Detection of HDV RNA in HBV PCR Positive and HBV PCR Negative Patients

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Abstract: HDV (Hepatitis D Virus) infects only patients that are previously infected by HBV (hepatitis B virus). It proliferates only in the presence of HBV because it is considered as a sub satellite virus which is dependent on HBV. As HDV is an incomplete RNA virus which requires the assistance of HBV to propagate in host. HDV causes co-infection or super infection along with harsh complication as compared to only HBV infection. This study was to found out the presence of HDV in both HBV Positive as well as HBV Negative cases. To find out either HDV is always dependent on HBV for causing infection or there may be some possibilities of such out of character infection, without Co-infection with HBV. For the study total 109 HBsAg ELISA positive samples from different geographical regions of the Pakistan were received. HBV DNA PCR positive samples were only further utilized to detect the presence of HDV RNA. For which, HBV DNA and HDV RNA were extracted and amplified using RT-PCR, (reverse transcriptase polymerase chain reaction), nested PCR and real-time PCR. Total 109 HBsAg positive samples, Out of which HBV DNA was detected in total 52 (47.7%) samples of different patients. Of these 52 patients, HDV RNA was observed in 26 (23.8%) patients. Out of these 26 positive cases of HDV, 10 (9.1%) were females and 16 (61.5%) were male patients. The dual infection percentage was higher in males as compared to female patients. Total 86(78.8%) patients were under 40 years and 23(21.1%) were over 40 years of age. 17 were dual infection cases. And 9 were such cases in which HDV was Positive but HBV was Negative which we named them as Rare infectious cases. In Conclulsion HDV is not all time dependent on HBV but there may be such exceptional cases, in which HDV acts as a complete virus. another possibility that may be HBV was present there but in suppressive form.

Key words: Hepatitis B • Hepatitis D • Ribonucleic Acid • Polymerase Chain Reaction • Enzyme Linked Immunosorbant Assay • Deoxyribonucleic Acid

INTRODUCTION

HDV is known as delta agent that is misshapen and imperfect RNA virus its replication and expression is reliant on the presence of HBsAg. HDV is considered to be dependent on HBV because it can promulgate only in its existence [1]. HDV is mostly present in Russia, South America, Africa, Romania, Southern Italy including Mediterranean region [2] In 1980 Rizzetto first discovered HDV in those patients who were already infected by HBV [3]. As HDV infects liver cells, thus creating a lot of medical complications which ultimately leads to hepatocellular carcinoma. It is Pandemic, predominantly in Pakistan. HDV’s particle size is about 36-nm and for completing its envelope and transmission it requires hepatitis B (HBsAg) surface antigen [4]. The genome of HDV is a circular, negative sense single-strand RNA, which is more or less 1700 nucleotides in length [5]. These nucleotides amass with two viral proteins HDAg-L and HD Ag-S to figure a ribonucleoprotein [6]. RNA editing is a process in which these Two Hepatitis D Ag proteins are translated from viral mRNA [7]. The dual infection of HDV and HBV occurs in the type of co-infection or as a super-infection. In union with HBV, HDV produces significantly more strict illness than HBV alone [8]. HDV is now quite well known in inducing a spectrum of acute and chronic liver diseases [9]. Individuals having co-infection of HBV-HDV might have more severe acute disease and privileged risks of fulminant hepatitis, cirrhosis and hepatocellular carcinoma (HCC) than those having HBV infection alone [10, 11].

Several studies proposed that the majority of the people acquired HDV infections through parenteral and sexual routes [12, 13]. In addition to this intravenous drug
users (IDU) as compared to the non-IDU population the reported seroprevalence of HDV infection along with HBV carriers is considerably higher [14]. The present study was to find out the presence of HDV in both HBV Positive as well as HBV Negative cases. To find out either HDV is always dependent on HBV for causing infection or there may be some possibilities of such rare infections of HDV without Co-infection with HBV.

MATERIALS AND METHODS

Sample Collection: Total 200 patients (from different regions of the Pakistan at Genome Centre for Molecular Diagnostics and Research, Lahore for HBV routine diagnosis were registered. Blood samples (5mL) were collected in sterile blood collecting tubes.

Separation of Serum: Serum was separated out and ELISA was done for the presence of HBV antigen. All HBV-ELISA positive samples were selected and presence of HBV DNA and HDV RNA were analysed by PCR.

Analysis of HBV DNA

Extraction of HBV DNA: For the detection of HBV DNA in the serum samples viral DNA was extracted and checked on agarose gel for the good capitate. This was used as a template for the PCR Amplification of HBV genome.

PCR Amplification of HBV Genome: For the amplification of HBV genome, specific primers were designed by the primer designing software “Primer 3” and amplification cycle parameters were optimized by following the standard protocols.

Analysis of HDV RNA: Presence of HDV RNA was analyzed in both HBV-PCR-positive and HBV-PCR-negative serum samples.

