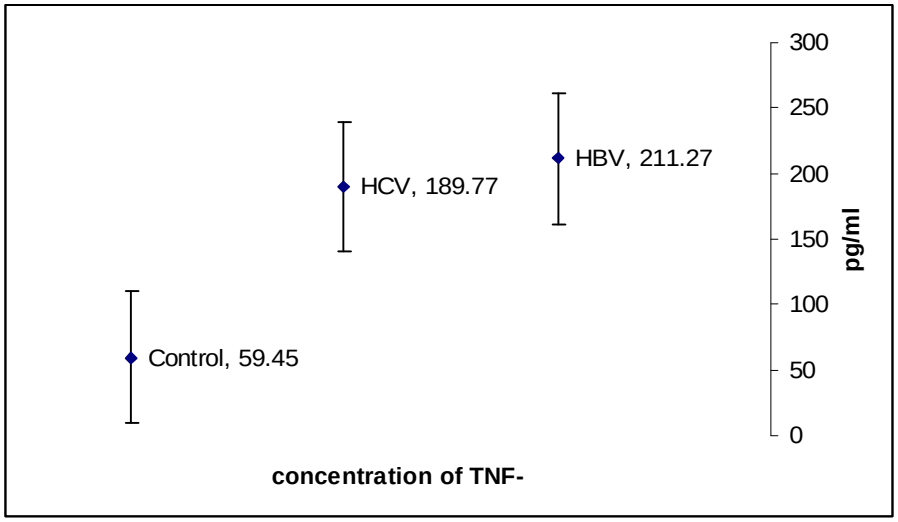

Fig.1 : Serum levels of TNF-α in patients of HCV and HBV.

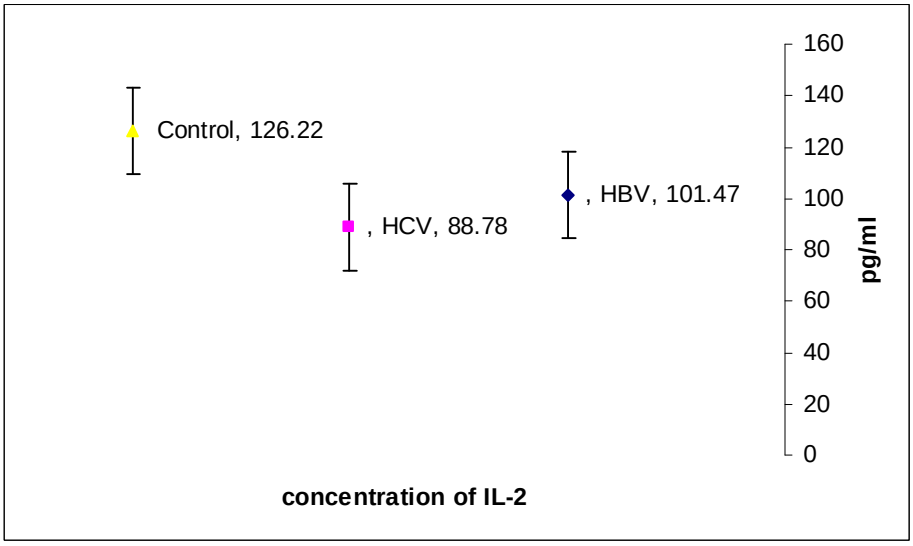

Fig (2) : Serum levels of IL-2 in patients of HCV and HBV.