Role of Interferon –Gamma and Interleukin -4 in Immunepathogenesis of Chronic Hepatitis C Virus

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Abstract

This study was carried out to investigate some immunological aspects in Iraqi patients with hepatitis C virus infection compared to patients and healthy control groups.The study included (50) patients with chronic hepatitis C infection referred to ((The gastroenterology and hepatology center) (Medical City- Baghdad)), with age range from (8-75) years , (20) patients with non viral chronic liver diseases and (20) healthy control groups. classified the HCV patients into five stages according to their fibrosis stage: five patients in stage 0, (14) patients in stage 1, (12) patients in stage 2, (12) patients in stage 3, (7) patients in stage 4. With application of ELISA technique, serum levels of Interleukin -4(1L-4), and Interferon –gamma (IFN-γ) were measured in (50) patients with HCV infection compared to that of control groups which include (20) patients with non viral liver pathologies (patients control) and (20) healthy control groups. The mean serum level of 1L-4 and IFN-γ showed a significant increase in patient with HCV as compared with patients control and healthy control groups. In addition, significant linear correlation between IFN-γ with fibrosis stages, while inverse correlation between 1L-4 and fibrosis stages in HCV patients.

الخلاصة

تم القيام بهذه الدراسة من اجل الكشف عن بعض المظاهر المناعية لدى مرضى عراقيين مصابين بالتهاب الكبد الفايروسي المزمن نمط ج بمقارنتها مع أفراد المجاميع الضابطة.

شملت الدراسة (50) مريض مصاباً بالتهاب الكبد الفايروسي المزمن نمط ج بالاضافة إلى (20) مريضاً مصابين بامراض التهاب الكبد الغير فايروسي و (20) شخصاً سليماً انضموا إلى الدراسة لغرض المقارنة كمجاميع ضابطة.

قسم مرضى التهاب الكبد الفايروسي نوع ج إلى خمسة مراحل من تليف الكبد: (5) مرضى في مرحلة الصفر و(14) مريضاً في المرحلة الأولى و (12) مريضاً في المرحلة الثانية و في المرحلة الثالثة (12) مريضاً أيضا واخيراً (7) مرضى في المرحلة الرابعة.

وباستخدام تقنية اختبار الروز المناعي المرتبط (ELISA) تم تحديد المستويات المصلية لكل انترلوكين 4، ،و انترفيرون كاما لدى لـ (50) مريضاً بـ التهاب الكبد الفايروسي المزمن نوع ج مقارنةً بالمجموعة الضابطة والتي شملت (20) مريضاً مصاباً بالتهاب الكبد غير الفايروسي و (20) شخصاً سليماً.

سجلت المستويات المصلية للانترفيرون كاما ارتفاعاً معنوياً لدى المرضى بالتهاب الكبد الفايروسي نوع ج مقارنةً بالمجموعة الضابطة (التهاب الكبد غير الفايروسي والاشخاص السليمين). في حين سجلت مستويات الانترلوكين 4 ارتفاعاً معنوياً في مرضى التهاب الكبد الفايروسي نوع ج ومرض التهاب الكبد الغير فايروسي مقارنة بالاشخاص السليمين.

بالاضافة الى ذلك لوحظت علاقة خطية موجبة بين مستوى المصلي للانترفيرون كاما من جهة ومراحل التليف من جهة اخرى. بينما توجد علاقة عكسية بين مستوى انترلوكين 4 من جهة ومراحل التليف من جهة اخرى لمرض التهاب الكبد الفايروسي نوع ج.

Introduction

Hepatitis C virus (HCV) has been shown to be an etiologic agent responsible for chronic liver disease, with eventual progression to cirrhosis and hepatocellular carcinoma while the immunologic mechanisms in chronic HCV infection have not been clearly defined, it is believed that cytokines are

involved [1]. In addition, the function of T-helper type 1 (Th1) and Th2 cytokines are wide ranging and include regulatory signals for activation, growth and differentiation of cytotoxic lymphocytes, macrophages, natural killer cells and granulocytes . Previous studies have examined the role of cytokines, including tumor necrosis factor, IL – 1, IL2, IFN- α and IFN – γ, in other viral infections such as hepatitis B and HIV. However, there have been little studies that specifically analyzed the levels of immunoregulatory cytokines in chronic HCV infection [2].

