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 developing hepatocarcinoma (1). Structural studies of the HCV genome have shown that the virus has a positive-strand RNA virus related to flaviviruses family, The high rate of mutation in the RNA genome of this 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 positive-stranded 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.

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 Immunosorbsent Assay (ELISA):

Principle

Employing a second-antibody sandwich principle, diluted patient sample or control were added to microtite 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. 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-1-naphthol 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).

**RESULTS AND DISCUSSION**

<table>
<thead>
<tr>
<th>Table 1. Sex distribution of studied group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C Patients</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>M/F ratio</td>
</tr>
</tbody>
</table>

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 undergo 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 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 any positivity to anti –HCV antibody and recombinant protein. Additionally, these results agreed with (16) who reported seventy 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. 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), 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

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

<table>
<thead>
<tr>
<th>Patients number</th>
<th>Anti–HCV antibody</th>
<th>Immuno-reactive to one or more HCV recombinant protein</th>
<th>Control group</th>
<th>Anti–HCV antibody</th>
<th>Immuno-reactive to one or more HCV recombinant protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>88</td>
<td>88</td>
<td>100</td>
<td>88</td>
<td>100</td>
<td>34</td>
</tr>
<tr>
<td>88</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Patients Number</th>
<th>Anti-capsid</th>
<th>Anti-NS3</th>
<th>Anti-NS4</th>
<th>Anti-NS5</th>
<th>Control group</th>
<th>Anti-capsid, Anti-NS3, Anti-NS4, Anti-NS5</th>
</tr>
</thead>
<tbody>
<tr>
<td>88</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>68</td>
<td>77.2</td>
<td>57</td>
<td>64.7</td>
<td>49</td>
<td>55.6</td>
<td>41</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Patients Number</th>
<th>Capsid protein</th>
<th>NS3 protein</th>
<th>NS4 protein</th>
<th>NS5 protein</th>
<th>Mixed proteins</th>
<th>Control group</th>
<th>Mixed proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>88</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>+ve</td>
<td>%</td>
</tr>
</tbody>
</table>
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.

**REFERENCE**


