UTILITY OF HCV CORE ANTIGEN ELISA IN THE SCREENING FOR HEPATITIS C VIRUS INFECTION IN PATIENTS ON HEMODIALYSIS

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Abstract

An enzyme immuno assay for hepatitis C core antigen was recently developed and its performance was compared with that of the hepatitis C virus (HCV) RNA in the screening of HCV infection in patients on hemodialysis. One hundred and eleven chronic renal failure patients undergoing hemodialysis between May 2003 and October 2004 were included in the study. All the patients were tested for anti HCV antibody, core antigen and RNA. Fifteen patients were anti HCV antibody positive, three patients were positive for HCV core antigen and RNA, three patients were positive for HCV RNA, while two patients were positive only for core antigen but negative for RNA. In anti HCV antibody positive patients, the core antigen was negative while the viral RNA continued to be present. Hence, relying solely on a single HCV core antigen assay may not be useful for a definite diagnosis of early HCV infection. The sensitivity and specificity of the assay were 60% and 83% respectively, while the positive predictive value was 14.3%, negative predictive value was 97.7% and the efficiency was 81.9%.

Key words: HCV, core antigen, RNA, chronic renal failure

Hepatitis C virus (HCV) is the major cause of parenterally transmitted non-A non-B hepatitis. Patients receiving renal replacement therapy, especially haemodialysis (HD) are recognized as a high risk group for HCV infection compared with the general population. HCV infected patients are at increased risk for morbidity and mortality and serve as a reservoir of HCV to haemodialysis staff. The risk for death in HCV infected haemodialysis patients compared with non-HCV infected haemodialysis patient is greater.

Virological diagnosis and monitoring of HCV infection is based on two categories of laboratory tests, namely serologic assays detecting specific antibodies to HCV (anti-HCV) and assays that can detect, quantify or characterize the components of HCV viral particles such as HCV RNA.

Early stages of the infection are missed because the antibodies develop only after one and half months of infection and the tests for anti HCV antibody may be negative in the initial period before the seroconversion phase. This window phase can be longer in haemodialysis patients as these patients are severely immunocompromised. In such situations, the HCV RNA detection by polymerase chain reaction (RT-PCR) is highly sensitive and is a reliable test in the early diagnosis of HCV infection. However, since the test is expensive and needs certain amount of expertise, it is not used routinely in the diagnostic laboratories, especially in developing countries.

The latest breakthrough in diagnosing early HCV infection is by detecting HCV core antigen (HCV cAg) that is present during the early stage or before seroconversion. A positive correlation between the concentration of the HCV cAg and HCV RNA in anti-HCV negative specimens has been shown in earlier studies. The aim of the present study was to assess the utility and performance characteristics of HCV cAg ELISA in early detection of HCV infection in haemodialysis patients.

Materials and Methods

One hundred and eleven chronic renal failure patients undergoing haemodialysis between May 2003 and October 2004, at the Nizam’s Institute of Medical Sciences, Hyderabad, Andhra Pradesh, India, were included in the study. All the patients were tested for anti HCV antibodies, HCV cAg and HCV RNA and were further followed for two to five months.

Blood samples were collected from all the patients before the initiation of the dialysis and monthly thereafter for 6 months. Serum for anti HCV and core antigen and plasma for RNA were separated from blood samples and stored at -20°C and -70°C respectively, until tested. The assays that were used in the screening of HCV infection included HCV antibodies by Ortho HCV 3.0 ELISA system (Ortho clinical diagnostics), HCV cAg by Ortho Antibody to HCV CAg ELISA Test System (Ortho clinical diagnostics) and HCV RNA by AMPLICOR HCV test version 2.0 (Roche diagnostics). The tests were performed as per the manufacturers’ instructions. Any positive result was confirmed by repeat testing.

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Results

Fifteen out of 111 patients were anti HCV antibodies positive. They were also positive for HCV RNA while the core antigen was negative. Of the remaining 96 patients, five were positive for core antigen three of which were also positive for HCV RNA. The remaining two core antigen positive patients were negative for HCV RNA, indicating a false positive result with core antigen. Three out of 96 patients were detected positive for HCV RNA and negative for core antigen during the follow up. Consolidated results of all the three assays are shown in table 1. Performance parameters of the core antigen were calculated taking PCR as a gold standard. Performance parameters of the HCV antigen are shown in table 2.

Discussion

Antibody tests fail to identify HCV infected subjects before seroconversion or during the window period, when specific antibodies have not yet been produced or are in low titres. However, the virus continues to replicate and RNA can be detected in the plasma. Window period may extend up to two months in immunocompetent subjects or as long as 6 to 12 months in immunodeficient patients. Patients on haemodialysis or immunocompromised patients infected with HCV produce fewer antibodies. Direct measurement of the HCV virus in the serum of the infected individual remains the gold standard in the diagnosis of HCV infection. HCV RNA is detectable in the serum within one to two weeks after the infection.

A new test has recently been developed by Ortho clinical diagnostics, to detect the HCV core protein (HCV core antigen), which is coded by one of the most conserved regions of the virus genome. HCV core antigen testing permits the detection of HCV infection about 1.5 months earlier than the HCV antibody screening tests and an average of only two days later than quantitative HCV RNA detection in individual specimens. HCV core antigen becomes undetectable with the appearance of anti HCV in the serum. These antibody positive specimens remain RNA positive. In our study, 15 anti HCV positive patients were HCV RNA positive and core antigen negative.

A false positive core antigen result was obtained in two patients in our study. Core antigen may not be detected in all the preseroconversion viraemic specimens in the blood donors. Peterson et al observed that only 87% of HCV RNA positive specimens were positive for core antigen. In another study, core antigen was present only in 88% HCV RNA positive preseroconversion specimens of patients on hemodialysis and in 83% of HCV RNA positive but antibody negative blood donors. In our study, three patients were positive for HCV RNA and negative for core antigen indicating that core antigen may not be sensitive enough to detect low levels of antigens in early viraemic preseroconversion phases. Hence, relying solely on a single HCV core antigen assay may not be useful for a definite diagnosis of early HCV infection.

References


Table 1: Consolidated results of RT - PCR, core antigen ELISA and anti HCV ELISA for detection of HCV infection among dialysis patients (n = 111)

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab+RNA+/Ag-</td>
<td>15</td>
<td>13.5</td>
</tr>
<tr>
<td>Ag+RNA+/Ab-</td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>Ag+/Ab-RNA-</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>RNA+/Ab-Ag-</td>
<td>3</td>
<td>2.7</td>
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</tbody>
</table>

Ab: Antibody, Ag: Antigen

Table 2: Performance parameters of the core antigen ELISA using HCV RT PCR as gold standard (n = 111)

<table>
<thead>
<tr>
<th>Core Antigen</th>
<th>positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR positive</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>PCR negative</td>
<td>2</td>
<td>88</td>
</tr>
</tbody>
</table>

Sensitivity-60%, Specificity -83%, Positive Predictive Value - 14.3%, Negative Predictive Value -97.7%, Efficiency -81.9%.


