Prevalence of hepatitis B and C in internally displaced persons of war against terrorism in Swat, Pakistan

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Background: Hepatitis B and C are the most common blood-borne liver infections worldwide. According to the recent estimates, 270–300 million people worldwide are infected with hepatitis C virus (HCV) and more than 2 billion people have been infected with hepatitis B virus (HBV). Transmission of these viruses is carried out by exposure to infectious blood or body fluids containing blood. Methods: Five-hundred and ninety (290 males and 300 females, 5–65 years) internally displaced persons (IDPs) from Swat area of northern Pakistan were screened for hepatitis B surface antigen (HBsAg) and anti-HCV antibodies using immune-chromatography kits. The subjects, positive for virus-related antibody, were further confirmed for viral RNA (for HCV) and DNA (for HBV) in the blood by polymerase chain reaction (PCR) amplification. The virus of PCR-confirmed HCV-individuals was further genotyped and the prevalence of HCV infection was determined with respect to age, sex, history of exposure to blood or surgical operation and different types of liver diseases. Results: The HCV infection was found to be the predominant liver infection in the population which was 91% of the positive cases, as against 9% of HBV. Among the HCV-positive subjects (68% females, 32% males) 56% were asymptomatic. No co-incidence of HBV and HCV was found in any subject. Genotype 3a was the dominant strain of HCV followed by 2a > 3b > 1b > 1a, 2b. Conclusion: The viral hepatitis among the apparently healthy population of a relatively natural and pollution-free environment refers to an alarming condition about liver infections, particularly of HCV, in Pakistan.

Keywords: hepatitis B, hepatitis C infection, victims of war of terrorism

Introduction

Viral hepatitis is a serious health problem around the world. These viruses are transmitted through blood and blood products, although sexual transmission and intrafamilial transmission have also been reported.1 It has been estimated that about 2 billion people have been infected with hepatitis B virus (HBV) and 350 million have chronic lifelong infection. The prevalence of hepatitis C virus (HCV) is also high and it is estimated that about 170 million people are chronically infected while 3–4 million people are newly infected every year.2–4 A considerable proportion of these patients will progress onto cirrhosis and hepatocellular carcinoma.5,6

Generally, the epidemiological studies concerning the prevalence of HBV and HCV are restricted to the hospitalized patients,7,8 whereas there are very few population studies to estimate the exact infected population in different areas.9 Alam and Ahmad10 have reported 15.6% of the blood donors in Sialkot as carrier of hepatitis, while Bangash et al.11 found 5% of the healthy blood donors at Kurram Agency, Northern Area, Pakistan, positive for hepatitis B surface antigen (HBsAg) and 1.1% positive for HCV antibody. Agboatwalla et al.12 reported 94% healthy Pakistani children out of 258 with HAV IgG antibody.

In the present paper, we present the prevalence of HCV and HBV infection among the male and female population of internally displaced persons (IDPs) of Swat during the war against terrorism in Pakistan in 2009. The study also provides an alarming analysis of disease epidemiology in the areas that have comparatively pollution-free environment, focusing on the need for strategies for timely screening and treatment.

Methodology

A hepatitis-C and hepatitis-B screening study was conducted among the apparently healthy male and female population of IDPs from Swat, Pakistan, sequel to war against terrorism in the Northern Areas of Pakistan. These IDPs were temporarily housed in the camps of Baghicha Dheri area of district Mardan and Ghazi Kot district Manshera, North Frontier Province, Pakistan. This screening was conducted during the first 3 weeks of the massive displacement of Swat population. Besides screening, other symptoms of viral hepatitis such as pain in upper right part of abdomen, anorexia, nausea, dyspepsia, vomiting, fever and jaundice were also recorded.
**HCV and HBV screening**

For primary screening, the blood samples were obtained from venous punctures, and the sera were separated from the coagulated blood by centrifugation at 5000 r.p.m. for 10 min at room temperature. The HCV and HBV screening was based on the detection of antibodies against the related viruses in the sera. Screening kits (ICT) were used for the detection of anti HBsAg and anti HCV (ACON®, ACON Laboratories Inc., San Diego, CA 92121, USA).

