Genotypes and Viral Load of Hepatitis C Virus among HIV Patients Visiting ART Center of Nepal

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Abstract

HIV patients are prone to various other complications, disorder and coinfections mainly HCV coinfection. HCV coinfection increases up the frequency and severity of liver disease. The aim of this study was to find the prevalence of HCV coinfection among HIV patients, HCV viral load and HCV genotypes in Nepal. This cross-sectional study was conducted from Oct 2021 to May 2022 in the HIV seropositive patients attending the Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. All the demographic and blood samples were collected from the patients. The RNA was extracted and estimated the viral load and HCV genotypes by standard Q-PCR protocols. A total 203 PLHIV were enrolled in the study out of which 115 (56.6%) had HCV co-infection by Q-PCR. Most commonly HCV infection was higher in male 110 (95.6%) than female 5 (4.4%). HCV viral load < 34 IU/ml was observed as higher number 56 (27.6%) then 31 (15.3%) had among 34-3000 IU/ml, 17 (8.4%) had 3000–30000 IU/ml, 8 (3.9%) had 30000–300000 IU/ml and only 3 (1.5%) had >3000000 IU/ml HCV viral load. Genotype 3 were most prevalent 36 (61.1%) then genotypes 5a, 6, 1 and 1a as 13 (22.1%), 6 (10.2%, 2 (3.3%) and 2 (3.3%) respectively. Genotype 3a was most prevalent genotypes and the presence of HCV genotype 6 in Nepalese patients may indicate a shift of HCV genotypes.

Introduction

Due to immune suppression, Human Immunodeficiency Virus (HIV) patients are prone to various other complications, disorder and coinfections (Jarrar & Song, 2018; Kinkel et al., 2015). Hepatitis C Virus (HCV) coinfection has emerged as a major cause of non-AIDS-related morbidity and mortality in HIV-positive patients (Mandorfer, Schwabl, Steiner, & Reiberger, 2016). Hepatitis C is a liver inflammation caused by the virus of the HCV. As opposed to the other hepatitis viruses A, B, D, or E, infection with the HCV leads, in a high number of cases, to chronic liver disease and may not be symptomatic for a relatively long period of time. For this reason, most patients are not aware of their HCV infection (BERMAN, Alter, Ishak, Purcell, & Jones, 1979).

HIV-HCV coinfection is common since both share the same transmission routes (Sulkowski, 2008). Globally, it is estimated that of the 36.7 million people infected with human immunodeficiency virus (HIV), 6.3% are co-infected with hepatitis C virus (HCV) (Mayanja, Luboobi, Kasozi, & Nsubuga, 2020). Coinfection adds more severity for the two diseases involved. HIV accelerates the progression of HCV co-infected patients (Carvalho & Pinto, 2014). Besides hepatic damage, which is accelerated in the presence of HIV-associated immunosuppression, HCV may contribute to disease in co-infected individuals by potentiating immune activation and chronic inflammation, which ultimately account for an increased risk of cardiovascular events, kidney disease, and cancers in this population (Mayanja et al., 2020). Compared with HCV infection alone, co-infection with HIV increases HCV levels in plasma (Suzman et al., 2008). Furthermore, HAART regimens have been found to be hepatotoxic and are associated with transient flares of HCV replication thus increasing liver damage in chronic hepatitis C patients often leading to rapid progression of liver fibrosis (Macías et al., 2004).
HCV is currently classified in seven genotypes (HCV-1 to HCV-7) and multiple subtypes according to their genetic sequence and the occurrence of the HCV genotype is variable globally (Scheel et al., 2012). Another nomenclature for the classification of HCV is proposed which defines three major types, 1, 2 and 3, with each type being divided into two subtypes, a and b (Chan et al., 1992; Simmonds et al., 1993). HCV-1 is predominant in Australia, Europe, Latin America, and North America (53–71% of all cases), whereas HCV-3 occurs predominantly in Asian countries (40% of all infections) (Petruzziello, Marigliano, Loquercio, & Cacciapuoti, 2016). In Nepal, predominance of HCV-3 (55% − 60%), followed by HCV-1 (36% − 42%) and others (0–8%) ( (Kinkel et al., 2015; Mishra et al., 2020; Poudel & Poudel-Tandukar, 2021). Patients with lower pre-treatment viral load were more likely to respond positively to antiviral therapy than those patients with high pre-treatment viral load.

