P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2013,(7), (1) :36-41

Comparison between Recombinant Immunoblot assay 3rd generation and enzyme linked immunosorbent assay for detection hepatitis C virus .

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ARTICLE INFO

Received: 21 / 9 /2012
Accepted: 29 / 1 /2013
Available online: 30/11/2013
DOI: 10.37652/juaps.2013.83124

Keywords:
Immunoblot assay,
Immunosorbent assay,
Hepatitis C Virus.

ABSTRACT

This study was carried out to compare between recombinant immunoblot assay 3rd generation and enzyme linked immunosorbent assay for identify hepatitis C virus. Seventy six Iraqi patients undergo hepatitis C were involved in this study. The study was carried out from July 2010 to April 2011 were followed up in public health center- Baghdad. These patients included 54 males and 22 females as well as their ages ranged between (8-68) year with a mean age of 47.1±13.79, besides, the majority of patients are at the age between 31-60 year (54 %), while children elicit less frequency of infection (2 %). Additionally the ratio between male to female was 2.45:1. There are two methods which are used for HCV diagnosis. The first method by using ELISA technique for detection of anti HCV antibody. 61 out of 76 sera samples of hepatitis patients (i.e. 80.2%) were found to be positive for this test. Another advanced method such as recombinant immunoblot assay 3rd generation (RIBA) has been applied, all these specimens gave positive results (100 %) with significant difference was noticed between them (P<0.05), therefore the current results confirm that a RIBA 3rd generation is more sensitive manner to detect hepatitis C virus than ELISA.

Introduction

Hepatitis C virus (HCV) is a small (55-65 nm in size), enveloped, positive-sense single-stranded RNA virus of the family Flaviviridae, hepatitis C virus is the cause of hepatitis C in humans , The hepatitis C virus particle consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin, two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope (1). Hepatitis C is an infectious disease affecting the liver, the infection is often asymptomatic, but once established, chronic infection can progress to scarring of the liver (fibrosis), and advanced scarring (cirrhosis) which is generally apparent after many years, in some cases, those with cirrhosis will go on to develop liver failure or other complications of cirrhosis, including liver cancer (2). Hepatitis C virus is a blood borne virus that is most efficiently transmitted through large or repeated percutaneous exposure to blood, such as transfusion or transplants from infected donors, inadvertent contamination of supplies shared among patients undergoing chronic heamodialysis or sharing of equipments among injection drug users, transmission of HCV may also occur through non-percutaneous route include sexual transmission, perinatal transmission and intra-familial transmission (3). The primarily serologic screening assay for infection is the enzyme linked immunoassay (ELISA), employing a second-antibody sandwich principle, patient sample (antibody) 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 (4). The recombinant immuno-blot assay (RIBA) has uses

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antigens similar to those for enzyme immuno-assay (EIA) but in an immunoblot format, so RIBA is considered as supplemental specific reactivity (5). The first generation (RIBA-1) gave additional indication that reactivity by EIA was specific for HCV antibody, this test was developed to second generation (RIBA-2) by FDA, RIBA-2 includes four recombinant antigens:- [5-1-1 from *Escherichia coli*, C100-3 and C22-3 from yeast, C33c from *E. coli* and super-oxide dismutase (SOD)], a positive assay is defined by the detection of antibodies against two or more antigens and an in determinant test by the detection of antibodies against a single antigen, the third generation of RIBA was used the same antigen that found in RIBA-2 but with new band representing NS5 region recombinant proteins and (5-1-1 is not found) (6,7), therefore the aim of this study was to compare between RIBA 3rd generation method and ELISA method in the detection of hepatitis C virus.

Subjects and Methods

Patient Study Group.

Seventy six Iraqi patients undergo hepatitis C were involved in this study. The study was carried out from July 2010 to April 2011 were followed up in public health center- Baghdad. Their ages ranged between (8-68) year. These patients included 54 males and 22 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.

Control group.

Thirty four healthy individuals with age range from (9-64) year were studied as control group. This group included 23 males and 11 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

Note :- all the samples obtained from control group didn't show any reactivity to hepatitis C virus

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 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

Detection of Antibodies to Hepatitis C Virus (Anti-HCV) by :

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).