**Polymerase chain reaction-based confirmation of HBV in positively screened people**

For confirmation of HBV in the above-screened positive samples, 250 μl of blood sample was mixed with 500μl of DNAzol® BD. Cat. No.DN129 (a Product of Molecular Research Centre, Inc., Cincinnati, OH, USA) for DNA isolation. Further protocol as described by the manufacturer was followed. The isolated DNA was dissolved in 50 μl of nuclease-free water and used for simple and nested PCR.

For the first round of PCR, following primers were used:

- **Forward:** 5′-CATCCTGTGCTATGCTCATCT-3′
- **Reverse:** 5′-CAGAACCAGTAGGAAATGGCATC-3′,

whereas for the nested PCR (i.e. second round of PCR) the primers used were as follows:

- **Forward:** 5′-GGTATGTTGGCCGTCTGTCTCT-3′
- **Reverse:** 5′-GGGACTAGTAAACTGAGCCA-3′

For the first round of PCR, the 20-μl PCR reaction mixture contained 1 μl of 2.5 mM dNTPs, 1.5 μl of 25 mM MgCl₂, 1 μl each of 10 μM forward and reverse primers, 2 μl 10× Taq buffer, 1.0 μl of Taq polymerase (5 U μl⁻¹), 3.3 μl nuclease-free water and 10 μl of DNA template. The reaction mixture for the second round of PCR was almost the same except for 9.5 μl of nuclease-free water and 4 μl of DNA template.

PCR-thermocycler was adjusted at 94°C for denaturation for five minutes, followed by 30 cycles, each of 30 s at 94°C (denaturation), 40 s at 53°C (annealing) and 30 s at 72°C (extension) with final extension at 72°C for 5 min. The nested PCR was carried out under the same PCR conditions as above.

**PCR-based confirmation of HCV in positively screened people**

For the detection of HCV RNA among the positive subjects in the antibody screen test, RNA was isolated from blood samples with Trizol-reagent kit (Invitrogen) method. cDNA was generated using gene-specific reverse primers with reverse transcriptase polymerase chain reaction (RT-PCR) involving Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase at 37°C for 50 min. The viral RNA was detected with nested PCR.

The primers used for the first PCR-amplification were as follows:

- **Forward:** 5′-CCCTGTGAGAAGCTACTGTCTTCAAGC-3′
- **Reverse:** 5′-ACTGCCAAGGACCCCTAAGGAGGCTAC-3′,

whereas the primers used for amplification of the internal region were:

- **Forward:** 5′-GAAAGCGTCTAGCCATCGGC-3′
- **Reverse:** 5′-CACAAGGCGCTTTTCGAGAC-3′

The 20-μl reaction mixture for the first and the second round of PCR was prepared just as was done above. The PCR-thermocycler was adjusted at 94°C for denaturation for 5 min, followed by 30 cycles, each of 30 s at 94°C (denaturation), 40 s at 64°C (annealing) and 30 s at 72°C (extension) with final extension at 72°C for 5 min. The nested PCR was carried out under the same PCR conditions with an annealing temperature at 53°C. The cDNA was visualized on 1.5% agarose gel for determining the presence or absence of hepatitis C virus in the blood serum.

**Genotype identification of hepatitis C strain among PCR-positive individuals**

Fresh serum samples were used for isolation of RNA as described above for HCV PCR. Viral cDNA was generated with RT-PCR using reverse transcription PCR method with core-gene specific reverse primer. The genotype was identified with multiplex-PCR. Two reaction mixtures, containing two sets of primers each, were processed in parallel in the nested PCR.

PCR-thermocycler was adjusted at 94°C for denaturation for 5 min, followed by 30 cycles, each of 30 s at 94°C (denaturation), 40 s at 64°C (annealing) and 30 s at 72°C (extension) with final extension at 72°C for 5 min. The nested PCR was carried out under the same PCR conditions as above, except for the annealing temperature which was 53°C.

