PREVALENCE OF HEPATITIS C VIRUS (HCV) COINFECTION IN HIV INFECTED INDIVIDUALS IN SOUTH INDIA AND CHARACTERIZATION OF HCV GENOTYPES

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Abstract

Purpose: To determine anti-HCV antibodies and genomic subtype of HCV in 1487 confirmed human immunodeficiency virus (HIV) positive samples. Methods: A total of 1487 confirmed HIV-positive samples were tested for anti-HCV antibodies by using a third generation ELISA kit (Ortho 3.0) and by RT PCR for HCV. HIV and HCV coinfected samples were selected for HCV genotyping by RFLP and subtyping with NS5-type specific primers. Results: A total of 1487 HIV-infected serum samples were screened for HCV infection, of which, a 1443 (97.04%) were negative and 45 (3.02%) were coinfected. HIV–HCV coinfection was predominant in the age group 41–50 years (51.1%). HCV genotyping and subtyping was done for the 45 HCV RNA-positive specimens of which genotype 1 was observed in 31 (68.8%) and genotype 3 was observed in 14 (31.1%) subjects. Further subtyping analysis showed the genotype 1b in 23 (51.1%), 1a in eight (17.7%), 3a in 10 (22.2%) and 3b in four (8.8%) subjects. Conclusion: HIV and HCV seroprevalence is higher in South India, and the most prevalent genotype in coinfection was genotype 1b.

Key words: Coinfection, genotyping, human immunodeficiency virus, hepatitis C virus

India is the second largest populated country in the world with more than 1.1 billion people.[1] Globally, a total of 39.5 million were living with HIV in 2006, of whom approximately 5.7 million (3.4–9.4 million) were in India.[2] Acquired immunodeficiency syndrome has grown more rapidly than the scientific progress of understanding how to control the main causative agent. Globally, hepatitis C virus (HCV) has infected more than 170 million people[3] and thus represents a viral pandemic seven times more widespread than infection with the HIV. It is estimated that in India approximately 1.8–2.5% of the population is presently infected by HCV[4] and about 20 million people are already having HCV infection.[5] Prolonged survival of HIV-infected patients coinfected with HCV may become an important clinical problem. In the United States and in European countries, it is estimated that approximately the prevalence of HIV/HCV coinfection in the HIV population ranges from 30 to 50%.[6] HIV and HCV show some common biological features like both are RNA viruses and both show a large heterogeneity of their viral genomes producing various genotypes. These viruses also have some differences, like HCV belongs to the Flaviviridae family and HIV to the Retroviridae family. Filoviruses have a single RNA strand whereas retroviruses have double RNA strands. The HIV-RNA, transcripted to DNA by the reverse transcriptase (RT), integrates in the infected cell’s genome, constituting the integrated provirus; this integration is the cause of the irreversibility of HIV infection. In contrast, the HCV genome does not integrate into the cell’s genome and the replication of the virus takes place in the liver cell’s cytoplasm. This non-integration makes it easier to eradicate HCV and hence to cure the infection. These viruses share similar routes of transmission like through blood and blood products, sharing of needles to inject drugs and sexual route.

Previously, blood transfusion was a major mode of HCV transmission but now that donor blood is thoroughly screened for the virus, majority of the cases are injectable drug users. HCV is also transmitted perinatally, by improperly sterilized dialysis equipment (68%[7] of the cases) and by unprotected sex with an infected partner. Cohort studies report that men who have sex with men (MSM) and those with other sexually transmitted infections are at a greater risk of contracting HCV from unprotected sex. An estimated 20% of people with chronic HCV infection will progress to cirrhosis over a 20–50-year interval.[8] A greater proportion of HIV/HCV coinfected people may progress to cirrhosis (serious liver scarring) and liver disease than those with HCV alone.[9] HIV-infected individuals have a high probability of getting coinfected with HCV. HIV disease progression and enhanced immunosuppression has a direct bearing on the natural history and pathogenesis of these infections. Although there have been some reports of the prevalence of HCV infection and HIV infection in various populations in India, none have looked specifically at the prevalence of active HCV coinfection (i.e. HCV RNA positive) and HCV genotype and subtype in HIV-positive...
individuals.