Aim of the study

The present study analyzed serum levels of IL-4, IFN-γ as indicators of the immune response in HCV patients and it specifically measure Th1 and Th2 cytokines in sera of patients infected with HCV and in control groups to better understand the antiviral immune response, or lack thereof, in these patients.

Patients and methods

Patients:

Hepatitis C Virus (HCV) Group:

A total of 50 patients (23 male and 27 female), with a mean age (38) years, age range (8-75) years with HCV infection referred to (The gastroenterology and hepatology teaching hospital- Medical City-Baghdad) were included in this study . Initial diagnosis was by a positive anti HCV Antibody in serum by using third generation enzyme linked immunosorbant assay (ELISA-3) test , a positive recombinant immunoblot assay (confirmetary test), and histological evidence of chronic hepatitis on liver biopsy which was performed before initiation of treatment. None of the patients studied had concurrent infection with the hepatitis A, B, or D viruses, or the human immunodeficiency virus (HIV) these were proved by negative serological test . Pregnant patients and those with alcohol consumption were also excluded from study.

Patient Control Group:

To compare the immunological parameter results obtained for HCV patients , 20 patients (9 male and 11 female with a non – viral pathologies were included as a patient control group , they were among the patients attending the above centre and they were seven patients with alcoholic liver disease (ALD), six with primary biliary cirrhosis (PBC), three liver abscess and four with autoimmune hepatitis (AIH).

Healthy Control Group:

A total of twenty volunteers who where age matching to the patient groups , were selected as a healthy control group .

Methods

Laboratory Investigations:

The experimental part involved in the present study includes:

1. Detection of antibodies to HCV by third generation ELISA (ELISA-3) screening test .
* Patients positive for the screening test were further examined for confirmatory immunobolt – test for determination of anti HCV antibodies.
2. Determination of serum IL-4 level by enzyme immunoassay method.
3. Determination of serum IFN-γ level by enzyme immunoassay method.

Results & Discussion

Study Groups:

As shown in table (1), a total of fifty patients with HCV infection (23 males and 27 females), their age ranged from 8-75 years with a mean age (37.5±18.4) years were included in the study.

In addition, a forty subjects were included in study as control groups as follows:

1. Twenty patients with non-viral liver pathologies which include (9 males and 11 females), their age range 19-71 years

with a mean age (43.3±16.1) years were included as a patients control group.

2. Twenty healthy subjects who were age matched to the patients group were also included as a healthy control group

Table 1. Age and gender distribution of the studied groups

groups / parameters		Healthy controls (N =20)	Patients control (N =20)	HCV patients (N =50)
		No. (%)	No. (%)	No. (%)
Age (years) X ± SD Range		31 ± 17.7 (9-67)	43.3 ± 16.1 (19-71)	37.5 ± 18.4 (8-75)
Gender	Male	10 (50%)	9 (45%)	23 (46%)
	Female	10 (50%)	11 (55%)	27 (54%)
	Total	20 (100%)	20 (100%)	50(100%)

Histological characteristics in HCV patients:

Hepatitis C can cause a spectrum of liver pathology ranging from mild non-specific to changes of end stage liver disease including Cirrhosis and hepatocellular carcinoma. At present, the best tool for diagnosis and prognosis of chronic viral hepatitis is based on histological examination of liver biopsy. However, there are no pathognomonic features of hepatitis C, but the biopsy may be helpful in ruling out addition pathologic conditions [3]. In addition, there is consensus that one parameter need to be measured in liver biopsies (:Fibrosis Stages): Fibrosis assessment also has prognostic significance, because it allow to differentiate the patients to those with early disease or slow progressor and those with advanced disease and high risk of developing cirrhosis and even hepatocellular carcinoma [3].Therefore, as demonstrated in Figure (1), the present study classified HCV patients according to their fibrosis score into 5 groups: 0 to stage 4 these are 5 (10%): 14 (28%); 12 (24%); 12 (24%); and 7 (14%), respectively.

