Distribution Pattern of Various Genotypes of HCV Circulating in District Mardan, Khyber Pakhtunkhwa, Pakistan

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Abstract: Hepatitis “C” is an infectious liver disease, the infection of HCV is often asymptomatic and the chronic infection comes to be hepatocellular carcinoma, cirrhosis and end-stage liver disease. Six main genotypes of HCV and several subtypes are recognized worldwide. HCV genotypes play important role in distribution of frequency, clinical management and development of vaccine. Current article shows distribution frequency of HCV genotypes present in various tehsils of district Mardan of Khyber Pakhtoonkhaw, Pakistan. Sum of 375 patients were positive for HCV RNA by PCR and were screened using genotyping assay. Data analysis showed that 241 out of 375 PCR positive patients were male and 134 were female. 190, 110 and 75 patients came from tehsils of Mardan, Takhat Bhai and Katlang respectively. Results showed that: Out of the total screened patients, genotype specific PCR fragments were seen in 322 (85.8%) patient. The distribution of typeable genotypes was as follows: 39 patients (10.4%) with genotype 1a, 11 (2.9%) patients with genotype 1b; 227 (60.5%) patients infected with genotype 3a; 24 (6.4%) patients genotype 3b; and 21 (5.6%) patients were with mixed genotypic infection. 53 (14.1%) serum samples were found un-typeable by the molecular genotyping assay used. In Conclusion It was concluded from the current research work that different genotypes of HCV such as 1a, 1b, 3a and 3b present in different tehsils of Mardan district and genotype 3a is most common genotype among general population of district Mardan.

Key words: Khyber Pakhtoonkhaw • HCV • Mardan • GCMD

INTRODUCTION

Hepatitis “C” is an infectious liver disease, the infection of HCV is often asymptomatic and the chronic infection comes to be hepatocellular carcinoma, cirrhosis and end-stage liver disease [1]. It is also expected that the cirrhotic patients may also get other problems of cirrhosis, hepatic cancer and liver [2-4]. All over the universe 200 million people are infected with HCV and in Pakistan there are 17 million people infected [1, 5]. The Hepatitis C virus was first discovered in 1989 [6]. Approximately 3.3% of the population worldwide (minor in Europe 1.03% and maximum in Africa 5.3%) and in Pakistan 10% of the people are infected with chronic hepatitis C virus [5, 7-9].

Hepatitis C virus has been classified as the single member of the genus Hepacivirus of family Flaviviridae, which have further more species the Pestivirus and the Flavivirus [10-12]. The size of Hepatitis C virus is approximately 55-65 nm, enveloped, with positive sense single strand RNA virus and in human the main causative agent of hepatitis, hepatocellular carcinoma (HCC) and liver cirrhosis, was isolated in 1989 [2, 3, 6, 13].

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HCV can be transmitted from infected individual to healthy individuals through various routes i.e. through contaminated blood transfusion, sharing of razors, toothbrushes and shaving from barber. It is also transmitted through contaminated dental apparatus, unsafe sex and drug abusers [14, 15, 16]. In Pakistan principal source of HCV transmission is contaminated blood transfusion because of lack of proper screening of blood, weak infrastructure and untrained medical staff [16].

Due to genomic variation HCV is divided into six main genotypes and multiple subtypes [17]. Genotypes Distribution of HCV varies geographically. HCV subtype 1a and 1b is common in Europe and USA [18-20]. In Japan most commonly circulated subtype is 1b [21]. In North America, Japan and Europe subtype 2a and 2b of HCV are distributed. Subtype 2c is mostly distributed in Italy while Genotype 4 is present in Middle East and North America. Genotype 5 and 6 are present in South Africa and Hong Kong [18-22].

The present research was conducted in Mardan, a district in the Khyber Pakhtunkhwa (KP) province of Pakistan. The total area of the district is 1632 square kilometers. According to 2008 survey the population of district Mardan is 1.96 million. Mardan city is the headquarters of the district. Few studies are available related to the prevalence of HCV genotypes circulating in different tehsils of Mardan. Therefore, the aims of this study was designed for finding out different and the most prevailing genotypes among the population of Mardan.

