Comparative Efficacy of Elisa and Pcr for the Diagnosis of Hepatitis C in Various Areas of Pakistan

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Abstract: Hepatitis C Virus is one of the major causes of hepatitis C all over the world specially countries with poor health standards. HCV infection has become one of the common infection in Pakistan since last few years. Patients are routinely screened by antibody assays. This study was conducted to determine the results obtained by ELISA and PCR techniques in the detection of HCV in serum samples. Serum of the patients from different regions of Pakistan was tested by anti-HCV antibodies and HCV RNA by ELISA and PCR. Out of the 300 serum samples, 183 (61%) were ELISA positive and 117 (39%) were ELISA negative samples. Out of total ELISA positive patients, 89 (48.6%) were females and 94 (51.3%) were males. Out of 89 ELISA positive females, 37 (41.57%) were PCR positive and 52 (58.4%) were PCR negative. Out of 94 ELISA positive males, 32 (34%) were PCR positive and 62 (68%) were PCR negative.

Key words: Hepatitis C • Polymerase Chain Reaction • Enzyme Linked Immunosorbant Assay • Ribosnucleic Acid

INTRODUCTION

Human hepatitis C is an infectious disease, caused by the Hepatitis C Virus (HCV) and mainly affects the liver. The infection shows no symptoms in start, but once it is developed, it can lead to the fibrosis and cirrhosis of liver. Moreover, cirrhosis will further go on to develop liver failure and also liver cancer in many cases [1]. HCV is the only known member of the Hepacivirus genus in the family Flaviviridae. It is singlestranded 50 nm positive sense RNA virus with six majorgenotypes causing hepatitis C [2, 3]. It is reported that small number of cases infected with HCV clear the virus from theirbodies during the acute phase of infection but majority of the patients infected with HCV developschronic hepatitis C [4], which further develops livercirrhosis with an high risk of the progress of hepatocellular carcinoma [2, 5, 6].

There are about 170 million patients with HCV in the world and three to four million individuals are diagnosed every year [7, 8]. Pakistan, a developing nation of 170 million people has alarming rate of outbreaks of hepatitis C virus [9, 10] which need proper detection by using advance techniques laboratory diagnosis of HCV infection is usually made on the basis of the detection of circulating antibodies. Serological tests for the detection of antibodies to HCV are generally classified as screening tests or confirmatory tests. The most widely used screening test is ELISA but it detects only the anti-antibodies in the blood that does not indicate the status of the infection i.e. either the infection is in active form or not. So for the confirmation PCR technique must be used to detect the HCV RNA.

MATERIALS SAND METHODS

Sampling: The study included individuals from different regions of Pakistan. A total of 300 individuals were tested for HCV. Two techniques were used for this purpose i.e. ELISA and PCR.

ELISA: Sera were tested for anti-HCV antibodies by ELISA. All the ELISA positive samples were processed for RNA extraction.

RNA Extraction and PCR: HCV RNA was extracted from 100 µl serum sample by using Ana-gen RNA extraction kit (Ana-gen, USA) according to manufactures’ instructions. cDNA was prepared by Reverse transcription PCR using M-MLV reverse transcriptase (Fermentas, USA).
The amplified cDNA was further subjected to two rounds of PCR amplifications using nested primers [11]. The conditions for the first round PCR were as follows: An initial denaturation step at 94°C for 2 minutes followed by 35 cycles of 94°C for 40 seconds, 55°C for 40 seconds and 72°C for 1 minute performed in a thermal cycler (Eppendorf, Germany). The conditions for the 2nd round PCR were the same except that a different set of inner primers was used and the annealing temperature was lowered to 50°C in order to amplify the 1st round product.

**Gel Electrophoresis:** All the PCR products (first and second rounds) were analyzed on 2% agarose gel prepared in 0.5% TAE buffer, stained with ethidium bromide (10 mg/ml) as fluorescent dye. Gels were photographed using Alpha quant (Alpha Innotech). A 100-bp DNA ladder (Fermentas Technologies USA) was used as DNA size marker.

**RESULTS**

Total 300 individuals were registered for the current study. Out of these, 183 (61%) were ELISA positive and 117 (39%) were negative. Out of total ELISA positive patients, 89 (49%) were females and 94 (51%) were males. These ELISA positive samples were further analyzed by PCR. Out of 89 ELISA positive females, 41 (46%) were PCR positive and 48 (54%) were PCR negative. Out of 94 ELISA positive males, 32 (34%) were PCR positive and 62 (68%) were PCR negative (Table 1).

