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Interleukin -6 and C- reactive protein profiles in Hepatitis C virus patients

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الخلاصة:

شملت هذه الدراسة (44) اربعة واربعون شخصا من المصابين بالتهاب الكبد الفايروسي نمط سي وعشرون من غير المصابين لتقدير تراكيز الانترليوكين–6 والبروتين الفعال سي CRP. تم تحليل النتائج المستحصلة إحصائيا لمعرفة معنوية الفروقات والعلاقة بين المتغيرات المدروسة.

أظهرت النتائج أن إنتشار البروتين الفعال سي كان متساويا في الافراد الايجابيين والسلبيين لأضداد التهاب الكبد الفايروسي نمط سي بينما وجد بأن مستوى الانترليوكين–6 مرتفعا في 50% من المرضى الايجابيين لأضداد التهاب الكبد الفايروسي نمط سي، وأن عدوى التهاب الكبد الفايروسي نمط سي تقترن إيجابيا في أغلب الحالات بارتفاع مستوى الانترليوكين–6.

تقترح هذه النتائج أن مستويات الانترليوكين–6 والبروتين الفعال سي يتفاوتان أثناء الاصابة بالتهاب الكبد الفايروسي نمط سي وأنهما لا يعكسان درجة التهاب الكبد في مرضى التهاب الكبد الفايروسي نمط سي.

Abstract:

Forty four anti-HCV positive and twenty anti-HCV negative individuals were included in this study for the assessment of interleukin (IL)-6 concentrations and the frequency of C-reactive protein (CRP). Data obtained were statistically analyzed for the significance of differences and correlation between parameters studied.

The results showed that CRP frequency was equal in anti-HCV positive and negative individuals, whereas IL-6 found to be elevated in 50% of anti-HCV positive patients. HCV infection found to be positively associated in most cases with increased levels of IL-6.

The results suggested that il-6 and CRP levels varied during the course of HCV infection and do not reflect the extent of liver inflammation in HCV patients.

Key words: Hepatitis C virus (HCV), Interleukin (IL)-6, C- reactive protein (CRP).

Introduction:

Hepatitis C is a disease with a significant global impact. According to the World Health Organization there are 170 million people infected with the hepatitis C virus (HCV), corresponding to 3% of the world's total population. There are considerable regional differences [1].

It is difficult to determine the number of new HCV infections, as most acute cases will not be noticed clinically. Fewer than 25% of acute cases of hepatitis C are clinically apparent. In addition, the age of infection upon diagnosis is not possible to determine in most cases [2].

The spectrum of clinical manifestations of HCV infection varies in acute versus chronic disease. Acute infection with HCV is most often asymptomatic. It leads to chronic infection in about 80% of cases. The manifestations of chronic HCV range from an asymptomatic state to cirrhosis, and hepatocellular carcinoma. HCV infection usually is slowly progressive. Thus, it may not result in clinically apparent liver disease in many patients if the infection is acquired later in life [3].

The risk of chronic HCV infection is high. 80-100% of patients remain HCV RNA positive after acute hepatitis C [4]. It is unclear why infection with HCV results in chronic infection in most cases [3].

The risk of developing cirrhosis within 20 years is estimated to be around 10 to 20%, with some studies showing estimates up to 50% [5-7]. Due to the long course of hepatitis C the exact risk is very difficult to determine, and figures are divergent for different studies and populations[4].

Therefore, studies with short observation periods sometimes fail to show an increase in mortality. In addition, survival is generally not impaired until cirrhosis has developed. On the other hand, there is no doubt that patients with chronic hepatitis C have a high risk of cirrhosis, decompensation, and hepatocellular carcinoma in longterm follow-up. It is not completely understood why there are such differences in disease progression. An influence of host and viral factors has to be assumed [8].

