Sero-Prevalence of HCV and HIV antibodies among different groups of general population of Peshawar Cantonment, KPK, Pakistan

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Abstract

Hepatitis C and AIDS are serious health problems worldwide. Hepatitis C is a major cause of liver disease, while AIDS results in progressive failure of the immune system that allows life threatening opportunistic infections and cancers to thrive respectively. The objective of this study was to analyze the prevalence of HCV, HIV and HCV/HIV co-infection among different groups of apparently healthy people of Peshawar cantonment and to know the risk factors. The study was conducted at Pathology department, Khyber Teaching Hospital Peshawar Cantonment from February 2013 to June 2013. A total of 400 people from general population were screened for HCV and HIV by ELISA method. The overall prevalence of HCV shows that out of 400 samples for HCV, 340 subjects were found negative and 60 were positive for HCV. Age of the subjects was 19-60 years. 40 samples were screened for each group and out of 40 samples each 16(40%) shopkeepers, 10(25%) barbers, 10(25%) drug users, 8(20%) sweepers, 8(20%) food workers, 2(5%) out of 40 each among scrap merchants, mechanics, government employs and health care workers were positive for HCV. However, all the samples from students were HCV negative. 28% HCV positive cases were below the age of 40 (<40 years), while 51% of the HCV positive cases were above the age of 40 (>40 years). 95% of the HCV positive individuals were uneducated and 60% of HCV positive individuals were married. The total prevalence of HCV in Peshawar cantonment was 15% while the prevalence of HIV and HCV/HIV co-infection was 0 %. The majority of study groups were uneducated and belonged to low socioeconomic status. The risk factors include commercial sex and non-marital sex, sharing blades and shaving kits, exposure to infected blood or infected needles, using unsterile equipment’s, injection drug use and unawareness. It is concluded that chronic hepatitis C is common in asymptomatic healthy population and they are the possible candidates for chronic liver disease and potential sources of spread of infection, while HIV or HCV/HIV co-infection is not prevalent in the studied region.
Key words: HCV, HIV, Infection, ELISA, Sero-Prevalence.

Introduction

Hepatitis C virus (HCV) was first discovered in 1989 by Houghton and coworkers. HCV is important cause of chronic liver disease and hepatocellular carcinoma [1, 2]. The hepatitis C virus is a small (50nm in size), enveloped, single stranded, and positive sense RNA virus [2, 3]. It is the only known member of the hepacivirus genus in the family Flaviviridae. There are six major genotypes of the HCV, which are indicated numerically (e.g., genotype 1, genotype 2 etc.). WHO estimated the global prevalence of Hepatitis C is 3%. HCV is transmitted through contaminated blood transfusion, surgery, surgical instruments, dental surgery and excessive dental consultations, sexual contacts, drug abuses, sharing of the house hold items such as razors, toothbrushes and shaving from the barber [4, 5]. Primary sources of HCV infection are unsterilized injections, equipment’s and infusion of inadequately screened blood, needles and syringes, hemodialysis equipment and oral hygiene instruments. HCV transmission is mainly parenteral [6].

Vertical transmission (mother-to-child) of HCV is estimated to be less than 5%, but in case of human immunodeficiency virus (HIV) co-infection, the risk of mother-to-child transmission can reach 15% to 20% [7]. Similarly, HCV/HIV co-infection promotes the progression of hepatitis to cirrhosis [8]. Pakistan, a developing nation of 180 million people has alarmingly rate of outbreaks of HCV [9], which need proper survey and genotyping. Sero-prevalence studies of anti-HCV antibodies in the general population of Pakistan have been recorded as 5.31% to 7.5% [6, 8]. HCV prevalence is in the range of 4.1 to 36% reported from various parts of Khyber Pakhtunkhwa (KPK) province of Pakistan [10], but no data has been reported till now on the prevalence of HCV from general population of Peshawar cantonment. As no study has earlier been conducted to figure out the prevalence of anti-HCV antibodies among the general population of district Peshawar (cantonment), we for the first time conducted this study to find out prevalence of active HCV infection among different groups in general population of Peshawar (cantonment).