The objective in this study, therefore, was to determine the prevalence of HCV antibodies in the HIV-infected Indian population. This is aimed at providing the baseline data on HIV/HCV coinfection. In order to gain a better understanding of the public health issues in these countries, we evaluated the anti-HCV antibody and genotype and subtype of HCV infection in 1487 confirmed HIV-positive individuals.

Materials and Methods

A total of 1487 confirmed status of HIV-positive samples (as per the World Health Organization strategies) were collected from different parts of South India and were anonymously tested for HCV markers. The study period was from January 2005 to February 2008.

Virological assays

The sera were screened for anti-HCV antibodies using a third generation ELISA kit (Ortho 3.0; Ortho Clinical Diagnostics, Raritan, NJ, USA). The Ortho HCV 3.0 ELISA test is a qualitative assay, each microwell being coated with a combination of recombinant HCV antigens c22-3, c200 and NS5. The amino acid sequence of the three HCV recombinant proteins are c22-3 AA # 2–120, c200 AA # 1192–1931, NS5 AA # 2045–2995. The assay was carried out as per the standard test procedure mentioned by the manufacturer.

Nucleic acid extraction

HCV RNA was isolated from the serum by the guanidinium isothiocyanate (GITC)-acid–phenol method. Briefly, 200 µL of serum was mixed with 500 µL lysis solution (4M GITC, 0.75 M sodium acetate, 0.5% Sarcosyl and 0.1 M 2-mercaptoethanol), 500 µL water-saturated phenol and 200 µL chloroform–isoamyl alcohol (24:1). RNA was precipitated from the aqueous phase sequentially with isopropanol and resuspended in 25 µL of diethyl pyro carbonate treated water. Before RT PCR the RNA was denatured by heating at 95°C for 2 min, followed by rapid chilling. Amplification by PCR was carried out essentially by the method of Das et al. Briefly, the 50 non-coding region amplification was carried out with 25 pmole each of the primers, 10X Taq buffer (Mg2+ free), 2.5 mM MgCl2, 200 mM dNTPs, 25 units of ribonuclease inhibitor (RNAsin), 4 units of AMV reverse transcriptase (Promega, Madison, WI, USA), 1 unit of Taq DNA polymerase and RNA template and was made up to a volume of 50 µL. The RT-PCR step was carried out in a single tube using a programmable thermocycler (MJ Research, MA, USA) at 42°C for 1 h, followed by 95°C for 2 min, 35 cycles at 94°C for 30 s, 50°C for 45 s, 72°C for 1 min and a final extension at 72°C for 5 min. First-round PCR was performed using the forward primer 5'-ACTGTCTTCACGCAGAAAGCGTCTAGCCAT-3' and the reverse primer 5'-CGAGACCTCCCGGGCACTCGCAAGCACCC-3'; 10 µL of the first-round PCR product was re-amplified with internal primers (forward primer 5'-ACGCGAGCGTGTCAGCC-ATGGCCTAGT-3' and reverse primer 5'-TCCCCGGGCACCTCGCA-AGCACCCCTATCAGG-3') for another 35 cycles under the same conditions. A negative control, a positive control and a water blank were tested during RNA extraction, reverse transcription and amplification for quality control and to exclude false-positive results in the PCR due to cross contamination. PCR products were analysed on 2% agarose gels followed by staining with ethidium bromide and visualized under a UV trans-illuminator. A 100-bp ladder (Promega) was used as a size marker. Detection of a 256 bp PCR product indicated that the sample was positive for HCV (Fig. 1).

Restriction fragment length polymorphism (RFLP) analysis of the 5` UTR amplified product

The amplified product obtained by RT-PCR was subjected to RFLP analysis by digestion with Hae III, Hinf I and Bst NI enzymes (New England Biolabs, Beverly, MA, USA). The size of the undigested amplicon is 256 bp, which is cleaved separately with these three enzymes. After treatment, the restriction fragments are separated by agarose gel electrophoresis and visualized in gel doc. The restriction patterns of the samples are compared with the predicted patterns to determine the genotype. For generating the above patterns, representative full-length sequences of various genotypes [HCV-1 (1a); HCV-J (1b); HC-G9 (1c); HC-J6 (2a); HC-J8 (2b); Bebel (2c); NZIL (3a); TR (3b)] were selected and the electrophoretypes predicted using RESTRI and DIGEST programs of PCGENE software package. The Hae III and Mva I enzymes were originally used for typing of the HCV isolates from India.