C-reactive protein (CRP) belongs to pentaraxin family [9]. It is produced by the liver in response to several inflammatory mediators, the most important of which is interleukin-6 (IL-6) [10]. C-reactive protein is a sensitive but nonspecific inflammatory marker [11, 12]. During inflammation, levels of CRP can be increased up to 1000 folds [13] and as soon as inflammation subsides it comes to normal levels. CRP has been documented as a predictor of cardiovascular disorders, myocardial infarction, stroke, and sudden heart attack [14].

IL-6 is a cytokine of hematopoetin family[15] which is synthesized by mononuclear phagocytes, vascular endothelial cells, and fibroblasts in response to IL-1 [16] which is an inflammatory marker, and its level increases in hemodialysis

patients [17, 18]. It is well documented that the immune system of HCV-infected individuals is suppressed, further, in HCV patients, there is macroglobulinemia, which leads to immune complex deposition in various organs. Therefore, inflammatory markers are raised, i.e., CRP and IL-6 [19]. There is a synergetic effect of CRP and pro-inflammatory cytokines like IL-6 and IL-10 that serves as a strong predictor of cardiovascular disease mortality [20]. All these changes in HCV-infected individuals may directly or indirectly affect cardiovascular disease and especially the heart because there is increased mortality and morbidity in these patients [21]. The present study was designed to determine the link between HCV, inflammatory markers. Therefore, it was planned to determine the levels of CRP and IL-6 in HCV-positive patients.

Materials and Methods:

44 anti-HCV positive patients were included in this study. 21 patients were from kidney dialysis unit in Baghdad teaching hospital, 13 blood donors from the national center for blood transfusion, and 10 other patients from visitors of special clinics. Another twenty anti-HCV negative persons were included as control for comparison.

Venous blood (5 ml) were collected from each subject and sera were used for virological and serological assays.

Third Generation Bio Elisa HCV Screening Kit (supplied by biokit, Spain) was used as a screening method for detection of hepatitis C virus infection. Positive results were confirmed by using Recombinant Immunoblot Assay (RIBA). RecomBlot HCV IgG (supplied by Microgen, Germany).

Sera of all individuals were tested for determination of the levels of C-reactive protein (the kit is supplied by SAS™ CRP-Latex, USA) and Interleukin (IL)–6 Determination Test (IL ELISA IM1120, Cell COM, supplied by Beckman Coulter, FRANCE). The calculated maximum normal average of IL-6 was (100 pg/ml).

Results subjected for Statistical Analysis using spss software (version 17). Correlation between parameters were calculated according to Spearman's correlation coefficient (significance 2-tailed) ie ($p \leq 0.05$).

Results and Discussion:

Equal frequency of CRP found in HCV patients and HCV negative groups. Moreover, 11 out of 44 (25%) of HCV patients showed raised CRP level. This group was distributed as follows; 5/13 blood donors, 4/21 kidney dialysis patients, 2/10 of special clinics patients (as shown in table-1).

From 44 HCV positive patients, 22 showed increased IL-6 level, and 8 out of them were with high CRP level, 14 were with normal IL-6 level and raised CRP. Other results showed that three patients had raised CRP and normal IL-6 level and nineteen without an increase in both markers.

The average of IL-6 concentrations for anti-HCV positive samples was 198.07 pg/ml, t test revealed a significantly high concentration of IL-6 in comparison with the maximum normal average (100 pg/ml) at the level of 0.05 (t test value = 2.452, significance (2-tailed) = .018, 95% confidence intervals from 17.417 to 178.718).

A high standard deviation was found for anti-HCV positive samples (265.27) because of the wide range of recorded concentrations (0 to >1000 pg/ml).

Spearman's rho correlation coefficient was used to measure the closeness of association between variables studied in HCV patients.

Table 2 showed that IL-6 correlated positively with HCV infection frequency and CRP (significant with both) but no correlation found between HCV infection frequency and CRP level.