Human immunodeficiency virus (HIV) is a lentivirus, part of the family Retroviridae lentiviruses that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which progressive failure of the immune system allows life threatening opportunistic infections and cancers [11]. HIV is lethal and at present is an incurable disease having remarkable social repercussions [12]. Previous studies in general population of Pakistan have shown a sero-prevalence of HIV to be 0.23% [13]. All the modes of transmission, homosexuals, heterosexual contact, blood transfusion, intravenous drug users, from mother to child are all prevalent in Pakistan [14]. The data from the point-prevalence studies suggest that foreign and Pakistani nationals who have either worked abroad (in the Gulf states or elsewhere) or have worked as seafarers may make up a sizeable portion of the recognized HIV/AIDS cases [15]. The studies also documented cases of HIV infection among individuals with multiple sexual partners, blood transfusion recipients, and prisoners [15]. A total of 744 HIV/AIDS cases have been reported in KPK, in which Peshawar district is the most vulnerable with 75 patients, whereas 98,000 people are affected by the disease in the country. According to Express Tribune report, most AIDS cases had been reported in Bannu district, but the ratio has declined with the passage of time. There were 30 AIDS cases in Khyber Agency, 60 in Kurram Agency and 100 in Afghan refugees. As there was no data available about prevalence of HCV and HIV in this region so the aim and objective of the study was to study and analyze the prevalence of HCV and HIV and HCV/HIV co-infection in Peshawar cantonment, to provide data to health planner /policy makers for the control of infections, to ensure the health education activity among the population and to find out the risk factors for
HCV, HIV and HCV/HIV co-infection in Peshawar cantonment.

Materials and Methods

Area of study

This study was conducted to analyze the prevalence of HCV, HIV and HCV/HIV co-infection among different groups that includes shopkeepers, barbers, drug users, sweepers, food workers, scrap merchants, mechanics, government employs, health care workers and students. Other parameters like age, educational level and marital status of apparently healthy people of Peshawar cantonment were also included to know the risk factors. The majority of study groups were uneducated and belonged to low socioeconomic group. The age of the subjects under study was 19-60 years. This cross sectional experimental study was carried out at Pathology department, Khyber Teaching Hospital Peshawar cantonment from February 2013 to June 2013. A total of 400 blood samples from the general population were collected and screened for HCV and HIV antibodies by ELISA method.

Study design

Data was collected on the structure Performa/questionnaire. After an informed written consent, brief clinical history was recorded. The questionnaire consists of 18 different questions, which contained personal information (age, education level, occupation and marital status), clinical history and other related questions. Blood samples were collected from different groups of 400 people from the general population and screened for HCV, HIV and HCV/HIV co-infection.

Blood collection and serum isolation

Blood samples were collected from general population of Peshawar in regard to their laboratory request form and were labeled properly according to their serial number, name and date. Blood samples (3cc) were taken by using sterilized disposable syringes and were immediately transferred into vacutainer tubes. These samples were allowed to clot and then centrifuged at 3000 rpm for 5 min within two hours of collection. The sera were collected in autoclaved Eppendorf tubes and were stored at -20°C for testing.

Detection of Anti-HCV by ELISA Method

All the collected samples were confirmed by performing ELISA. The HCV ELISA kit employs solid phase, indirect ELISA method for the detection of IgG-class antibodies to HCV in two steps incubation procedure. Polystyrene micro well strips were pre-coated with recombinant, highly immuno reactive antigens corresponding to the core and non-structural regions of HCV (3rd generation).

ELISA plate reading

The stop solution was added to the wells to stop the enzyme reaction. After addition of the stop solution, the color developed was observed on micro titer plate reader. 3rd generation ELISA plate reading was performed with ELISA plate reader within 15 minutes after dispensing the stop solution and the cut–off value was determined and then the samples were interpreted. Colored wells show positive result for HCV (Figure 1).

