

Molecular detection of the Hepatitis C virus genotypes circulating among both sexes of Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Background: Hepatitis C Virus (HCV) is the major cause of viral hepatitis. A few studies revealed that HCV genotypes; 3a, 3b, 1a and 1b are the most common genotypes found in the general population of Khyber Pakhtunkhwa. **Aims and Objective:** The current is designed to find out the prevalence of HCV genotypes among both sexes of Khyber Pakhtunkhwa. **Study Methods:** The present study was carried out in various area of Khyber Pakhtunkhwa during the period of July, 2012 to July, 2013. All the HCV positive samples were genotyped. The statistical analysis of the data was done by using Statistix 9.0 software. **Result:** The results showed that the male, 53.1% (n=340) were more infected as to female, 46.8% (n=300). The overall result shows that in male patients; the high number of cases (216) was reported of 3a whereas the lowest cases (8) were reported of 1b. The mixed genotype is recorded in 8 patients. In female patients; the 3a is detected in 170 patients whereas the lowest cases of genotype (3b) are reported in 5 patients. In female patients; the mixed genotype is reported in 11 patients. **Conclusion:** From the present study, it was concluded that the male were more infected as compare to female and the genotype 3a was the most common genotype in both sexes.

Key words: Hepatitis C Virus, Genotypes, Chi-square test, GCMD

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INTRODUCTION

Hepatitis C Virus is an envelope, single stranded RNA virus with a genome size of 9.6 kb. HCV was first time discovered by Choo in 1989 and was placed in family Flaviviridae and genus Hepacivirus.^{1,2} Hepatitis C Virus is similar to dengue, Yellow fever and Hepatitis G viruses.³ In human HCV can lead to chronic and acute hepatic inflammation.⁴ HCV is mostly responsible for chronic hepatitis, Hepatocellular carcinoma and liver cirrhosis.⁵ Globally, HCV infects about 170 million of the world population. Hepatitis C Virus infection is distributed globally but its prevalence varies from area to area.⁶ Because of the limited resources and low facilities, HCV infection rate is high when compare with

developed countries.⁷ From different regions of Pakistan, many uncontrolled outbreaks of HCV infection have been reported.⁸ Hepatocytes and B lymphocytes are targeted by Hepatitis C Virus and about 10 trillion new viral particles are produced per day.⁹

HCV can transmit through many sources, i.e., re-use of syringes, improper blood screening before transfusion, sharing of razors, unsafe sex, etc. In Pakistan, key factors that are responsible for Hepatitis C Virus infection are blood transfusion, re-use of syringes and needles used for nose and ear piercing, re-use of blades in the barber shop and contaminated surgical instrument.^{10,11} Due to genetic variation in nucleotide sequences, HCV is further classified

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into various genotypes.¹² Up to date six principal genotypes and almost 80 subtypes of Hepatitis C Virus are identified around the world.¹³ HCV genotype 1, 2 and 3 are circulating worldwide while the remaining genotypes vary from region to region. HCV subtypes 1a and 1b are most commonly distributed throughout the world especially in Europe and USA.^{14,15} Subtype 1b is responsible for 73% of HCV infection in Japan. Subtype 2a and 2b are most common in Europe and North America while 2c are mostly found in Northern Italy.¹⁰ HCV genotype 3 is most dominant genotype in South-East Asia while in Egypt, Central Africa and Middle East; genotype 4 is mostly distributed. Genotype 5 infects most of the population of South Africa¹⁶ while genotype 6 is most common in Hong Kong.¹⁷

A few numbers of studies on HCV genotype distribution are available from Khyber Pakhtunkhwa that based on small samples sizes.¹⁸ Recently, a less number of studies on the distribution of HCV genotypes is reported from Khyber Pakhtunkhwa which shows that 3a, 3b, 1a and 1b are the most common genotypes found in the general population in Khyber Pakhtunkhwa.¹⁹ The present research study was conducted to analyze the prevalence of HCV genotypes between both sexes of various areas of Khyber Pakhtunkhwa.