Figure 1: The PCR amplicons of the 5’UTR region from HCV infected patients done on a 2% agarose–TBE gel. Lanes 1–4: 256 bp fragment (5’ UTR); Lane 5: DNA size standards: 100-bp ladder.

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NS5 typing

An expected size of DNA fragments for each genotype is as follows: 1a, 3356 bp; 1b, 143 bp; 2a, 240bp; 2b, 309bp; 3a, 143bp; 3b, 201 bp.

Primer sequences used in the study

1a: 5’-GAGTCACTGAGAGCGSACATCCGTACG-3’
1b: 5’-AGGCCACTGCGGCCTGTCGAGCTGCGAA-3’
2a: 5’-TATGTTCACACGCAAGGCGCCAGA-3’
2b: 5’-GGCTTGTTCCCTGCCTCAAGAGGCCA-3’
3a: 5’-CTCGGACCCCTGACCTTTCT-3’
3b: 5’-CGCGCTAGCGGCGTCTTGC-3’
CA: 5’-CCTGGTCACTAGCCTCCGTGAA-3’ (anti-sense primer for all genotypes).

Results

Of the total 1487 HIV-infected test samples, 1443 (97.04 %) were negative in the Ortho 3.0 EIA assay and 45 (3.02%) were coinfected with HCV. The male predominance was more with 61.5% (915 males) compared to females by 38.4 % (572 females) and the median age was 37 years, ranging from 20 to 55 years. The risk factors were assessed for all the 1487 patients, of whom 1183 (79.5%) were heterosexual, 69 (4.64%) were intravenous drug users (IVDs), 45 (3.02%) were blood transfusion recipients, 115 (7.73%) were haemophiliacs and 75 (5.04%) were unnoticed (Table 1). The majority of the HIV alone-infected patients were from the age group 31 to 40 years (44.2%), followed by the 41–50 years (29.2%), while HIV–HCV coinfection was predominant among patients in age group 41–50 years (51.1%) (Fig. 2).

Of the 45 HIV/HCV coinfected patients, 44 were anti-HCV positive. The remaining 1443 anti-HCV seronegative cases along with 44 anti-HCV positive cases (total n= 1487) were selected for qualitative HCV RNA testing by PCR and one sample (0.06%) was found to be positive for HCV RNA in 1443 anti-HCV negatives cases and all 44 anti-HCV were HCV RNA positive. HCV genotyping and subtyping was done for 45 HCV RNA-positive samples of which genotype 1 was observed in 31 (68.8%) and genotype 3 was observed in 14 (31.1%) subjects. Further subtyping analysis shows the genotype 1b in 23 (51.1%), 1a in eight (17.7%), 3a in 10 (22.2%) and 3b in four (8.8%) subjects (Fig. 3, Table 2).

Among the coinfected patients, the predominant risk was observed among haemophiliacs (42.2%) followed by IVD’s (37.77%), heterosexual (13.3%) and blood transfusion recipients (6.6%). HIV-1 or HIV-2 status was determined by using the HIV TRI-DOT for the 1487 samples of which 1445 (97.1%) were HIV 1, followed by HIV 1 and 2 dual positives 23 (1.5%) and HIV-2 in 19 (1.2%).

Discussion

All individuals infected with HIV should be screened for the presence of HCV infection.[13] Screening is done with anti-HCV EIA followed by a confirmatory test or qualitative RNA test for positive patients. HCV RNA is usually detectable within 2 weeks after infection. HCV antibodies usually develop 6 weeks to 6 months after infection. All

Table 1: Characteristics of human immunodeficiency virus and HIV-HCV coinfected patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>Sexual</th>
<th>IVDs</th>
<th>BT</th>
<th>Haemophilic</th>
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<td>876</td>
<td>566</td>
<td>1177</td>
<td>52</td>
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<td>6</td>
<td>6</td>
<td>17</td>
<td>3</td>
<td>19</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 2: Age distribution of HIV infected and HIV-HCV coinfected patients

