

**Study of proteins encoded by different fragments of C virus
gene for detection hepatitis C infection**

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Abstract

This study was carried out to evaluate proteins encoded by different fragments of HCV genes for detection hepatitis C infection. Eighty eight Iraqi hepatitis C patients were involved in this study, these patients attended the Gastroenterology Department of Baghdad Teaching Hospital during the period from December 2009 to March 2011. These patients included 58 males and 30 females with ages ranged between (16-64) year. The results of this study showed that 88 specimens were immunoreactive to one or more of the 4 HCV recombinant proteins, besides that 34 serum samples were collected from apparently healthy individuals (included 21 males and 13 females) they served as a control group, all the samples obtained from control group didn't show any reactivity to hepatitis C virus. Additionally, the positive rates of anti-capsid ,anti-NS3,anti-NS4 and anti-NS5 were 77.2%,64.7%,55.6% and 46.5% respectively ,while 13.6 % reacted to capsid protein , 5.7% reacted to NS3 protein , in addition , 3.4% reacted to NS4 protein , furthermore , 2.3% only reacted to NS5 protein . Finally, all the 88 specimens (100%) were immuno-reactive to the mixed proteins. In conclusion , Our results sustain different immunoreactivity of the HCV recombinant proteins encoded by different fragments of C virus gene, therefore the mixture of all the protein derived from HCV different regions should be applied in further HCV diagnostic test to detect hepatitis C infection.

Key words: hepatitis C infection , C virus genome , NS proteins

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دراسة البروتينات المشفرة من القطع المختلفة لجين الفيروس ج للتحري
عن الإصابة بالتهاب الكبد الفيروسي ج.

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الخلاصة

أجريت هذه الدراسة لتقييم البروتينات المشفرة من القطع المختلفة لجين الفيروس ج للتحري عن الإصابة بالتهاب الكبد الفيروسي ج. تضمنت الدراسة 88 مريض عراقي مصابين بالتهاب الكبد الفيروسي ج من المراجعين لقسم الجهاز الهضمي في مستشفى بغداد التعليمي خلال الفترة الممتدة من كانون الأول 2009 إلى آذار 2011. المرضى مؤلفين من 58 ذكر و 30 أنثى وبمعدل عمرى يتراوح من 16-64 سنة. أثبتت نتائج الدراسة بان 88 نموذج أعطوا تفاعل مناعي لواحد أو أكثر من بروتينات فيروس ج الأربعة. من جانب آخر تم اخذ 34 نموذج مصلي من أشخاص أصحاء ظاهريا وتم استخدامهم كمجموعة سيطرة، جميع النماذج التي أخذت من مجموعة السيطرة لم تعطي أي نتيجة تفاعلية مع فيروس سي. من جهة أخرى أكدت نتائج الدراسة أن معدل الموجبية لكل anti-capsid و anti-NS3 و anti-NS4 و anti-NS5 كان 77.2%، 64.7%، 55.6%، 46.5% على التوالي، بينما 13.6% أعطى نتيجة موجبة مع بروتين الغلاف capsid، 5.7% أعطوا نتيجة موجبة مع بروتين NS3 وبالإضافة إلى 3.4% أعطوا نتيجة موجبة مع NS4 و 2.3% أعطوا نتيجة موجبة مع NS5 وأخيرا فإن جميع النماذج 88 (100%) أعطوا نتيجة موجبة مع البروتينات المخلوطة. نتائج الدراسة أثبتت وجود اختلاف مناعي تفاعلي لبروتينات الفيروس ج المشفرة من القطع المختلفة لجين الفيروس ج لذلك فإن خليط البروتينات المشتقة من المناطق المختلفة لفيروس التهاب الكبد ج يجب أن يوضع ضمن الفحوصات التشخيصية لفيروس سي لغرض التحري عن الإصابة بالتهاب الكبد الفيروسي ج.