MATERIALS AND METHODS

Study area

This study was conducted at the Mardan medical complex from July, 2012 to July, 2013. Khyber Pakhtunkhwa is the beautiful place on the earth that consists of 24 districts with an area of 74521 sq km, and a population of 0.22 billion. HCV positive males and females patients from 10 to 75 years of various districts of Khyber Pakhtunkhwa were selected in our study. Out of the total 640 patients, 267(41.7%) belong to district Peshawar, 219(34.2%) to district Mardan, 96(15%) to district Swat, 20(3.1%) to district Malakand, 15(2.3%) to district Dir lower, 12(1.8%) to district Abbottabad and 11(1.7%) to district D. I. Khan. Blood samples were collected from all the patients and serum were separated from it and send to Genome Center for Molecular Based Diagnostics & Research for screening of HCV infection.

Viral RNA isolation

Viral RNA was isolated from 150 µl of patient's samples by using Nucleo Spin DNA Virus/Virus F kits by Machery and Nagel, isolation kit according to instruction provided by manufacture.

HCV genotyping

For genotyping of HCV Ana Gen (USA) Kit was used. First of all complementary DNA was synthesized from

10 µl of extracted RNA at 37°C in 50 min with the help of MMLV(Molony-murine leukemia virus) enzymes. Complementary DNA was further subjected into two round of PCR amplification. About 2 µl of cDNA was used to amplify 470-bp region from HCV 5'NCR+Core region in 1st round PCR. After that, 1st round PCR product were subjected into two round of nested PCR. First with mixed-A primers and 2nd with mixed-B primers. Mixed A primers consist of 1a, 1b, 1c, 3a, 3c and 4 and mixed-B primers consist of 2a, 2c, 3b, 5a and 6a. Finally the amplify PCR product were run on 2% agarose gel, stained with ethidium bromide and visualized under UV transilluminator. The genotype-specific cDNA bands identified the HCV genotype in each sample.

Statistical analysis

Statistical analysis was done by using Statistic 9.0 software. Chi-square test was used for variable categorization. P-value less than 0.05 were considered as significant.

RESULTS

A. Prevalence of HCV genotype circulating in different area of Khyber Pakhtunkhwa

In the present study, only those patients were included that were found positive for HCV RNA through PCR. Out of 640 HCV positive patients, 46.8% (n=300) were females and male 53.1% (n=340). All the patients belong to different regions of Khyber Pakhtunkhwa. Ages of these patients are from 10 years to 80 years. Table 1 analyzed distribution of HCV genotypes in various regions of Khyber Pakhtunkhwa. In total of these 640 HCV positive patients, 267 patients belong to District Peshawar in which 136 patients were found with genotype 3a, 40 patients with genotype 1a, 9 patients with 1b, 2 patients with 3b, 3 patients with mixed genotypes and 77 patients were found untypable. From District Mardan 219 patients were selected where 149 patients were found with 3a genotype, 25 with 1b, 10 with 1b, 6 with 3b, 8 with mixed genotype and 21 were untypable. From District Swat, 96 HCV positive patients were analyzed where genotype distribution were 3a; (68), 1a; (13), 1b; (3), 3b; (2), mixed genotype; (1), while 9 patients were found untypable. Sum of 20 patients from district Malakand were screened for HCV genotypes in which genotype 3a is most common that is found in 7 patients, 1a was found in 2 patients, 3b in 3 patients, 3 patients found with mixed genotype and 2 patients were found untypable. Fifteen patients from District Dir lower were screened for HCV genotyping in which distribution pattern of HCV genotypes was 3a; (7), 1b; (1), mixed genotype; (3) and untypable; (4). In district Abbottabad, 12 patients were checked for HCV genotyping in which genotype 3a is mostly distributed, 3b was detected in 2 patients, 1 patient with mixed genotypes

and 2 patients was found untypable. In district D. I. Khan 3a genotype was found in 9 patients, 1a in 1 patient and 1 patient was untypable.