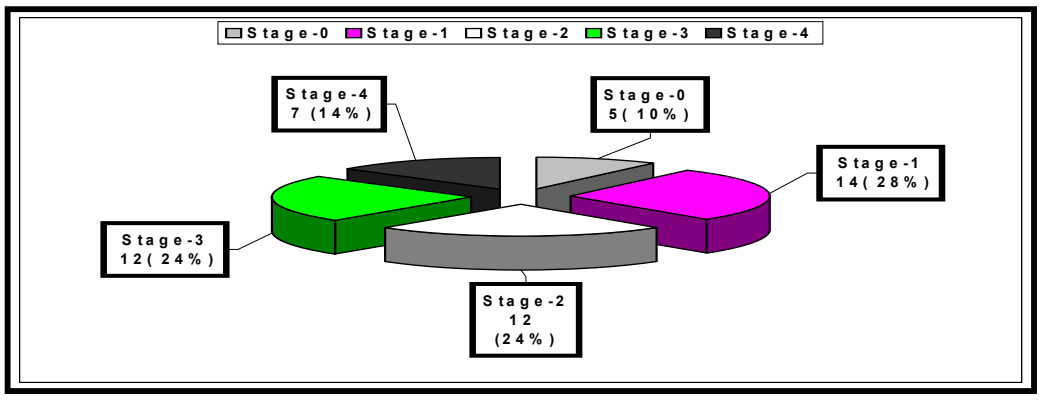

Fig 1. Frequency distribution of HCV patients by fibrosis stages of liver biopsies

Of interest, most cases are diagnosed in relatively early stages, which may indicate advanced health education of Iraqi patients and on the other hand, the presence of high

skilled physicians and laboratories that capable to diagnose this disease early in this country.

Estimation of serum levels of Interleukin - 4:

Table (2) and figure (2) revealed high levels of IL-4 in sera of HCV patients (114 ± 81pg/ml) and patients control (115 ± 99pg/ml) as compared to healthy control (8.7 ± 3.9 Pg/ml) with P<0.001.

Table 2. The difference in mean serum interleukin 4 level (pg/ml) between the three studied groups

Study groups / values	Healthy control (N =20)	Patients control (N =20)	HCV patients (N =50)
Minimum	4	4	4
Maximum	17	261	285
Mean	8.71	115	114
***SD**	3.91	99	81
****SE**	0.88	22	11.5

* Standard deviation
** Standard error
P value (ANOVA) = 0.001

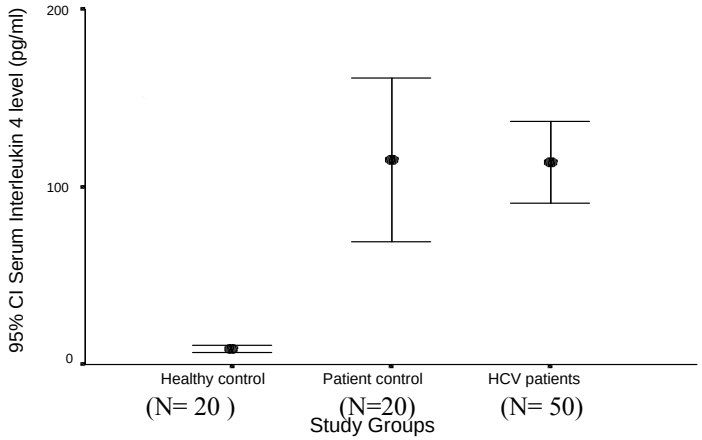

Fig 2. Error bar chart showing the mean with its 95% confedance interval of serum Interleukin 4 level (pg/ml) in the three study groups

These results were in accordance with the previous results reported by Cacciarelli *et al.* [4] and Fan *et al.* [5] who found that HCV patients display elevated level of circulating IL-4 when compared with healthy donors. In both studies, the increase of Th2 type cytokines such as IL-4 and IL-10 is greater than that seen for the Th1 counterpart, implying the involvement of Th2 lymphocytes in immunopathogenesis of HCV, possibly through the inhibition of Th1-mediated antiviral response. Reiser *et al.*, [6], also reported elevated IL-4 levels in sera of patients with HCV and found Th2 cell markers in inflammatory infiltrates within the liver, although Th2 cells represented a majority of the infiltrating mononuclear cells In addition to that, there was evidence suggesting that circulating IL-4 levels are also elevated in other types of human liver diseases such as autoimmune hepatitis (AIH), liver abscess and alcohol liver disease(ALD) [7, 8], this suggestion could explain the finding

study of high serum IL-4 levels in patients control group were detected.

On the other hand, the results obtained from this present study are in contrast to that reported by Woites *et al*. [9], who found an elevated IL-4 serum level only in HIV – coinfected hepatitis C patients while no change in its level in either HCV or HIV infected patients alone. As well as in disagreement with Sarih *et al*., [10] who reported that IL-4 production by phytohemagglutinin (PHA)- stimulated peripheral blood mononuclear cell (PBMC) was not detected in both HCV patients and controls.

