Measuring of C-reactive protein titer in patients with acute hepatitis-C Virus infection

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المستخلص

الهدف: قياس عيارية بروتين (C) المنشط في التشخيص التنبؤي لالتهاب الكبد الفيروسي نمط (C) الحاد.

المنهجية: شملت الدراسة ٢١٠ من المرضى المصابين بإلتهاب الكبد الفيروسي نمط (C) الحادّ و٢٣٤ شخصاً من الأصحاء ظاهرياً كمجموعة ضابطة. تضمنت مجموعة المرضى ٧٤ (٣٥،٢%) أناث و١٣٦ (٦٤،٨%) ذكور وبمعدل عمر (٢٧±١٦،٥) سنة. المجموعة الضابطة تألفت من ١١٤ (٤٨،٧%) أناث و١٢٠ (٥١،٣%) ذكور وبمعدل عمر(٥،٨ ±٢٦) سنة. جُمِعَت نماذج الدم وتمّ فصل الأمصال وتجزئتها في أنابيب صغيرة وحفظت في درجة حرارة – ٢٠ م . تشخيص حالات إلتهاب الكبد الفيروسي نمط (C) الحادّ اعتمد على وجود المستضد السطحي (HC Ag) والضدات النوعية IgM (Anti-HC –IgM) إجراء فحوص وظائف الكبد تحديد عيارية بروتين المنشط أجري بطريقة التلازن شبه الكمية جميع البيانات حللت إحصائياً.

النتائج: اعتماداً على نسبة ٩٥% فإنّ المستوى الأساس العيارية بروتين(C) المنشط بين الأشخاص الأصحاء كان ١: ٨ (١٦ ملغرام/لتر) وبين المرضى ١:٥١٢ (١٠٢٤ ملغرام/لتر). أظهرت النتائج الوسيط الحسابي العيارية بروتين (C) المنشط كان أعلى بشكل معنوي اححائيا مقارنة بالاشخاص الاصحاء (P<0.001) ان صلاحية عيارية بروتين(C) المنشط عند القيمة الفاصلة ١: ٦٤ للتنبؤ بإلتهاب الكبد الفيروسي نمط (C)الحاد اعطت حساسية خصوصية ١٠٠% و ٩٦% على التوالي ، وان نسبة الصواب كانت ٩٨%. فضلاً عن ذلك، أظهرت النتائج وجود ترابط معنوي إحصائياً بين عيارية بروتين (C) المنشط يمكن ان يكون ذا قيمة في التشخيص التنبؤي لاتهاب الكبد الفيروسي نمط (C) الحادّ عندما يشكل التشخيص السريري ٥٠%.

Abstract

Objective(s): To measure serum C-reactive protein (CRP) titer as a predictive diagnosis of acute hepatitis C virus (HCV) infection.

Methodology: Two hundred and ten patients with acute HCV infection and 234 apparently healthy individuals as control group were enrolled in this study in Baghdad medical city (Teaching Laboratories). The patents include 74(35.2%) females and 136 (64.8%) males with mean age (27±16.5) years. The control group includes 114 (48.7%) females and 120 (51.3%) males with mean age (26±5.8) years. Blood samples were collected from out patients from Alfadul in Baghdad city. Sera were separated and stored at 20 ^{0}C. The diagnosis of acute HCV infection was based on detection of HC Ag and anti- HCV IgM and standard liver function tests. Determination of CRP titer was assessed by semi-quantitative tube agglutination test. All data were statistically analyzed.

Results: Based on 95% percentile, the baseline CRP titer in healthy individuals was 1:8 (16mg/l) and for patients 1:512 (1024mg/l). There was a statistically significant increase in the mean CRP titer in patients with acute HCV infection compared to healthy individuals (P< 0.001) .The validly of CRP titer 1: 64 as a cut –off value to predict HCV infection provide a sensitivity and specificity of 100 % and 96% respectively. Furthermore, there was a significant correlation between CRP titer and liver function test values.

Recommendation:

Therefore, in further studies, we recommends the evaluation of C- reactive protein titer in patients with acute hepatitis B Virus infection and patients with non–infectious diseases such as cardiovascular disease, diabetes mellitus and hyperlipidemia infection, and compare between them.

Keywords*:* C-reactive protein, hepatitis-C virus

Introduction:

C-reactive protein (CRP) is a non-glycosylated polymeric protein belongs to the pentraxin family of calcium–dependent ligand–binding plasma protein. Plasma CRP is produced only by hepatocytes predominantly under control of interleukin 6(IL-6), although other sites of local synthesis have been suggested [(1)]. CRP production is part of non–specific acute–phase response to most forms of inflammation, infection, and tissue damage [(2)]. It has been suggested that the sole determinant of circulating CRP concentration is the synthesis rate, which thus directly reflects the intensity of the pathological processes stimulating CRP production [(3)].