Extraction of HDV RNA: For the detection of HDV RNA, viral RNA was extracted and cDNA was prepared with specific primer. This acted as template for PCR.

PCR Amplification of HDV: The viral gene was amplified by using the specific gene primers. These primers were designed by the primer designing software “Primer 3” by following standard protocols amplification cycle parameters were optimized. On agarose gel the PCR products was electrophoresed and stained with ethidium bromide and under UV transilluminator were visualized.

Statistical Analysis: The data was analyzed by statistical formulae in order to evaluate the significance of this co-infection.

Hepatitis B Virus (HBV) PCR

Sample Collection: A total 109 ELISA positive serum samples were used to evaluate the prevalence of HBV/HDV co-infection of hepatitis and for the detection of HDV in HBV Positive and HBV negative Patients. These serum samples were received at Genome Centre for Molecular Diagnostics and Research, 226-R1, Johar Town, Lahore for HBV viral load from various geographical regions of Pakistan. Serum samples were first quantified for the presence of HBsAg and HBV DNA. Only those samples showing HBsAg and HBV DNA positivity were further tested for the presence of HDV RNA in the current study.

Fig. 1: studied subjects and ratio of co infection
Table 3: Summary of studied subjects and results

<table>
<thead>
<tr>
<th>Total studied subjects = 200</th>
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<tr>
<td>HBV ELISA positive = 109</td>
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<tr>
<td>HBV ELISA negative = 91</td>
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<tr>
<td>HBV DNA PCR Positive = 52</td>
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<tr>
<td>HBV DNA PCR Negative = 57</td>
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<tr>
<td>HDV RNA PCR Positive = 17</td>
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<td>HDV RNA PCR Positive = 9</td>
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**DISCUSSION**

Hepatitis Delta virus (HDV) infection is present worldwide, it infects only those patients that are previously tainted by HBV [7]. As it is now a well-known fact that HDV is an imperfect virus, comprising RNA particles of nucleoprotein in virion-like form, present in patients with acute hepatitis B and chronic hepatitis that requires the presence of a hepadna virus (HBV) for full replication. That is why for its penetration into the hepatocytes and assembly of virion, it needs the assistance of HBV that provides the viral coat surface antigen and can survive only in the presence of HBV (co-infection or super-infection) [14].

The HDV is a small, spherical virus having diameter of 36 nm. It consists of an outer coat containing three HBV envelope proteins called (large, medium and small) hepatitis B surface antigens and host lipids adjoining the inner nucleocapsid. Distinct spikes on their outer surface are missing and are possibly icosahedral [15]. When the virus particle is disrupted with non-ionic detergents, an internal nucleocapsid is released and HDAg becomes detectable the 19nm nucleocapsid contains about 60 copies of HDAg in its two forms (24 and 27 kDa) and HDV genomic RNA [1].

Present study was conducted in 200 patients. PCR was performed for the detection of HBV DNA content and it was found that, out of total 109 ELISA Positive samples, 52 were HBV PCR positive and 57 were HBV PCR negative. Out of total HBV PCR positive patients 67% were males and 33% were females. Out of total HBV PCR negative patients 63% were males and 37% were females.

PCR was performed for the detection of Hepatitis D DNA content and it was found that. Out of total samples 24% were HDV PCR positive and 76% were HDV PCR negative. Out of total 26 HDV PCR positive 62% were males and 38% were females. Out of these 26 HDV PCR positive 65% were those with dual infection or Co-infection of both HBV and HDV positive. Out of these 26 dual cases 59% were males and 41% were females. Out of these 26 HDV PCR positive patients 35% were rare infectious cases of (HDV PCR postive but HBV PCR negative) and out of these 9 rare infectious cases 67% were males and 33% were females.

HDV has a defective RNA virus and it requires HBV for causing infection. So we can assume that there may be some rare cases in which HDV acts as complete virus and can cause infection by itself. It can also be assumed that circumstances and factors behind it, that HDV is suppressing the production of HBsAg, in these cases HBV viral content is below the threshold of detection. Since the diagnosis of patients with dual infections (HBsAg and HDV) is poor, screening for anti HDV in all HBsAg positive patients of chronic liver disease is suggested. In addition the poor predictive result of dual infections also points out the importance of carrying out widespread childhood immunization against HBV to avoid the development of a more severe disease due to associated HDV infection. HBV may be present in Hepatocytes but in suppressive form. We observed that the ratio of these rare infectious cases of HDV positive but HBV negative was greater in males than that of the females and mostly found in age group I (> 40).
Suggestions: We can suggest that patients with HBV DNA negative should be analyzed for HDV viral content by PCR, proper therapy should be applied. It is also suggested that molecular diagnosis of HDV RNA should be independent of any other viral infection, so person who already has HBV infection, exposure to blood should be strictly avoided.

REFERENCES