In Nepal, only a few studies report the co-prevalence of HIV and HCV with genotypes. The prevalence of HCV infection in the general population is 0.64%, dramatically lower than 4.1% among HIV-infected individuals (Karki, Ghimire, Tiwari, Maharjan, & Rajkarnikar, 2008; Supram, Gokhale, Sathian, & Bhatta, 2015). In this study we aimed to find the prevalence of HIV and HCV co-infection, genotypes and viral load of HCV in Nepal.

**Materials And Methods**

**Study design**

This cross-sectional study was conducted from Oct 2021 to May 2022 in the HIV seropositive patients attending the Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. The patients were diagnosed and confirmed HIV seropositive under the national HIV testing algorithm of the National Centre for AIDS and STD Control (NCASC). The patients were randomly selected and were of all ages, sex, and social classes with different ART statuses. Information regarding sex, date of HIV diagnosis, ART duration, and type of ART was obtained before sample collection. In addition, clinically relevant information was also obtained from the log book of the hospital.

**Inclusion and exclusion criteria:**

Patients of all ages were included in the study. HIV patient’s taking thyroid supplementation, and pregnant and lactating women were excluded from the study. Individuals who did not wish to participate in the study were also excluded from the study. Similarly, HIV patients with proven disorders before HIV diagnosis were also excluded from the study.

**Sample collection and processing:**

Blood samples from the HIV seropositive patients were used in this study. First, 5 ml of the venous blood sample were aseptically collected as per the standard guidelines and protocol. Next, the blood sample was centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum was then screened for anti-HIV antibodies for confirmation. The serum samples were separately analyzed for thyroid hormones (to
determine thyroid function) and HCV coinfection. These processes were carried out under the aseptic conditions in the BSL-2 laboratory following WHO guidelines.

**Screening of HCV coinfection**

The screening of HCV coinfection was performed by the HCV Rapid Test. First View HCV Rapid Antibody Test Kit was used for this purpose. It is a double antigen lateral flow chromatographic immunoassay for the quantitative determination of anti-hepatitis C virus antibodies (IgG, IgM, IgA) in human serum or plasma.

**Viral Nucleic Acid Extraction:**

Viral RNA was extracted using QIAamp DSP Virus Kit according to the manufacturer's instructions with slightly modification. Briefly, 25 µl protease was pipetted into a 1.5 ml microcentrifuge tube then 200 µl of plasma was added into it.

200 µl Buffer AL (containing 28 µg/ml of carrier RNA and internal control) was added and incubated at 56°C for 15 min in a heating block. 250 µl of ethanol (96–100%) was added to the sample then incubated for 5 min at room. Carefully all of the lysate were transferred onto the QIAamp DSP Virus column and centrifuge at 6000 x g for 1 min. QIAamp DSP Virus column was placed in a clean 2 ml collection tube, and discarded the collection tube containing the filtrate. Carefully QIAamp DSP Virus column was opened and 500 µl of Buffer AW1 added then centrifuged at 6000 x g for 1 min. Similarly, AW2 was also processed. QIAamp DSP Virus column was opened and 500 µl of ethanol (96–100%) were added and centrifuged at 6000 x g for 1 min. QIAamp DSP Virus column placed in a cleaned 2 ml collection tube then centrifuged at 20,000 x g for 3 min then membrane was dried at 56°C for 3 minute on heating block. QIAamp DSP Virus column placed in a clean 1.5 ml microcentrifuge tube and 60 µl of Buffer AVE was applied into it and centrifuged at 20,000 x g for 1 minute. Extracted RNA were stored at -20°C and −80°C based on its application.

**Quantitative PCR:**

Q-PCR was performed for the estimation of viral load of HCV RNA using QIAGEN Artus HCV QS-RGQ kit (QIAGEN, Germany) by quantitative RT-PCR using QuantStudio 5 (applied bioscience, USA). The QIAGEN Artus HCV kit included reagents and enzymes for the reverse transcription and quantification of HCV RNA by amplification of conserved sequence of HCV. Briefly, for one sample; Hep. C Virus RG Master A 12 µl and Hep. C Virus RG Master B 18, a total 30 µl of master mix and 20 µl of sample/positive control/negative control and standard control were mixed and briefly centrifuged. The amplification was performed at two steps: step 1 cDNA synthesis; 50°C for 30 min and 95°C for 15 min for cycle 1 and step 2 PCR for 50 cycles; Denaturation 95°C for 30 sec, annealing 50°C for 1 min and extension 72°C for 30 sec. Amplified HCV were detected by FAM and internal control by ROX reporter and there were NFQ-MGB quencher for both targets.