Detection of Anti-HIV by ELISA Method

All the collected samples were confirmed by performing ELISA. The HIV 1-2 sandwich ELISA kit was used for the in vitro detection of antibodies HIV Type 1 and Type 2 in serum or plasma. The HIV-1, 2 antibody enzymes linked immunosorbant assay (ELISA) kit employs a technique called a qualitative sandwich immunoassay. The micro titer plate provided in this kit is pre-coated with synthetic HIV polypeptides and recombinant HIV proteins which corresponds to highly antigenic epitopes consisting of essential sequences derived from both the envelop and core proteins of HIV-1 and HIV-2. Samples with optical density (OD) value greater than or equal to the cut-off value were considered reactive by the criteria of this HIV-1, 2 antibody ELISA Kit. Stop solution (Sulphuric acid) was added in each well (including the blank) to stop the enzymes reaction. The absorbance of the controls and test samples was determined within 30 minutes on ELISA plate reader at 450nm and the results were calibrated after all the readings and cut-off values.
Results and Discussion

From different areas of Peshawar cantonment, 400 blood samples were collected from the general population (40 samples each from shop keepers, barbers, drug users, sweepers, food workers, scrap merchants, mechanics, government employs, health care workers and students) and were screened for anti-HCV. Age of the subjects under study was 19-60 years. Majority of the subjects (65%) were below the age of 40 (<40 years), 80% of the subjects were uneducated and 55% of them were married. Among the screened subjects, 340 were negative and 60 were positive for HCV, so out of 40 samples each 16 (40%) shopkeepers, 10 (25%) barbers, 10 (25%) drug users, 8 (20%) sweepers, 8 (20%) food workers, 2 (5%) out of 40 each among scrap merchants, mechanics, government employs and health care workers were positive for HCV. However, all the samples from students were negative for HCV (Table 1). Majority of the HCV positive cases reported in subjects were above the age of 40 (>40 years), while 28% HCV positive cases were below the age of 40 (<40 years) (Fig. 2). 95% of the HCV positive individuals were uneducated, while 60% of HCV positive individuals were married. So the overall prevalence of HCV in Peshawar cantonment was 15% (Figure 2). The risk factors for HCV infection include commercial sex and non-marital sex, sharing blades and shaving kits, exposure to infected blood or infected needles, using unsterile equipment’s, injection drug use and unawareness.

For HIV, the result of the study showed that from 400 screened samples of the blood, from different areas of Peshawar cantonment (40 samples each from shop keepers, barbers, drug users, sweepers, food workers, scrap merchants, mechanics, government employs, health care workers and students) none was positive for HIV-1, 2. So the prevalence of HIV and HCV/HIV co-infection was 0% (Table 1).

Hepatitis C is rapidly emerging as a major health problem in both developed and developing countries including Pakistan [16, 17]. The study was conducted in specific region (Peshawar cantonment) of the Peshawar city. The main ethnic group in Peshawar is Pakhtuns, followed by a number of smaller ethnic groups. In Pakistan more than 10 million people are living with HCV, which is a reason of high morbidity and mortality. HCV prevalence is in the range of 4.1 to 36% reported from various parts of KPK Province of Pakistan [10]. In our research the prevalence of HCV was 15% in which different groups of the community were studied. Prevalence of HCV may be different in different regions and various groups of the same community [18]. For HCV, 400 samples were collected from different groups of the general population of Peshawar cantonment in which 340 subjects were found negative, while 60 were positive for HCV, which indicate quite high prevalence of HCV positive people in the area. The HCV virus was most prevalent among shopkeepers 16 (40%), while no HCV was detected in students. The reason could be more awareness among the students about disease and its mode of transmission and less exposure. Hospital based studies revealed prevalence rates of HCV as 5.31% in Islamabad [19], 2.45% - 20.89% in various parts of the Punjab province [20], 4-6% in Karachi [21], 5%-9% in North West Frontier