MATERIALS AND METHODS

Study Area: The present research was conducted in different areas of district Mardan during February-August, 2012. Mardan district is administratively subdivided into three tehsils that are: Mardan, Takht Bhai and Katlang.

Blood Sampling from HCV Positive Patients: A total of 375 HCV PCR positive patients were subjected for HCV genotyping. HCV positive samples were collected and data was recorded on data forms designed for study and distributed to all collection centers in different tehsils of district Mardan.

RNA Extraction: RNA was extracted from the serum of HCV positive patients by using RNA isolation Kit Gentra (Puregene Minneapolis, MN, USA) according to the manufacturer's recommended protocol.

Complementary DNA (cDNA) Synthesis: Complementary DNA was synthesized at 37°C in 50 min using reverse transcriptase PCR (RT-PCR) of total isolated viral RNA (20 µl) by using 100 units of Malany-murine leukemia virus reverse transcriptase Zenzyme (M-MLV R) transcribed 10 µl to cDNA 1 µM of downstream primer (outer antisense) according to manufacturing instructions (Gibco BRL).

HCV genotype specific PCR: For all the HCV RNA positive individual genotyping was performed by using HCV genotyping method described by Idrees [5]. As HCV has 12 different genotypes, type-specific primers were divided in two groups on the basis of differences in the sizes of the different bands to avoid overlapping of similar bands on agarose gel. Each first round amplified PCR products were subjected to two second rounds nested PCR amplifications. First with mix-A primer that are specific for 1a, 1b, 1c, 3a, 3c and 4 genotypes and second with mix-B primers that are specific for 2a, 2c, 3b, 5a and 6a genotypes.

Gel Electrophoresis: Round two amplification products were electrophoresed on a 2% agarose gel to separate type-specific fragment. DNA ladder of 100-bp (Invitrogen, Corp, California, USA) was used as size marker for DNA to compare genotype specific bands.

Statistical Analysis: The data were analyzed and summarize using statistical software Statistica version 9.0 for windows. The results for all variables were presented in percentage (%). Fisher's exact and Chi Square tests were used to find out positive association among the categorical variables. P<0.05 was considered as significant.

RESULTS

Demography of the HCV Patients: A total of 375 HCV positive infected patients were selected from different tehsils of district Mardan, Khyber Pakhtunkhwa, Pakistan for specific genotyping procedure. Demography of the diseased subjects was shown in Figure 1. Of 375 patients 64.2% (n=241) were males and 35.7% (n=134) were females of age ranged less 10 and above 60 years. Distribution breakup of the patients was 190, 110 and 75 from tehsil Mardan, Takht Bhai and Katlang, respectively. The selected samples in this setup that were HCV-RNA positive with high viral titer were subjected for specific genotyping assay.
Prevalence of Specific HCV Genotypes in Different Tehsils of District Mardan: Total 51 % (n=190) samples were investigated from tehsil Mardan, 29%(n=110) from tehsil Takht Bhai and 20%(n=75) samples from tehsil Katlang. The most prevalent genotype was 3a in all tehsils. In tehsil Mardan, 3a genotype was 64.7%(n=123), in Takht Bhai tehsil 3a genotype was 70.9%(n=78) and in tehsil Katlang 3a genotype was 34.6%(n=26), followed by 1a which was 9.4%(n=18) in tehsil Mardan, 6.3%(n=7) in tehsil Takht Bhai and 18.6%(n=14) in tehsil Katlang, 3b genotype in tehsil Mardan was 6.3%(n=12), in tehsil Takht Bhai 2.7%(n=3) and in tehsil Katlang 12%(n=9), 1b genotype in tehsil Mardan was 1(n=2)%, in tehsil Takht Bhai 4.5%(n=5) and in tehsil Katlang 5.3%(n=4), mixed genotypes in tehsil Mardan was 3.1%(n=6), in tehsil Takht Bhai 5.4%(n=6) and in tehsil Katlang 12%(n=9) with mixed genotypes and in all tehsils Mardan, Takht Bhai and Katlang, 15.2%(n=29), 10%(n=11) and 17.3%(n=13) patients have not shown genotype specific fragments, hence, they were called untypable (Table I).