Introduction

Hepatitis C virus (HCV) infection has become a major public health problem, about 170 million people considered to be infected worldwide. The disease progresses slowly and a chronic infection develops in 85% of the cases. Among patients with chronic hepatitis, 20 to 30% develop cirrhosis that, once established, carries a poor prognosis, with a high risk of 1). Structural studies of the HCV genome have shown that the (developing hepatocarcinoma virus

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have a positive – strand RNA virus related to flaviviruses family , The high rate of mutation in the RNA genome of this RNA virus may cause the variability of the envelope protein (2,3). HCV has been linked to a blood – borne , e. g. patients receiving organ transplants , blood product , or intravenous drug use , born to an infected mother , and sexual practices (4) . Infection with acute HCV is usually subclinical, but the likelihood of chronic is high. Infection with HCV is most typically diagnosed in the chronic phase of infection (5).Patients may be diagnosed coincidentally during the investigation of fatigue or abnormal liver function test, or with manifestation of chronic liver disease. The initial screening test for HCV is detection of circulating anti-HCV antibodies, if present; confirmation is required either by more specific antibody testing using immunoblot, or more often by using Polymerase Chain Reaction (PCR) for viral RNA. Quantitative PCR and viral genotyping are becoming more relevant as treatment strategies evolve (6). The HCV genome is a positivestranded RNA molecule of approximately 10,000 nucleotides, which contains a single uninterrupted open- reading frame that encodes a protein of about 3,000 amino acids . The structural region consists of core and envelope (E1 and E2) proteins, and a 3' region codes the nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B), which after processing constitute 10 mature viral proteins (7).The determining proteins which are encoded by different fragments of HCV genome are essential for diagnostic hepatitis C infection , therefore this research was aimed to study proteins encoded by hepatitis C virus gene for detection hepatitis C infection.

Subjects

Patients Study Group

Eighty eight Iraqi patients infected with hepatitis C were involved in this study , these patients attended the Gastroenterology Department of Baghdad Teaching Hospital during the period from December 2009 to March 2011. Their ages ranged between (16-64) year. These patients included 58 males and 30 females. They were sequentially selected from cases referred to the hospital at first presentation. They were diagnosis based upon the patient's medical history, physical examination and laboratory test.

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Control group

Thirty four healthy individuals with age range from (21-62) year were studied as control group. This group included 21 males and 13 females. Samples were collected from those individuals only if they were not receiving any medication and did not had a history of a chronic or acute illness

Samples collection

From each individual included in this study, 5 ml of blood was drawn by vein puncture using disposable syringes. The blood was placed in plastic disposable tubes, it was left to stand at room temperature (20-25°C) to allow it to clot, then the sera was separated by centrifugation 10000 r.p.m for 5 minutes, and divided into aliquots (250 µl) and stored at - 20°C till examination. Each aliquot of the serum was used once to avoid thawing and freezing. All sera and reagents were allowed to stand at room temperature before use in the test.

Methods

1-Enzyme Linked Immunosorbent Assay (ELISA):

Principle:

Employing a second-antibody sandwich principle, diluted patient sample or control were added to microtitre wells precoated with purified antigens mimicking the core, NS3, NS4 and NS5 gene segments of the HCV genome. These peptides have been shown to react and bind with the predominant classes of anti-HCV antibodies present in HCV positive serum. After incubation, peroxidase-conjugated anti-human IgG antibody was added to form a detectable complex. After washing, one shot substrate was added to form a colored complex. The intensity of the color that may consequently develop is proportional to the amount of anti- HCV present in the sample. The reaction was then stopped by the addition of acid and the resulting color intensity can be read spectrophotometrically at 450 nm (8).

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Procedure :

The detailed procedure was carried out as has been suggested in the leaflet supplied with the test kit (Randox, U.K)

2-Recombinant Immunoblot Assay

Principle:

The RIBA HCV 3.0 is a three-stage test which utilizes individual recombinant HCV antigens and the synthetic peptides immobilized as individual bands onto the test strips. In the first stage, the specimen or assay control is diluted and incubated with the strip. Antibodies specific to HCV, if present, will bind to the corresponding recombinant antigen and / or synthetic peptide bands on the strip. In the second stage, the strip is incubated in the presence of a peroxidase-labeled coat anti-serum IgG conjugate. In the third stage, a colorimetric enzyme detection system composed of hydrogen peroxide and 4-chloro-1naphthol is added.. After the development of color on the strip, the reaction is stopped by removal of the reactants and final wash steps. The visual band patterns which develop on each individual strip are the result of specific antibody being bound to each of the individual recombinant antigens and/ or synthetic peptides on that strip. The reactivity of specimens towards each antigen band is determined by visually comparing the intensity of the individual antigen band with that of the low and high human IgG internal control bands plotted onto each strip. (9).