Moreover, the present study assess the association of IL – 4 serum level of HCV patients to the degree of liver histopathologic lesions and hence disease progression according to so called – fibrosis stage, there is inverse correlation between serum level of IL – 4 and fibrosis progression in HCV patients ($P<0.001$, $r = -0.667$) as shown in Figure (3).

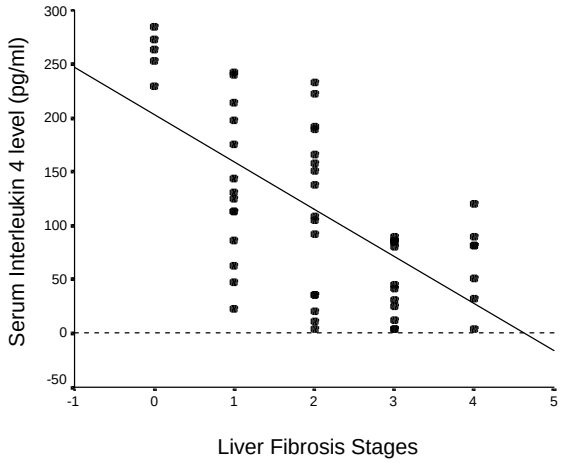

Fig 3. Correlation of interleukin 4 serum level and liver fibrosis stages in HCV patients.

These findings were in consistent with the previously published data. For example, Napoli *et al*., [11] looked at the role of cytokines in the mechanism of liver injury in chronic HCV patient. They found that patients with sever chronic hepatitis C or cirrhosis had a very low expression of IL – 4 mRNA, in contrast, controls and patients with milder inflammation had higher expression of IL- 4.

In line with these findings, Bertoletti *et al.* [12], who studied the intrahepatic $CD4^+$ response in sever HCV infected patients demonstrated that the majority of liver – infiltrating T cells were Th1 cells unable to secrete IL – 4, a cytokine that is commonly secreted by Th2 cells .

The present and previously reported results could be further supported by the findings of Nelson *et al*. [13] who reported that therapy with Th2 like cytokines such as IL-4 and IL-10, although have no apparent antiviral activity , they improve liver histology and reduce liver fibrosis in a large proportion of patients receiving treatment .As well as, they hypothesized that *in vivo* administration of these cytokine may tilt the balance away from Th1-like cytokines dominance , there by reducing the hepatocellular injury that characterizes HCV infection. Human IL-4

has both anti- inflammatory and immunosuppressive properties , of particular importance is the capacity of IL-4 to down regulate production of proinflammatory cytokines, such as TNF-α , IL-1 and INF-γ from T cells [14]. In fact , endogenous IL-4 reduces the intrahepatic inflammatory response and limits hepatotoxicity in several models of liver injury that characterizes HCV infection [15].

On the other hand, this study results are in disagreement with that of Tsai *et al* .[16]. Who found that activation of Th2 responses , and hence elevation of circulatory IL-4 and IL-10, in acute hepatitis C patients may play a role in the development of chronicity .They made their suggestion depending of their findings of elevated levels of Th2 cytokines in chronic HCV patients in comparison to patients with acute HCV infection .However, they give no explanation for their results and does not reported whether those patients with acute infection have been reached complete recovery thereafter .

Estimation of serum level of Interferon – gamma (IFN- γ):

As reported in table (3), figure (4), patients with HCV infection showed high levels of IFN-γ (63.5±60pg/ml) as compared to patients with non-viral liver pathologies (52.6 ±40pg/ml) and healthy controls (8.2 ± 4) as control groups (P<0.001).

These findings were coincide with the results of Tilg *et al*., Cacciarelli *et al*., and Hayakawa *et al*., [17,4,18] who found a significant elevation of IFN-γ levels in HCV patient compared to healthy controls.