ELISA positive patients were categorized into two groups: group-I the patients (< 40 years) and group-II (≥ 40 years) of age were included. Total 111 were observed in group I. Out of 111, 47 (42%) were females and 64 (58%) were males. Out of these 47 females, 20 (43%) were positive and 27 (57%) were negative for PCR. Out of 64 males, 22 (34%) were positive and 42 (66%) were negative for PCR. Total 72 were observed in group II. Out of 72, 40 (56%) were females and 32 (44%) were males. Out of these 40 females, 21 (53%) were positive and 19 (47%) were negative PCR. Out of these 32 males, 10 (31%) were positive and 22 (69%) were negative for PCR.

Area wise analysis showed that, out of totals 183 ELISA positive patients, 54 (29%) belonging to Swat were ELISA positive, 14 (26%) were PCR positive and 40 (74%) were PCR negative patients. Out of 183 ELISA positive patients, the patients belonging to Mardan, 15 (88%) of them were ELISA positive patients, 12 (80%) from those were PCR positive and 3 (20%) were PCR negative. Out of total 183 patients, the patients belonging to Lahore, 30 (16%) of them were ELISA positive patients, 13 (43%) from those were PCR positive and 17 (57%) were PCR negative.

Out of total 183 patients, the patients belonging to Faisalabad, 28 (15%) of them were ELISA positive patients, 12 (43%) from those were PCR positive and 16 (57%) were PCR negative. Out of total 183 patients, the patients belonging to Dir, 32 (17%) of them were ELISA positive patients, 14 (44%) from those were PCR positive and 18 (56%) were PCR negative.

**Table 1: Summary of the Studied Subjects and gender wise distribution.**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total</th>
<th>ELISA Positive</th>
<th>PCR Positive</th>
<th>PCR Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>183</td>
<td>89</td>
<td>32</td>
<td>57</td>
</tr>
<tr>
<td>Males</td>
<td>117</td>
<td>94</td>
<td>62</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>183</td>
<td>73</td>
<td>110</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Hepatitis C virus is one of the main causes of chronic hepatitis in developing countries. In this study 300 serum samples were tested by two techniques i.e. ELISA and PCR. The study revealed that out of these 61% was ELISA positive and 39% were ELISA negative samples. But for the active HCV infection the ELISA positive samples were retested by PCR which revealed that 23% have HCV RNA in their blood. Presence of anti-HCV and the absence of HCV RNA in the blood may be attributed to the self limiting nature of the disease in some people or it may be due to the presence of antibodies against HCV in treated subjects.

Out of total ELISA positive patients, 49% were females and 51% were males. Out of ELISA positive females, 46% were PCR positive and (54%) were PCR negative. Out of ELISA positive males, 34% were PCR positive and 68% were PCR negative. It means females have more chances of active HCV infection.

This study proposed that from total 300 individuals, 183 were ELISA positive, it means that these individuals have HCV anti-antibodies in their blood and 73 among them were PCR positive meaning that the disease is in its active infectious form.

ELISA positive patients were categorized into two groups: group-I the patients (< 40 years) and group-II (≥ 40 years) of age were included. Total 111 were observed in group I. Out of 111, 47 (42%) were females and 64 (58%) were males. Out of these 47 females, 20 (43%) were positive and 27 (57%) were negative for PCR. Out of 64 males, 22 (34%) were positive and 42 (66%) were negative for PCR. Total 72 were observed...
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Area wise analysis showed that, out of total 183 ELISA positive patients, the patients belonged to Swat, 54 of them were ELISA positive i.e. these having just antibodies in blood but no virus found, 14 were PCR positive i.e. having active HCV infection and 40 were PCR negative patients. This proposes that the individuals from Swat are at greater risk to both chronic and acute infection of HCV. Then the rest of the cities are at descending order having lower chronic and acute infection i.e. the patients belonged to Lahore, 30 of them were ELISA positive patients, 13 from those were PCR positive and 17 were PCR negative. The patients belonged to Faisalabad, 28 of them were ELISA positive patients, 12 from those were PCR positive and 16 were PCR negative. The patients belonged to Tohtak Singh, 24 of them were ELISA positive patients, 8 from those were PCR positive and 16 were PCR negative. The patients belonged to Dir, 32 of them were ELISA positive patients, 14 from those were PCR positive and 18 were PCR negative. The patients belonged to Mardan, 15 of them were ELISA positive patients, 12 from those were PCR positive and 3 were PCR negative. It was concluded from our study that ELISA should not be the only method considered for the diagnosis of HCV but rather PCR must also be done in order to confirm the state of infection.

REFERENCES