Procedure :

The detailed procedure was carried out as has been suggested in the leaflet supplied with the test kit (Chiron, Ireland).

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Results and Discussion

Table 1 : Sex distribution of studied group

Sex	hepatitis C Patients		Control group	
	Number	%	Number	%
Male	58	65.9	21	61.7
Female	30	34.1	13	38.3
Total	88	100	34	100
M/F ratio	1.93:1		1.61:1	

Table 1 shows the majority of patients and control groups are males (65.9% and 61.7%) respectively rather than females (34.1% and 38.3%) respectively. The ratio between male to female among patients group was 1.93:1, whereas 1.61:1 among control group . This high frequency of infection with HCV among males may be attributed to socio-community nature of Iraqi people which makes men undergone the responsibility of working and eventually are in great contact with the pathogens rather than the women. Most studies denoted to the prevalence of HCV was among men rather than women which revealed that the male: female is (2:1) (10,11,12,13). Some observed an equal ratio of males: females (1:1) in Iraq (14,15).

Table 2 : Distribution of patients and control groups according to anti-HCV Ab and recombinant proteins

Patients number	Anti -HCV antibody		Immuno-reactive to one or more HCV recombinant		Control group	Anti -HCV antibody		Immuno-reactive to one or more HCV recombinant	
	+ ve	%	+ ve	%		+ ve	%	+ ve	%
88	88	100	88	100	34	0	0	0	0

Table 2 revealed the distribution of patients according to anti-HCV Ab and recombinant proteins. According to this table, It was found that 88 anti-HCV-positive specimens were immunoreactive to one or more of HCV recombinant proteins, while control group didn't show

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any positivity to anti –HCV antibody and recombinant protein . Additionally, these results agreed with (16) who reported seventy-five out of the 90 known anti-HCV-positive were reactive to one or more of HCV recombinant antigens. Moreover (17) found that young adults, a considerable difference among anti –HCV antibody and HCV recombinant proteins for investigating hepatitis C infection .

Table 3: Frequency of patients and control groups according to antibody against viral recombinant proteins

Patients Number	Anti-capsid		Anti-NS3		Anti-NS4		Anti-NS5		Control group	Anti-capsid , Anti-NS3, Anti-NS4, Anti-NS5	
	+ ve	%	+ ve	%	+ ve	%	+ ve	%		+ ve	%
88	68	77.2	57	64.7	49	55.6	41	46.5	34	0	0

The positive rates of anti-capsid ,anti-NS3,anti-NS4 and anti-NS5 were 77.2%,64.7%,55.6% and 46.5% respectively in comparison to control group (0%)(table 3) .

Table 4: Frequency of patients and control groups according to positivity to viral recombinant proteins

Patients Number	capsid protein		NS3 protein		NS4 protein		NS5 protein		Mixed proteins		Control group	Mixed proteins	
	+ ve	%	+ ve	%	+ ve	%	+ ve	%	+ ve	%		+ ve	%
88	12	13.6	5	5.7	3	3.4	2	2.3	88	100	34	0	0

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while 13.6 % reacted with capsid protein besides , 5.7 % reacted with NS3 protein , in addition , 3.4% only reacted with NS4 protein , furthermore , 2.3 % only reacted with NS5 protein , while control group didn't show any positivity to mixed proteins , finally, all the 88 specimens (100 %) were immuno-reactive to the mixed proteins as presented in table 4 . The result of the current study is comparable to that of the (16) which mentioned that there was high positivity among anti-capsid, anti-NS3, anti-NS4 and anti-NS5 were 70.0%, 61.1%, 52.2% and 44.4% respectively, as well as 13 % reacted with capsid protein , 5.9 % reacted with NS3 protein , 3.2% reacted with NS4 protein and 1.9 % reacted with NS5 protein . However, all the specimens were reactive to the mixed proteins. Moreover, The result appeared in concurrence with study done by (17 ,18) who recorded a lower detection level of capsid , NS3, NS4 and NS5 proteins among patients with hepatitis C , whereas all patients gave positive reaction to the mixed proteins. Therefore the mixture of all the antigens derived from HCV different regions should be applied in further HCV diagnostic test to detect hepatitis C infection.

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