During the past few years, it has repeatedly shown that CRP is a strong predictor of cardiovascular disease [(4-6)], diabetes mellitus and hyperlipidemia [(7-8)] and various bacterial infection particularly septicemia [(9-10)]. Furthermore studies, C-reactive protein is a sensitive marker of systemic inflammation, and prospective data from population of apparently healthy men indicate that baseline levels predict risk of first myocardial infarction (MI) [(11)]. Specifically, among men free of prior cardiovascular disease participating in the physicians health study, many studies recently reported that those with baseline level of CRP in highest quartile had a three folds increase in risk of developing future MI compared with those with levels in the lowest quartile as such these data demonstrate that CRP is a marker of cardiovascular risk only among those with static and unstable angina, elderly and selected high risk patients, but also among individuals with on current evidence of cardiovascular disease [(12)]. The path mechanism of hepatic and extra hepatic manifestation of hepatitis C virus (HCV) infection has been reviewed extensively. Little is know, however, of the behavior of the acute-phase reaction during chronic hepatitis caused by HCV. Recently we found significant increase in serum concentration of C9 and C1-inhabetor (C1-INH), two acute-phases protein (AFP) belonging to treatment with (IFN-a) at least 50% drop of HCV RNA concentration (1). This observation has been corned Correspondence to: L. Kalabay [(13)].

Methodology:

Two hundred and ten patients with acute HCV infection and 234 apparently healthy individuals as a control group were sampling technique (of patient and controls or) enrolled in this study. This study was conducted from October, 1st, 2010 to August, 30th, 2011 in Baghdad Medical City (Teaching Laboratories). The patents were 74 (35.2%) females and 68 (64.8 %) males with mean age (27.5) years. The control group included 114 (48.7%) females and 60 (51.3%) males with mean age (25.8) years. Blood samples were collected; sera were separated and stored in aliqutes at -20 ^{0}C till use. The diagnosis of acute HCV infection was based on detection of HC Ag and anti–HCV IgM were diagnosed by ELISA technique and standard liver function tests (Total, direct, and indirect serum bilirubin, Alanine aminotransferase (ALT), Alanine

aspartate aminotransferase (AST), total serum protein, serum alkaline phosphatase). Determination of CRP titer was assessed by semi–quantitative tube agglutination test. All data were statistically analyzed using computerized SSP version 13.

Results:

Table (1): showed that 82(35% of the healthy control had the lowest CRP titer (zero), while the highest titer (1:16) was found in 10 (4.3%). The 95% percentile of CRP titer was 1:8 .In patients, the lowest titer 1:16 was found in 4(3.8%) and the highest titer 1:1024 was found in 4(1.9%). The 95% percentile of CRP titer was 1:512 the mean CRP titer in healthy control was 1:8, while the higher mean titer in patients with acute HCV infection was 1:64 54 (25.7%).

Table 1. C- Reactive protein (CRP) titer in patients with acute hepatitis C virus infection and controls

CRP titter	Healthy control		Patients	
0	Number	Percent	Number	Percent
	82	35	0	0
2	48	20.5	0	0
4	36	16.3	0	0
8	48	23.9	0	0
16	10	4.3	8	3.8
32	0	0	50	23.8
64	0	0	54	25.7
128	0	0	48	22.9
256	0	0	26	12.4
512	0	0	20	9.5
1024	0	0	4	1.9
Total	234	100	210	100
95% percentile	224(95.7)		206(98.1)	

Table (2) showed the range, median and interquartile range of CRP titer in the study groups. The statistical analyses (Mann-whitney test) revealed a significant difference in the median of CRP titer between the two study groups.

Table 2. Range, median and interquartile range of CRP titer in study groups

CRP titer	Healthy control	Patients
Range	(0-32)	16-1024
Median	4	64
Interquartile range	(0-16)	(32-256)

P (Mann-Whitney) < 0.001

The validity of CRP titer 1:8 as a cut–off value to differentiate between healthy control and patients with acute HCV infection when clinical suspicion was 50 %. The results showed that all patients give a titer 1:8 and more, whereas, 168 of the healthy control give a titer > 1:8 and 66 of them gives a titer 1:8 and more. The statistical analysis showed a significant difference between the two groups (P>0.001). The sensitivity and specificity of the test were 100% and 72% respectively. The positive and negative predictive values were 76% and 100% respectively. The test accuracy was 85%.Table (3).