The details of the primers and the expected sizes of the PCR products are shown in table 1.

**Results**

**HCV+HBV prevalence**

In the present study, 57 out of the 590 apparently healthy subjects (290 males and 300 females) of 5–65 years of age were found positive for the hepatitis B and C infections, thereby showing 9.66% prevalence, 37% males and 63% females (figure 1A). Out of 57 hepatitis-positive subjects, 52 (91.22%) were found positive against HCV antibody test and only five (8.77%) were positive for HBV antibody.

**HCV prevalence**

Considering the total population size screened for hepatitis, 8.81% subjects, 35% males and 65% females were positive for HCV antibody test.

The screened out individuals were further investigated for the presence of hepatitis C-related RNA using nested PCR method. Of the 52 HCV antibody-test-positive subjects, 25 individuals (48.07% of the antibody positive) were found positive for hepatitis C RNA in the serum after nested PCR, 17 (68%) of which were females and eight (32%) were males. Among the 25 HCV RNA-positive individuals, 11 (44%) were symptomatic and 14 (56%) were asymptomatic. Two individuals among the symptomatic group were diabetic.

Figure 1B shows the prevalence of HCV in the two sexes. Among the female subjects 11.3% were found positive for hepatitis C antibody and 5.65% were HCV-PCR positive. Among the males, 6.2% were found positive for HCV antibodies out of which 2.77% were positive for HCV-PCR.

Table 2 shows the prevalence of HCV in different age groups of Swat IDPs. Amongst 52 anti-HCV antibody positive individuals, 20 (38.5%) were from the age group 36–45 years, followed by 12 (23.1%) of 26–35 years and 10 (19.2%) of 46–60 years age group.

**HBV prevalence**

Considering the total population size screened for hepatitis, only 0.85% were found positive for HBV antibodies. Figure 1B also shows the prevalence of HBV in the two sexes. Among the female subjects, only 0.66% were found positive for hepatitis B antibody and none was HBV-PCR.
Among the males, 1.03% were found positive for HBV antibodies and 0.69% were positive for HBV-PCR. Of the five HBV antibody test positive individuals (60% males, 40% females), the viral DNA in the serum was found in only two, both males, by nested PCR. One of the two was symptomatic, while the other was asymptomatic. Two individuals were in the age group 36–45 years and one each in 26–35 and 46–60 years. There was none in the 5–15 years age group.

Genotypes of HCV

Figure 2 shows the genotypic analysis of hepatitis C virus of the 25-HCV positive IDPs. The genotype 3a has been found to be the most predominant strain in the population; 37% males and 46% females had this genotype. The other strains detected were 1a of which 12% were males and none was female, 2a of which 12% were males and 18% females, 1b of which 12% were females and none was male, 2b of which 13% males and none was female, and 3b of which 13% were males and 12% females. The HCV strains 4a, 5a and 6a were not detected in any of the HCV positive subject. An unknown strain was also detected which did not fall under any category. It included 12–13% of the total HCV-positive population.

Discussion

The present study describes the prevalence of viral hepatitis infection among the individuals of apparently healthy
population from a far-off mountain area where the environment is quite natural and pollution-free. Among the studied population, 9.7% were found to have antibodies against the hepatitis B- and C-related viruses and only 4.6% of the studied population was positive for viral DNA and RNA in the blood serum. About 56% of the subjects, confirmed with hepatitis C viral RNA, were asymptomatic indicating a high percentage of chronic disease. After genotype analysis 3a was found to be the most predominant HCV genotype in males and females followed by 2a > 3b > 1a, 1b and untypable (unknown) genotypes. The genotypes 4a, 5a and 6a were not found in any studied subject.