**Genotyping:**
HCV RNA positive samples that had viral load > 34 international unit per milliliter (IU/mL) were enrolled for genotyped using TRUPCR® HCV Genotyping Kit IVD (Bhopal, India); able to genotypes 1a, 1, 2 (2a/2b), 3, 4, 5a and 6. Briefly, 7 µl reaction mix (RT mix 6 µl and enzyme mix 1 µl) of kit and 10 µl of extracted RNA sample mixtures were used for the cDNA preparation for 60 min at 42°C and 5 min at 95°C for single cycle. The three different PCR tubes were used for the genotyping tube 1; for HCV detection, Genotype 1 and 5a, tube 2; genotypes 1a, 4 and IC and tube 3; genotypes 2 (2a/2b), 3 and 6 detections. Each tube contains multiplex master mix 10 µl, primer probe 10 µl mix separately and 5 µl of cDNA. The PCR were performed at 94°C for 10 min for single cycles and 40 cycles at 94°C for 15 sec, 58°C 45 sec and 72°C for 15 sec. The cut off value of this assay was 37 cycles.

Quality control

We used ISO and CE-IVD certified reagents and chemicals and performed the entire test including sample collection in highly aseptic conditions following standard operative procedures (SOPs). Positive and negative controls were used in each batch/step. The reagents and samples were stored and incubated at verified temperatures. The inclusion and exclusion criteria were monitored carefully.

Data management and analysis:

All the collected data were entered into the MS Excel sheet, and then statistical analyses were carried out using SPSS v20.0. A p-value < 0.05 confirmed statistical significance, frequency and percentage were calculated.

Results

Of the total 203 PLHIV out of them 148 (72.9%) were male and 55 (27.1%) were female. 115 (56.6%) and 112 (55.2%) had HCV coinfection by rapid diagnostics test and PCR assay (Table 1). There were 138 (68.0%) and 14 (6.9%) were in 25–50 years and < 15 year age group respectively (Table 1). HCV co-infection was higher in males 110 (95.6%) than females 5 (4.4%). HCV coinfection was the highest in the age group 25–50 while no HCV coinfection were seen in the < 1–25 year and > 50 years (Table 2).
Table 1
HCV status among people living with PLHIV and other parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Positive</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV detection by Rapid card</td>
<td>112 (55.2%)</td>
<td>91 (44.8%)</td>
</tr>
<tr>
<td>HCV Detection by PCR</td>
<td>115 (56.6%)</td>
<td>88 (43.4%)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 148 (72.9%)</td>
<td>Female 55 (27.1%)</td>
</tr>
<tr>
<td>Age Group</td>
<td>&lt;15 14 (6.9%)</td>
<td>15–25 29 (14.3%)</td>
</tr>
<tr>
<td></td>
<td>25–50 138 (68.0%)</td>
<td>&gt;50 22 (10.8%)</td>
</tr>
</tbody>
</table>
Table 2
HCV Viral load, genotypes, age, sex and HIV duration wise distribution.

<table>
<thead>
<tr>
<th>Viral load IU/ml</th>
<th>&lt;34</th>
<th>34-3000</th>
<th>3000-30000</th>
<th>30000-300000</th>
<th>&gt;3000000</th>
<th>Total</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>56 (27.6%)</td>
<td>31 (15.3%)</td>
<td>17 (8.4%)</td>
<td>8 (3.9%)</td>
<td>3 (1.5%)</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

| Genotypes | | | | | | |
|-----------------|-----|---------|------------|--------------|----------|-------|---------|
| 1 | 1 (50.0%) | 1 (50.0%) | 2 (3.3%) | 0.00 | |
| 1a | | | 2 (100%) | 2 (3.3%) | |
| 3 | 22 (61.1%) | 9 (25.0%) | 4 (11.1%) | 1 (2.8%) | 36 (61.1%) | |
| 5a | 4 (30.8%) | 7 (53.8%) | 2 (15.4%) | 13 (22.1%) | |
| 6 | 4 (66.7%) | 1 (16.7%) | 1 (16.7%) | 6 (10.2%) | |