This study found an equal low percentage (25%) of CRP in HCV patients and control groups. It is suggested that the low percentage of CRP in most HCV patients may be due to disease progression to chronicity, or to the absence of symptoms in which there is evidence that CRP is present in low levels in asymptomatic individuals [22]. In this study IL-6 concentrations varied largely between cases, although 50% of the samples were above the normal value. This variation may be due to the time in which the sample was obtained since IL-6 level reaches the peak at early morning and declines gradually towards low or normal during the day [23] or it may be due to the different stages of HCV infection since patients chronically infected with HCV exhibit immune dysfunction with a Th2-dominant cytokine profile, while Th1 cytokines are prominent in those with self-limited HCV infection [24].

Association of CRP with IL-6 found to be significantly positive. This association may be due to the role of IL-6 as the main stimulator of CRP production from hepatocytes at the acute stage of infection [25].

Absence of correlation between CRP and HCV infection do not abolish the relative increase in CRP level, however, equal percentage of CRP in HCV patients and anti-HCV negative individuals may be the cause.

In haemodialysis patients, Afzal etal., (2011) [26] found that HCV-positive patients had lower CRP levels as compared to HCV-negative patients, while IL-6 levels were higher in HCVpositive patients than in HCV-negative patients. He suggested that the difference in CRP production in HCV positive patients indicated that liver response to IL-6 stimulation might be changed due to HCV infection.

Therefore hepatic injury by HCV could be a reason for the disturbance in the production of CRP [16]. Nascimento etal., (2005) [17] also found that levels of CRP and IL-6 were significantly high in hemodialysis population. In Nascimento and colleagues study, HCV-positive patients had lower levels of highsensitivity CRP as compared to HCV-negative patients. There was a significant difference in highsensitivity CRP-IL-6 ratio in HCV-positive patients which might be due to hepatocellular injury that could affect CRP production.

Afzal etal. observed 56% of hemodialysis patients had a high level of CRP, while Panichi etal,[27] found that 47% of hemodialysis patients had high CRP levels, and in another study, 36% of hemodialysis patients showed high levels of CRP[28,29]. Hung etal., (2008) [30] also observed 25% of haemodialysis patients had CRP levels greater than 16.7 mg/L. The levels of both CRP and IL-6 were not significantly different between HCV-positive and HCV-negative patients. Low levels of CRP in HCV-positive patients indicated that liver was impaired by HCV, and therefore, CRP value did not give proper extent of inflammation.

Apparent increase in CRP levels in hemodialysis patients indicated inflammation, and therefore, it was designated as a sensitive and independent marker for malnutrition. These findings also matched with the findings of a study done by Nascimento etal., (2005) [17]. In Afzal study, IL-6 levels were high in HCV-positive patients compared to HCV-negative patients, and similar findings were found in the study of Nascimento and colleagues [17].

In this study, the frequency of CRP was less than that reported in the previous studies in patients on dialysis or even the group of blood donors (whom apparently asymptomatic), while IL-6 was elevated in most patients other than those on dialysis.

This study concluded that IL-6 and CRP did not give the proper extent of liver inflammation in HCV patients. However, more studies with a large number of patients are required.

Anti- HCV	Source of sample	IL-6	CRP	No. of cases
Positive N=44	SC N=10	+	+	2
		+	-	5
		-	-	3
	NCBT N=13	+	+	5
		+	-	4
		-	-	4
	KDU N=21	+	+	1
		+	-	5
		-	+	3
		-	-	12
Negative N=20		+	+	4
		+	-	2
		-	+	1
		-	-	13

Table-1: Concentrations of IL-6 and CRP in anti- HCV positive and negative individuals.

Notes: KDU= kidney dialysis unit, N= number of individuals, NCBT= national center for blood transfusion, SC= special clinics, + = above maximum normal average, - = equal or below maximum normal average.

PARAMETERS		IL-6	HCV
Correlation coefficient		.253*	
Significance(2-tailed)		.044	
HCV	**N**	64	
Correlation coefficient		.302*	000
Significance(2-tailed)		.046	1
CRP	**N**	44	64

Table-2: Non-parametric correlations between parameters studied (Spearman's rho).

* Correlation is significant at the 0.05 level (2- tailed).

N = number of individuals.

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