Table 3. Validity of CRP titer at 1:8 as a cut–off value

CRP titer at cut –off 8	Healthy control	Patients	P value
Negative <1:8	**168**	**0**	**<0.001**
Positive 1:8 and more	**66**	**210**	
Total	**234**	**210**	

Accuracy = 85%; False negative =0%; False positive =28%; Negative predictive value 100%; Positive predictive value = 76 %; Sensitivity = 100%; Specificity = 72 %;

The validity of CRP titer at 1:16 as a cut–off value revealed that 224 of the healthy control gives a titer > 1:16, while the remaining 10 gives a titer 1:16 and more .On the other hand, patients gives a titer 1:16 and more. There was a statistically significant difference between the two groups (P>0.001). The sensitivity and specificity were 100% and 96% respectively .The positive and negative predictive values were 95% and 100% respectively. The accuracy was 98% table (4).

Table 4. Validity of CRP titer at 1:16 as a cut off value

CRP titer at cut –off -16	Healthy control	Patients	P value
Negative < 1:16 and more	**224**	**0**	**<0.001**
Positive 1:16 and more	**10**	**210**	
Total	**234**	**210**	

Accuracy = 98 %; False negative =0%; False positive =4%; Negative predictive value = 100%; Positive predictive value =95 %; Sensitivity = 100%; Specificity = 96 %

Although there was no significant correlation between the CRP titer and liver function tests (total, direct, indirect serum bilirubin, ALT, AST, Total serum protein and serum alkaline phosphates) in the healthy group as assessed by Spearman's linear correlation.

However, CRP titer as highly correlated with liver function tests in patient group, table (5).

Table 5. Spearman's liner correlation between CRP and liver function tests

Liver function test	Spearman's linear correlation			
	Control		Acute hepatitis B	
	r	P value	r	P value
Serum Alkaline phosphatase	0.07	0.48[NS]	0.24	0.013
AST	0.09	0.34[NS]	0.47	<0.001
ALT	0.03	0.72[NS]	0.45	<0.001
Total serum protein	0.01	0.93[NS]	0.60	<0.001
Total serum bilirubin	0.06	0.51[NS]	0.57	<0.001
Indirect serum bilirubin	0.06	0.52[NS]	0.44	<0.001
Direct serum bilirubin	0.02	0.83[NS]	0.53	<0.001

ALT= Alanine aminotransferase; ; AST= Alanine aspartate aminotransferase; p= Level of probability; r= correlation

Discussion:

C- reactive protein is a test which measures the concentration in serum of a special protein produced in the liver that is present during episodes of acute inflammation or infection. In the body, CRP plays the important role of interacting with the complement system, an immunologic defense mechanism [14].

Hepatitis is an inflammation of the liver. Viral infections are among the most common cause of hepatitis [15-16]. Hepatitis C virus infection is the most common cause of acute viral hepatitis [17)]. Hepatitis C virus infection is endemic in Iraq; the chronicity rate among healthy blood donors in afadul city is 1.6% [18)].

The mean serum CRP concentration in healthy subjects obtained in the present stud was relatively higher than that reported by Shaya et al. (2002) [19]. In Al–Ramadi city who found that the CRP concentration among apparently healthy subjects was 6 mg/1. This may be due to the presence of asymptomatic infectious and /or non infectious disease that may have a role in elevating the CRP concentration. However, it is generally agreed that a CRP concentration above 10 mg/1 usually considered high [19-20].

The significant increase in serum CRP concentration in patients with acute HCV infection as compared to healthy control, may be due to the damage of hepatocytes as a result of direct viral replication and indirectly through induction of cytotoxic T-cell response that further destroy liver infected cells [22-24].

The results also revealed that the CRP titer 1:16 as a cut–off value provide sensitive and specific predictor for the diagnosis of acute HCV infection when the clinical suspicion was 50%. Unfortunately, no previous studies had been found in the literature regarding the utility of serum CRP concentration in the predictive diagnosis of viral hepatitis. However, our results were consistent with those obtained by utilization of CRP concentration in the predictive diagnosis of other infectious diseases such as community–acquired pneumonia [12-13, 25], and non–infectious diseases such as cardiovascular disease [7-9], diabetes mellitus and hyperlipidemia [10-11].

The significant linear correlation between the CRP concentration and candidate the CRP concentration as a surrogate marker in prediction of acute HCV infection beside the clinical picture.

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