| Sex | | | | | | |
|-----------------|-----|---------|------------|--------------|----------|-------|---------|
| Male | 53 (48.2%) | 29 (26.4%) | 17 (15.5%) | 8 (7.3%) | 3 (2.7%) | 110 (95.6%) | 0.783 |
| Female | 3 (60.0%) | 2 (40.0%) | 0 | 0 | 0 | 5 (4.4%) | |

| Age Groups (Year) | | | | | | |
|-------------------|-----|---------|------------|--------------|----------|-------|---------|
| 25–50 | 51 (50.0%) | 25 (24.5%) | 16 (15.7%) | 7 (6.9%) | 0 | 99 (86.1%) | 0.523 |
| >50 | 5 (48.7%) | 6 (46.2%) | 1 (7.7%) | 1 (7.7%) | 3 (2.6%) | 16 (13.9%) | |

| HIV/ART Duration (Year) | | | | | | |
|------------------------|-----|---------|------------|--------------|----------|-------|---------|
| 1–5 | 3 (60.0%) | 0 | 0 | 2 (40.0%) | 5 (8.5%) | 0.010 | |
| 6–10 | 20 (58.8%) | 8 (23.5%) | 5 (14.7%) | 1 (2.9%) | 20 (33.9%) | |
| 11–15 | 9 (45.0%) | 7 (35.0%) | 4 (20.0%) | 0 | 34 (57.6%) | |
Out of 115 HCV positive; viral load of HCV was high number 56 (27.6%) of patients had low viral load as < 34 IU/ml while 31 (15.3%) had among 34-3000 IU/ml, 17 (8.4%) had 3000–30000 IU/ml, 8 (3.9%) had 30000–300000 IU/ml and only 3 (1.5%) had > 3000000 IU/ml (Table 2). Genotype 3 36 (61.1%) were higher number of genotypes then genotypes 5a, 6, 1 and 1a as 13 (22.1%), 6 (10.2%), 2 (3.3%) and 2 (3.3%) respectively. The viral load and the HCV genotyping were significantly associated with each other (P = 0.00) (Table 2). High value of HCV viral load was observed in male than female which were not significantly associated (P = 0.783). Among age group 25–50 year higher viral load was observed while lo number were observed in > 50 year and also not significantly associated (P = 0.523) (Table 2). The proportion of HCV co-infected patients increased with the HIV duration up to 15 year. However, the rate decreased in the PLHIV having the duration below 5 years. HIV duration and HCV viral load were significantly associated with each other (P = 0.010) (Table 2).

Genotyping 3 were found more in both male 34 (59.6%) and female 2 (100%) and not significantly associated (P = 0.858). Age wise distribution of genotypes; genotypes 3 28 (54.9%) and 8 (100%) were observed among age group 25–50 and > 50 year respectively (Table 3); not significantly associated (P = 0.206). Whereas HIV/ART duration in higher number of genotypes 3 were observed in 6–10 years and 11–15 years (Table 3).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>1</th>
<th>1a</th>
<th>3</th>
<th>5a</th>
<th>6</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2 (3.5%)</td>
<td>2 (3.5%)</td>
<td>34 (59.6%)</td>
<td>13 (22.8%)</td>
<td>6 (10.5%)</td>
<td>0.858</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>0</td>
<td>2 (100%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Age Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25–50</td>
<td>2 (3.9%)</td>
<td>2 (3.9%)</td>
<td>28 (54.9%)</td>
<td>13 (25.5%)</td>
<td>6 (11.8%)</td>
<td>0.206</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>0</td>
<td>0</td>
<td>8 (100.0%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HIV/ART Duration (Year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–5</td>
<td>0</td>
<td>1 (20.0%)</td>
<td>4 (80.0%)</td>
<td>0</td>
<td>0</td>
<td>0.341</td>
</tr>
<tr>
<td>6–10</td>
<td>1 (2.9%)</td>
<td>1 (2.9%)</td>
<td>18 (52.9%)</td>
<td>10 (29.4%)</td>
<td>4 (11.8%)</td>
<td></td>
</tr>
<tr>
<td>11–15</td>
<td>1 (5.0%)</td>
<td>0</td>
<td>14 (70.0%)</td>
<td>3 (15.0%)</td>
<td>2 (10.0%)</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