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. Removal of unbound serum/ plasma components is accomplished by aspiration and washing. In the second stage, the strip is incubated in the presence of a peroxidase-labeled coat anti-serum IgG conjugate. The conjugate binds to the human IgG portion of the antigen-antibody complexes. Removal of the unbound conjugate is accomplished by

decantation and subsequent wash steps. In the third stage, a colorimetric enzyme detection system composed of hydrogen peroxide and 4-chloro-1naphthol is added. If bound conjugate is present, the enzymatic reaction will produce an insoluble blue-black colored reaction product at each specific HCV antigen, peptide or control band. The color reaction involves the initial divalent oxidation of the peroxidase enzyme by hydrogen peroxidase. Subsequent reduction of peroxidase to its initial state by two successive univalent interactions with soluble 4-chloro-1-naphthaol results in the insoluble blue-black colored reaction product. 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). Anti-HCV reactivity in a specimen is determined by comparing the intensity of each HCV band to the intensity of the human IgG (level I and level II) internal control bands on each strip.

The identity of the antibodies is defined by the specified location of the HCV band.

The intensity of the HCV bands was scored in relation to the intensities of the internal IgG controls as following:

Intensity of band	Score
Absent	-
Less than intensity of the Level I IgG control band	+ / -
Equal to intensity of the Level I IgG control band	1+
Greater than intensity of the Level I IgG control band and less than intensity of the Level II IgG control band.	2+
Equal to intensity of the Level II IgG control band.	3+
Greater than intensity of the Level II IgG control band	4+

A negative, intermediate or positive interpretation is based on the reaction pattern present on the strip. For valid runs the following criteria should be used for interpretation:

Antigen Band Pattern	Interpretation
-No HCV bands present having 1+ or greater reactivity. Or -hSOD band alone having 1+ or greater reactivity.	Negative
-Any single HCV band having 1+ or greater reactivity. Or -hSOD band having 1+ or greater reactivity in conjunction with one or more HCV bands having 1+ or greater reactivity.	Indeterminate
-At least two HCV bands having 1+ or greater reactivity.	Positive

Results and Discussion

It is clear from table (1) that the majority of patients are the males [54 out of 76 (i.e. 71%)] rather than the females [22 out of 76 (i.e. 29 %) with highly significant differences between both frequencies (P<0.001). The ratio between male to female was 2.45:1.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). The explanation for these variations may be attributed to the difference in sample's size in addition to the different time of blood collection.

Table (1) : Sex distribution of studied group

	hepatitis C Patients		P. value
Sex	**Number**	**%**	
Male	**54**	**71**	**0.01**
Female	**22**	**29**	
Total	**76**	**100**	
M/F ratio	**2.45:1**		

The effect of age on the frequency of HCV has been studied among the 76 patients. Table (2) revealed that the distribution of infection among different age groups. According to this table, It was found that the age of patients ranged between (8-68) year, with a mean age of 47.1±13.79 , besides, the majority of patients are at the age between 31-60 year (54 %), while children elicit less frequency of infection (2 %). There are significant differences between the incidences of the different age groups (P<0.05).The interpretation of these results depends on the degree of

viral exposure. This fact is related to working-age which may elevate the exposure chance for the virus, particularly men as mentioned previously. This finding explains generally the high frequency between the age of 31-60 years which comprises (54%), while the low frequency observed at the extremities of life span [i.e. among children and elderly people].

The result of the current study is comparable to that of the others which mentioned that there was high infectivity among Iraqi patients at age range between 27-60 years (12) as well as for the others which was between 40-60 years (13,14,16). On the other hand, aboard one declared that the highly infected age range between 13-82 years (62%) (17). This may be due to so many drug abusers among these communities in comparison with Iraqis. Moreover many bad costumes are prevalent among teenagers such as tattooing (18), besides intra-familial sharing razors, tooth brushes which enhance the disease transmission (19).