Gender Wise Frequency of HCV in District Mardan: Figure 2 shows gender wise distribution of different HCV genotypes in district Mardan. The 1a genotype was 10% in both genders; 1b was 3%, 3a was 61%, 3b 6%, mixed genotypes are 6% and the remaining 14% are untypable. In present study 3a genotype was the predominant genotype in both male and female in Mardan. In male, 3a genotype was 59% and in female 3a was in 63%, followed by 1a in both male and female, in male 1a was 10%, in female 11%, 3b genotype was 6% in male and 7% in female, 1b in male was 3% and 2% in females, 5% in male are mixed genotype and 7% in female, the remaining in male 17% are untypable and 10% in female.

Age Groups Wise Frequency of HCV in District Mardan: Now according to age groups distribution of different HCV genotypes in district Mardan are shown in Table II. In 20 age group totals of 1%(n=4) patients are present, in 21-40 years age group 48.5%(n=182) patients are investigated, in 41-60 years age group total 40.5%(152) patients are and above 60 years age group 9.8%(n=37) of patients are in the present study. The 3a genotype is dominant in Mardan, according to age groups, in < 20 year’s age group, 75 % (n=3) patients with 3a genotype and 25% (n=1) are untypable. In 21-40 year’s age group, 58.2 %(n=106) are 3a genotype, 12%(n=22) are 1a genotype, 1.6%(n=3) are 1b genotype, 7.1 %(n=13) are 3b genotype, 5% are mixed genotypes and 16% are untypable. In 41-60 years age group, 58.5%(n=89) patients have 3a genotype, 9.2%(n=14) patients have 1a genotype, 4.6 %(n=7) are 1b, 7.8%(n=13) patients have 3b genotype, 6.5%(n=10) patients have mixed genotypes and 13.1%(n=20) are untypable. In > 60 years age group, 78.3%(n=29) patients have 3a genotype, 8.1%(n=3) patients have 1a genotype and 2.7 %(n=1) patients with 1b, 2.7%(n=1) with mixed genotype and 8.1%(n=3) are untypable. In age groups wise distribution total 1a genotype is present 10.4%, 1b is 2.9%, 3a is 60.5%, 3b is 6.6%, mixed genotypes are 5.3% and the remaining 14.1% are untypable.

Table I: Prevalence of specific HCV genotypes in different tehsils of district Mardan

<table>
<thead>
<tr>
<th>Genotype/Sub-type</th>
<th>District</th>
<th>Isolated from Mardan</th>
<th>Isolated from Takht bhai</th>
<th>Isolated from Katlang</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1a</td>
<td>1b</td>
<td>3a</td>
<td>3b</td>
</tr>
<tr>
<td>Isolated from Mardan</td>
<td>18</td>
<td>2</td>
<td>123</td>
<td>12</td>
</tr>
<tr>
<td>Isolated from Takht bhai</td>
<td>7</td>
<td>5</td>
<td>78</td>
<td>3</td>
</tr>
<tr>
<td>Isolated from Katlang</td>
<td>14(18.6%)</td>
<td>4</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>39</td>
<td>11</td>
<td>227</td>
<td>25</td>
</tr>
</tbody>
</table>