HIV weakness the immune system leading to AIDS. Due to the weakened immune system, HIV patient is prone to new infections and various complications and others secondary co-infection.
HCV coinfection is common among PLHIV due to their common routes of transmission. Such co-infection increases up the frequency and severity of liver disease (Shrestha & Yadav, 2022). It is reported that the co-infected patients are likely to have cirrhosis of the liver 6 times more with those compared infected solely with HCV (Fuster et al., 2004). In this study more than half (56.6%) of the PLHIV had HCV coinfection which is higher than (43.3%) study conducted in Nepal (Poudel et al., 2014). Our prevalence of coinfection is much higher than the previous reports from India (Nagmoti, Patil, Jyoti, Mutnal, & Mallapur, 2012) and a recent report from Malasiya (Nagmoti et al., 2012). In this study HCV-infected individuals were in the age group of 25–50 years. Similar finding were also reported from Nepal in which age group ranges from 30–39 higher (Ionita et al., 2017).

In this study, the distribution of HCV genotypes in Nepalese patients attending TUTH, Kathmandu. Genotype 3 was the most prevalent followed by 5a, 6, 1a, and 1 genotypes. Majority of the HCV patients and genotypes 3 were at the age group of 25–50 years and male.

Higher prevalence of HCV Genotype 3 were also found to be dominant in some study and prevalent among intravenous drug users (Ionita et al., 2017; Kinkel et al., 2015). HCV genotypes (1, 1a, 5a, and 6) have also been reported earlier in studies from Asian countries, such as India, Pakistan and Nepal, where 3a was also dominant genotype (Singh, Verma, & Verma, 2004)

We had also found genotypes 1, 1a, 5a, and 6. Thirteen patients were found with genotype 5a infection also found which was also previously found in Nepal (Mishra et al., 2020) and six patients of genotype 6 were most probably fir report from Nepal whereas in India already reported (Panyala et al., 2019). The presence of 6 in Nepalese patients may indicate a shift of HCV genotypes. Shift of HCV genotypes may require attention in the diagnosis and treatment of HCV patients.

HCV genotype and viral load were significantly observed (P = 0.00). High number Genotypes 3 were observed in viral load 34-3000 IU/mL (61.1%), similarly genotype 6 were also higher among this range whereas genotypes 5a (53.8%) was observed in 3000–30000 IU/mL viral load range while genotypes 1a and 3 were found in > 3000000 IU/mL range. The finding of this research were also similar to the study conducted from Nepal, India and Pakistan (Ali et al., 2011; Mishra et al., 2020; Panyala et al., 2019).

For the proper treatment of HCV infection; viral load of HCV and genotypes play very crucial role for excellent outcome of antiviral therapy. Interferon therapy play very weak response towards the higher HCV viral load, which increases the probability of relapse compared to lower HCV viral load (Dalgard et al., 2004). Although HCV treatment is rare in Nepal, timely diagnosis of HCV would be beneficial in order to educate patients regarding HCV transmission, risk of reinfection, and liver disease progression.

**Conclusion**

HIV patients mainly males have higher risk of HIV coinfection. The risk of coinfection also increases with the age. The prevalence of thyroid dysfunction is similar in HCV co-infected patients and patients infected with HIV or HCV alone. This study provides additional information on the distribution of various
types of HCV genotypes. Genotype 3a was most prevalent genotypes and the presence of 6 in Nepalese patients may indicate a shift of HCV genotypes.

**Declarations**

**Acknowledgements**

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**Author’s contribution**

Sah UK designed the study, collected the samples and performed lab work. Anil K Sah: Manuscript writing, data analysis; Ansari M performed data analysis and drafted the manuscript. All authors read and approved the final version of the manuscript to be published and agreed to be accountable for all aspects of the work.

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Null

**Ethical approval and consent**

Nepal Health Research Council (NHRC), Regd. No. 644/2020. Written consent was taken from all the participants.

**Consent for publication**

Not applicable

**Competing interest**

The authors have no relevant financial or nonfinancial interests to disclose

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