Table 3. The difference in mean serum interferon – γ level (pg/ml) between the three studied groups

	Study groups		
Values	Healthy control (N =20)	Patients control (N =20)	HCV patients (N =50)
Minimum	4	4	4
Maximum	16	130	195
Mean	8.2	52.6	63.5
SD	4	40	60
SE	0.9	9	8.6

P value (ANOVA) =0.008

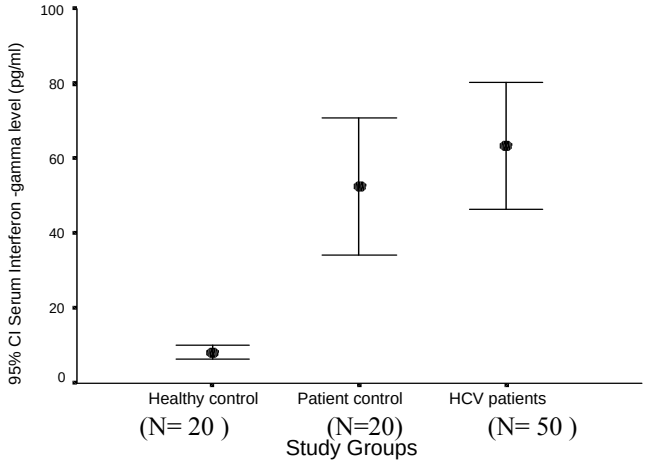

Fig 4. Error Bar chart showing the mean with its 95% confedance interval of serum interferon - γ level (pg/ml) in the three study groups.

As well as, this study results can be supported by *in vitro* studies which showed an increased production of IFN-γ and its mRNA by intrahepatic and peripheral blood $CD4^+$ T-cells in chronic hepatitis C virus infection [12, 19].

On the other hand, this study disagree with Pirovino *et al*. [20] and Woitas *et al*., [9] who reported no significant differences in serum levels of IFN-γ between HCV patients and healthy controls.

Interferon-γ is one of the important cytokines that known to play a role in host defense against viral infections[21]. While IFN-γ has the potential to trigger the activation of intracellular antiviral pathways
after its binding to specific receptors on the surface of the infected cells, other cytokines such as 1L-1, IL-2, 1L-6, 1L-12, 1L-13, and 1L-18, are believed to contribute to antiviral response indirectly by modulating various aspects of the immune response, including autocrine and paracrine upregulation of IFN-γ .

In addition, most cell types in the body respond to an incoming viral infection by secreting IFN-α/β. Conversely the production of IFN- γ is predominantly confined to cells of the immune system (NK cells, NKT cells. Th cells, CTL, macrophages and dendritic cells (DC) [21].

These results are concordant with those of (Cerny and Chasera, Cacciarelli *etl*). who showed that, in patients with HCV infection, as liver injury worsens there is an increased in serum levels of Th1-like cytokines such as 1L-2 and IFN- γ, though some other authors did not confirm these results. [24,4]

IFN- γ is an important proinflammatory cytokine with pliotropic effects on viral replication and the regulation of several immune cells and it has also shown to possess antifibrotic properties [25],
though the findings in this study suggest that in chronic hepatitis C, its actions as a proinlammatory cytokine negate any role that it may play in limiting the fibrotic process.

This assumption could be further supported by the results of several previous *in vitro* studies. For example, Morshed *et al*., [26], in a semiquantitative PCR analysis have shown IFN- γ mRNA to be increased in hepatitis C cirrhosis and chronic active hepatitis compared with chronic persistent hepatitis. In line with this, Napoli *et* al., [14] showed that in liver biopsy specimen obtained from patients with chronic HCV infection, intrahepatic mRNA expression of Th1 cytokines was significantly upregulated, and Bertoletti *et al*., [12] reported that the majority of T-cell clones established by liver-infiltrating lymphocytes in chronic hepatitis C were Th1 cells, suggesting that the Th1-type cytokines and Th1 cells have a strong relation to the liver damage caused by HCV infection.

Moreover, it is unknown exactly how the Th1-type cytokines (IFN- γ and 1L-2) or Th1 cells contribute to the liver damage. According to previous studies, HCV-specific CD8+ cytotoxic T lymphocytes (CTL) response has been observed both in peripheral blood and among liver infiltrating lymphocytes in patients with chronic HCV infection. These results suggest that HCV-specific CTL may be implicated in the pathogenesis of liver damage because of chronic HCV infection [27, 28,29].

As well as, in transgenic mouse model in which the IFN-γ gene is specifically expressed in liver, chronic active hepatitis was induced spontaneously [30], therefore, IFN-γ may possibly cause liver injury by activating monocytes/macrophages in subjects with chronic HCV infection.

Conclusion

1. There were significant increases in mean serum levels of (IL-4 and IFN-γ) in patients with chronic hepatitis C.
2. There were a significant correlation between the elevation of the mean serum level of (IFN – γ) and the progressive liver injury in chronic hepatitis C infection.

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