Figure 3: Agarose gel electrophoresis showing the PCR amplicons of different genotypes of HCV in the NS5 region. Lane 1: DNA size standards: 100-bp ladder; Lane 2: genotype 1a; Lane 3: genotype 3a; Lane 4: genotype 3b; Lane 5: genotype 1b; Lane 6: genotype 3b
positive HCV antibody results should be confirmed by testing for HCV RNA. Anti-HCV test is not adequate to diagnose chronic HCV infection, however, as some patients spontaneously clear the virus without treatment but remain antibody positive, a HCV viral load (HCV RNA) test is necessary to confirm or rule out chronic HCV infection. Studies have reported spontaneous viral clearance rates from 15 to 45% in HIV-negative persons.\cite{7} Although spontaneous viral clearance is less likely to occur among people who are HIV positive, some, particularly those with higher CD4 cell counts, do spontaneously clear HCV infection.\cite{14}

In Indian studies, there is a paucity of information of HIV/HCV coinfection. The global coinfection studies reported so far have been variable, depending on the geographical area, type of exposure and the risk behaviour groups. The present investigation was a prevalence study, not an incidence study. In this study, we have utilized 5’UTR and NS5 markers for HCV genotyping. There have not been any investigations reporting the presence of HCV genotypes and subtypes in HIV/HCV coinfected cases in India. In our study, prevalence of coinfection with HCV among known HIV patients was 3.02%, which is very low in comparison with the western data.\cite{15} This study also indicates that HIV-infected patients are at a higher risk (3.02%) of HCV infection, which is higher than the overall prevalence of HCV in the general population of South India.\cite{4}

Details of interaction between HIV and HCV still remain controversial and need a better understanding. HIV and HCV coinfection is emerging as an important and frequent finding in patients seeking therapy for one or the other viral entity. The fact that both viruses share a similar route of transmission and mechanisms of epidemic spread appears to be the most important reason for the growing nature of the coinfection. Coinfection with HCV has resulted in chronic liver damage in HIV patients, and more than 15% have developed severe liver damage or cirrhosis.\cite{15}

Within India, HCV coinfection among HIV-infected patients has been reported infrequently from region to region. A study by Bhattacharya et al.\cite{16} has shown a 6% prevalence of HCV infection and a 21% seroprevalence of HCV among HIV-infected individuals. These numbers probably do not represent the general population, but are of concern. Another study by Baveja et al.\cite{17} has shown a prevalence rate of 9.64%. However, our study indicates that HIV-infected patients are at a high risk of coinfection, as evident from the high prevalence of HCV (3.02%).

Uncertainties remain regarding the real effect of coinfection with HIV and HCV on the progression and outcomes of these viral infections. The situation is largely due to difficulties in performing accurate natural history studies, particularly in the constantly developing field of HIV medicine.

Knowledge of genotypes helps in predicting therapeutic response and the choice of treatment duration.\cite{18} In our study, genotyping and subtyping of HCV in HIV/HCV coinfection has been assessed and genotype 1b was found to be the most common, which correlates with studies reported in the United States,\cite{15} followed by 1a, 3a and 3b. The different genotypes are relevant to epidemiological questions, vaccine development and clinical management of chronic HCV infection. The geographical variations in the genotypes could be related to the way patients have acquired the HCV infection. Genotype 1b is spread more frequently by blood transfusion while genotype 1a is more frequently related to the high risk behaviour. Previous studies have found that patients coinfected with HCV genotype 1 had more liver-related deaths than patients coinfected with the other genotype. In our study, all the coinfected patients were HIV 1 infected; none were HIV 2 or dual HIV 1 & 2 positive. Another interesting finding was that none of them had infection with more than one HCV genotype.

The present study had some limitations. First, this was a prevalence study, not an incidence study. Thus, the real disease duration could not be estimated. Second, it could not evaluate the relationship between HCV genotypes and anti-HCV therapy outcome because none of the coinfected patients reported the use of antiviral therapy for HCV. Third, liver function tests were not done.

Where there is a scarcity of information on HIV/ HCV prevalence, particularly in developing countries like India, our study on the prevalence and characterization of prevailing HCV genotypes among HIV-infected individuals is sure to provide a useful insight to researchers working on HIV/HCV coinfection. Finally, further studies of HIV/HCV coinfection are needed to explore in more detail the current prevention strategies and the therapeutic management of this condition.

References


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