P= 0.0.1637 (>0.05) Non significant

Table II: Age groups wise frequency of HCV in district Mardan

<table>
<thead>
<tr>
<th>Genotype/Sub-type</th>
<th>Age</th>
<th>0------20</th>
<th>21----40</th>
<th>41----60</th>
<th>Above 60</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1a</td>
<td>1b</td>
<td>3a</td>
<td>3b</td>
<td>Untypable</td>
<td>Mixed</td>
</tr>
<tr>
<td>0------20</td>
<td>0</td>
<td>0</td>
<td>3 (75%)</td>
<td>0</td>
<td>1 (25%)</td>
<td>0 (4)</td>
</tr>
<tr>
<td>21----40</td>
<td>22</td>
<td>3</td>
<td>106(58.2%)</td>
<td>13(7.1%)</td>
<td>29(16%)</td>
<td>9 (5%)</td>
</tr>
<tr>
<td>41----60</td>
<td>14</td>
<td>7</td>
<td>89(58.5%)</td>
<td>12(7.8%)</td>
<td>20(13.1%)</td>
<td>10 (6.5%)</td>
</tr>
<tr>
<td>Above 60</td>
<td>3</td>
<td>1</td>
<td>29(78.3%)</td>
<td>0</td>
<td>3 (8.10%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>39</td>
<td>11</td>
<td>227 (60.5%)</td>
<td>25(6.6%)</td>
<td>53(14.1%)</td>
<td>20 (5.3%)</td>
</tr>
</tbody>
</table>

P=0.0015 (<0.05) significant
DISCUSSION

Commonly six HCV major genotypes and several subtypes have been recognized globally [18]. Detection of HCV genotype and its subtype is of great clinically importance before recommending treatment, for the reason that genotypes 1 and 4 show more resistance to PEG interferon plus ribavirin treatment as compared to genotypes 2 and 3, thus different HCV genotype needs different period and varying anti-viral treatment dose [23]. PEG interferon plus ribavarin therapy for genotypes 1 and 4 are 48 weeks while for 2 and 3 is 24 weeks [24].

This study was conducted to analyze the HCV genotypes circulating in various tehsils of district Mardan. The data analysis shows that the most predominant genotype in the studied population was 3a, 227 (60.5%) followed by 1a 39 (10.4%), 3b 24 (6.4%) 1b 11 (2.9%), 21 (5.6%) mixed genotypes and 53 (14.1%) were untypable. The present findings were similar to previous studies conducted in different areas of Pakistan that, 3a is most common genotype circulating in this area [5, 14, 25, 26]. The distribution pattern of HCV genotypes of this study is similar to that reported from other South Asian states such as in India [27, 28] and in Nepal [29] where the mostly circulating genotype was 3a but contradict from other regions of the globe such as in Thailand [30], Japan [31], Western Europe Vietnam, USA and where genotype 1 is the common HCV genotype [32].

In our data interpretation 21 (5.6%) individual were infected by mixed genotypes. Factor common to these patients was thalassemic subjects who had previously transfused unscreened blood. These findings are supported by other published reports indicating that mixed genotypes were common where blood transfusion is most prevalent particularly in thalassemia patients [33]. Furthermore individuals with mixed genotypes might influence the antiviral treatment response and infection progression [34]. In this study we were incapable to see a single 4 genotype from any HCV positive patient, that is supposed to be partially absent from Pakistan and it is the most predominant genotype of Middle East. [35] None of the patients were seen infected with genotype 6a and 5a; these are common in Hong Kong and South Africa [22].

This setup showed that high incidence rate of HCV infection (p=0.0015) was found in age group of 21-40. These results were similar with the findings of Inamullah et al. [26] that prevalence was seen in age group ≤ 40 years and similar findings were observed by Ali et al. [25] On the other hand our results deviated from Muhammad et al. [36] that most prevalent HCV incidence rate in Pakistan was seen in old age group. Thus these findings suggest that untimely detection of HCV may be due to the alertness of general population about HCV infection in our studied population.

The important implication of current study was the isolation of 53 (14.1%) isolates that were untypable and no genotype specific PCR fragments were found for these patients. These 53 serum samples with untypable genotypes were positive for hepatitis C virus by qualitative PCR. The rate of untypable genotypes observed in the recent study might be due to the pedestal that our untypable patients had already been through standard interferon plus ribavirin therapy. Moreover patients maybe non-responders or were relapsed subsequently.

CONCLUSION

In the current study we conclude that hepatitis C virus genotypes 1a, 1b, 3a and 3b are distributed in different tehsil of Mardan. Genotype 3a is the predominant genotype circulating in district Mardan. Furthermore majority of the infected population belong to age group 21-40 years.

Competing Interests: The authors declare that they have no competing interests.

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