Table (2): Distribution of hepatitis C Patients and control group according to age

	Hepatitis C Patients		P. value
Age groups (years)	Number	%	
< 10	2	2.6	0.05
11-20	4	5.3	
21-30	8	10.5	
31-40	24	31.6	
41-50	19	25.0	
51-60	11	14.5	
61+	8	10.5	
Total	76	100	
Mean age (years)	47.1±13.79		

There were two methods were used for HCV diagnosis in this study . The first method by using ELISA technique for detection of anti HCV antibody. 61 out of 76 sera samples of hepatitis patients (i.e. 80.2%) were found to be positive for this test. Another advanced method such as recombinant immunoblot assay 3rd generation (RIBA) has been applied. All these specimens gave positive results (100 %) with significant difference was noticed between them ($P<0.05$) as shown in table 3 . These findings reflected highly sensitivity and specificity of RIBA 3rd generation for detection of HCV infection (i.e. more accurate method for detection C virus) , therefore the current results confirm that a RIBA 3rd generation is more sensitive manner to detect hepatitis C virus than ELISA .our result is in agreement with the result obtained by (20) . Additionally, the results of this study agreed with (21) who reported that a significant difference has been noticed between RIBA and ELISA for detection hepatitis C infection . Moreover (22) has found that in young adults, a considerable difference among RIBA and ELISA for investigating hepatitis C infection and the percentage of positivity were lower when using ELISA test.

Table (3) : Comparison between Recombinant Immunoblot assay 3rd generation and enzyme linked immunosorbent assay according to hepatitis c patients .

Methods	Number of hepatitis C patients	Frq . positive cases	Percentage (%)	P. value
ELISA	76	61	80.2	0.05
RIBA 3rd	76	76	100	

In conclusion : In the present study, the comparison of the two methods of HCV identification revealed that the RIBA 3rd generation was able to detect very low amount of HCV in serum of patients, therefore this method is regarded as the most efficient technique. As a final point, the current results confirmed the importance of RIBA 3rd generation which leads to increase the chance of diagnosis and decrease the development of disease to end-stage of liver disease and hepatocellular carcinoma, therefore the high sensitivity and specificity of RIBA 3rd generation permits virus detection soon after infection and even before the onset of disease, as well as early detection may give physicians a significant lead in treatment.

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P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2013,(7), (1) :36-41

المقارنة بين التهجين المناعي البقعي الجيل الثالث و مقايسة الأنزيم المرتبط الممتز المناعي في التحري عن التهاب الكبد الفيروسي سي.

هناء ناجي عبدالله محمد عبد الدايم صالح خالد جمعة خليل

الخلاصة

أجريت هذه الدراسة للمقارنة بين التهجين المناعي البقعي الجيل الثالث و مقايسة الأنزيم المرتبط الممتز المناعي في التحري عن التهاب الكبد الفيروسي سي. أجريت الدراسة في مختبر الصحة المركزي– بغداد للفترة من حزيران 2010 ولغاية آب 2011. تضمنت الدراسة 76 مريض عراقي مصاب بالتهاب الكبد الفايروسي سي و مؤلفين من 54 ذكر و 22 أنثى و بعمر يتراوح من 8–68 سنة وبمعدل عمري قدره 13.79±47.1 وكذلك أوضحت الدراسة بان أكثر من نصف المرضى يقعون ضمن الفترة العمرية الممتدة من 31–60 سنة (54 %) وبينما سجل الأطفال اقل إصابة وبنسبة (2%). وبالإضافة إلى ذلك كانت نسبة الذكور أعلى من الإناث 2.45:1. تم استخدام طريقتين لتشخيص التهاب الكبد الفايروسي سي ، الطريقة الأولى باستخدام مقايسة الأنزيم المرتبط الممتز المناعي في التحري عن التهاب الكبد الفيروسي سي ، حيث أوضحت النتائج بان 61 من مجموع 76 نموذج مصلي لمرضى التهاب الكبد الفيروسي اعطو نتيجة موجبة باستخدام هذه الطريقة وبنسبة (82%) وبينما أعطت جميع النماذج المصلية نتيجة موجبة باستخدام طريقة التهجين المناعي البقعي الجيل الثالث وبنسبة (100%) لذلك فان نتائج الدراسة اثبت بان طريقة التهجين المناعي البقعي الجيل الثالث أكثر حساسية من طريقة مقايسة الأنزيم المرتبط الممتز المناعي في التحري عن التهاب الكبد الفيروسي سي.