Table 1: Prevalence of anti-HCV and anti-HIV antibodies in general population of Peshawar cantonment

<p>| Table 1: Prevalence of anti-HCV and anti-HIV antibodies in general population of Peshawar cantonment | August 2015, 2(1):1-8 | International Journal of Microbiology and Allied Sciences, August 2015, Volume 2 Issue 1 |</p>
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Job title</th>
<th>Samples</th>
<th>HCV +ve</th>
<th>Percentage (%)</th>
<th>HIV +ve</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shop Keepers</td>
<td>40</td>
<td>16</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Barbers</td>
<td>40</td>
<td>10</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Drug Users</td>
<td>40</td>
<td>10</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Sweepers</td>
<td>40</td>
<td>8</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Food Workers</td>
<td>40</td>
<td>8</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Scrap Merchants</td>
<td>40</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Mechanics</td>
<td>40</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Govt. Employs</td>
<td>40</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Health Care Workers</td>
<td>40</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Students</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total Screened</td>
<td>400</td>
<td>60</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 2:** Graphical representation of HCV positive individuals in Peshawar cantonment

Province (NWFP) [22, 23] and 25.7% in Northern Areas of Pakistan [24]. Slightly higher prevalence of HCV was recorded in the earth quake affected areas of Pakistan in 2005 [17]. Sero-prevalence studies of anti-HCV antibodies in the general population of Pakistan have been recorded as 5.31% to 7.5% [6-8]. The highest active HCV infection was 40% in shopkeepers. The shopkeepers have variable history of exposure to HCV risk factors such as major/dental surgery, blood transfusion and because of the interaction with large number of people on daily basis. Second highest active HCV infection of 25% was observed in barbers and drug users and this could be because of exposure to high risk factors such as sharing blades and shaving kits, using unsterile equipment’s, injection
drug use and educational level. Third highest active HCV infection of 20% was observed in sweepers and food workers. The reason of this high prevalence could be unfavorable polluted environment and their direct interaction with it. In merchants, mechanics, government employs, and health care workers the active HCV infection was 5%. The comparatively less prevalence in these groups could be due to least exposure to HCV risk factors. 28% HCV positive cases were below the age of 40 (<40 years), while 51% of the HCV positive cases were above the age of 40 (>40 years) (Figure 3). 95% of the HCV positive individuals were uneducated and 60% of HCV positive individuals were married and they could be source of infection to their partners and kids. Our study is in agreement with the fact that chronic hepatitis C is common in asymptomatic healthy population of Pakistan. They are the possible candidates for chronic liver disease and potential sources of spread of infection both horizontally and vertically and prevalence of HCV may be different in different regions and various groups of the same community. There is need to do a national level prevalence study for hepatitis to know the exact prevalence of HCV in Pakistan [25].

Figure 3: Percentage of the HCV positive (HCV +ve) subjects according to the age groups
There is high prevalence of HIV globally and the disease is very concentrated in some countries and regions of the world [11]. However, according to current estimates, using the various latest prevalence estimation models; indicate that the HIV prevalence among general adult population is still below 0.1%, in Pakistan. Previous studies in general population of Pakistan have shown a sero-prevalence of HIV to be 0.23% [13]. For the investigation of HIV, we relied on highly sensitive detection method based on ELISA. Among the 400 collected samples of blood from general population, none of sample was positive for HIV, so the overall prevalence of HIV and HCV/HIV co-infection was 0%. It was because of the less or no exposure to risk factors for HIV and less prevalence of HIV in Pakistan.

Conclusion
The prevalence of HCV among the general population of Peshawar cantonment is 15%, which is quite high. Chronic hepatitis C is common in asymptomatic healthy population so an immediate large scale prevalence study should be done in Pakistan. HCV positive asymptomatic people are possible candidates for chronic liver disease and potential sources of spread of infection both horizontally and vertically. The Prevalence of HIV and HCV/HIV co-infection was 0% among the study subjects of present study.

Conflict of interest statement
There are no conflicts of interest regarding the contents of this article.

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