The prevalence of viral hepatitis reported in this study is less compared with other previous studies with populations of homeless veterans in California and homeless people in Czech Republic, with lack of hygienic conditions.15,16

In order to combat this major health problem, it was necessary to consider the risk factors that contribute to this situation so that a framework can be established to reduce the infection rate. In the present study, hepatitis C infection was more prevalent in females and among the PCR-positive HCV females 50% were found to be infected during caesarean sections. The disease can be transmitted by various sources and the surgical paraphernalia may genuinely be the main source of transmission in these females. Ali et al.17 have implicated contaminated needle use in medical care and drug abuse and unsafe blood and blood product transfusion as the major causal factors for world’s highest burden of chronic hepatitis and mortality due to liver failure and hepatocellular carcinomas in Pakistan. Aslam et al.18 and Raja and Janjua19 have suggested the widespread prevalence of hepatitis C infections in Pakistan may be due to unintended consequence of country’s small pox vaccination programme, blood transfusion and use of community barber shops for face and armpit shaving.

This is the first study of its type on a section of general population, which got displaced en masse from Swat during war on terrorism and were temporarily housed in camps, and hence depicts the prevalence of hepatitis B and C in general population of Swat, which is comparatively less polluted area of North Frontier Province. Most of the studies which have been done earlier on the prevalence of hepatitis are based on patients visiting hospitals and blood banks.

None of the subjects from Swat IDBs had both the HBV and HCV co-infections, though one of the HCV males out of the studied population was positive for *Mycobacterium tuberculosis*. Zuberi et al.20 reported 11% out of 246 HBV patients positive for anti HBe, IgG, anti HDV and HCV. Likewise, Baig et al.21 have recently reported highest frequency of super infection of hepatitis C and D in HBV cirrhosis patients in Pakistan.

This high prevalence of hepatitis C in apparently healthy population like that of Swat is quite alarming. The situation becomes more alarming when 56% of the HCV infected individuals were found to have no symptoms of HCV infection, as against 29.4% asymptomatic carriers reported in hospitals.21 There is an urgent need to undertake such surveys systematically on general population and devise ways and means not only to cure but also to minimize its proliferation.

### Table 2 Hepatitis C prevalence in different age groups of internally displaced persons of Swat Valley, Pakistan during war against terror

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Anti HCV antibody Positive individuals</th>
<th>Percentage prevalence</th>
<th>HCV PCR Positive individuals</th>
<th>Percentage prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–15</td>
<td>3</td>
<td>5.8%</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>16–25</td>
<td>7</td>
<td>13.5%</td>
<td>2</td>
<td>8%</td>
</tr>
<tr>
<td>26–35</td>
<td>12</td>
<td>23.1%</td>
<td>6</td>
<td>24%</td>
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<td>36–45</td>
<td>20</td>
<td>38.5%</td>
<td>11</td>
<td>44%</td>
</tr>
<tr>
<td>46–60</td>
<td>10</td>
<td>19.2%</td>
<td>5</td>
<td>20%</td>
</tr>
</tbody>
</table>

### Figure 2 Hepatitis C genotypes in male and female subjects. In female subjects 3a genotype was found to be 46%, 2a 18%, 1b and 3b 12% each and untypable 12%. The genotypes 1a, 2b, 4a, 5a and 6a were not detected. In male subjects, genotype 3a was 37%, 2b and 3b 13% each, 1a and 2a 12% each, whereas the strains 1b, 4a, 5a and 6a were not detected.
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Conflicts of interest: None declared.

Key points
- This is the first study on prevalence of hepatitis B and C in a section of population of Swat, which was displaced en masse as a result of war on terrorism and were housed temporarily in the Camps.
- Of the hepatitis-positive cases, 91% were infected with HCV, 66% of which were females and 34% males.
- Of the HCV RNA-positive individuals 56% were asymptomatic, which indicates alarmingly high prevalence of HCV infection.
- Genotype 3a was the most dominant strain of HCV, prevailing among hepatitis C subjects.
- There is an urgent need to undertake screening of general population in a systematic manner to devise ways and means to control and prevent spreading of hepatitis in Pakistan.

References