B. Presence of HCV genotypes in both sexes of Khyber Pakhtunkhwa

Presence of different HCV genotypes in both sexes (males; n=340 and females; n=300) were analyzed in Table 2. Results show that 3a is the most common genotype among both sexes. In male patient, genotype 3a was found in 216 patients, 1a in 39 patients, 1b in 8 patients, 3b in 10 patients and 8 patients were found infected by two genotype (mixed genotype) while in the remaining 59 patients, none of the genotype were observed. It was considered untypable genotype according to Ohno et al [20]. Our results show that the total 300 females patient, HCV genotype 3a was detected in 170 patients, 1a in 43 patients, 1b in 14 patients, 3b in 5 patients while 11 patients was found with mixed genotype and 57 patients were found untypable.

C. Distribution of HCV mixed genotypes in both sexes of various area of Khyber Pakhtunkhwa

In our study, it is observed that 19 of the patients were found infected by combination of two genotypes. Table 3 indicated that 11 patients were infected by combination of two genotype 3a+1a, 5 were infected by 3a+1b and 3 were infected by 3a+3b. In total of 19 patients, 3 patients belong to district Peshawar, 8 to district Mardan, 1 to district Swat, 3 to district Malakand, 3 to district Dir lower and 1 to district Abottabad.

DISCUSSIONS

Khyber Pakhtunkhwa is one of the four Provinces of Pakistan that is located in the north-west of the country.

It has the second poorest economy after Balochistan.²¹ So it is comprehensible that health facilities like screening and sterilization are not up to par, leading to spread of blood-borne infections like HCV. HCV has been divided into six major types and several minor subtypes.²² Knowledge of its subtypes is crucial for clinical management, ie., for estimation of prognosis and duration of treatment and vaccine development. According to the WHO estimates, 3% of the worldwide population is infected with the Hepatitis C Virus.²³ HCV genotypes have heterogeneous geographical distribution. Genotypes 1, 2 and 3 have a global distribution while 4, 5 and 6 are restricted to more precise geographic regions, i.e., South Africa and Southeast Asia, Egypt and Africa respectively.²² Pakistan has a high prevalence rate of Hepatitis C ranging from 4.5% to 8%.²⁴ A detailed study from Pakistan showed the prevalence of genotype 3a (62%), 3b (9%), 1a (3%), 2a (2.144%), mixed (4.718%) and untypable (17.16%).²⁵ A study from KPK done on IDUs in 2011 showed that genotype 2a was the most prevalent followed by 3a, and that a considerable number of the IDUs were infected with genotype 4 also.²⁶ A study from Swat also showed similar results.²⁷ Similar pattern was observed in Sindh and Punjab provinces with Baluchistan showing disparity in that genotype 1a was found to be most predominant.²⁸

Our study is pertinent because all areas of KPK have been covered, including Swat, Mardan, Peshawar, Malakand, Dir (Lower), Abbotabad and D. I. Khan. This is in contrast to most other KPK studies which were limited to just one region. Such kind of extensive study was therefore needed and would give a far better picture of the over-all prevalence of genotypes in KPK, and differences in its region-wise, so that a more advanced study can be

Table 1: Molecular detection of HCV genotypes in both gender/sexes of different regions of KPK

Genotype/ Sub-types	District Peshawar	District Mardan	District Swat	District Bathkhela	District Dir lower	District Abottabad	District D.I. Khan	P-value
1a	40 (14.9%)	25 (11.4%)	13 (13.5%)	02 (10%)	01 (6.6%)	00	01 (9%)	0.0000 significant
1b	09 (3.3%)	10 (4.5%)	03 (3.1%)	00	00	00	00	
3a	136 (50.9%)	149 (68%)	68 (70.8%)	10 (50%)	07 (46.6%)	07 (58.3%)	09 (81.8%)	
3b	02 (0.7%)	06 (2.7%)	02 (2.08%)	03 (15%)	00	02 (16.6%)	00	
Mixed genotypes	03 (1.1%)	08 (3.6%)	01 (1.04%)	03 (15%)	03 (20%)	01 (8.3%)	00	
Untypable	77 (28.8%)	21 (9.5%)	09 (9.3%)	02 (10%)	04 (26.6%)	02 (16.6%)	01 (9%)	
Total	267	219	96	20	15	12	11	

Table 2: Distribution of HCV genotypes in both sexes of KPK

Genotypes	Subtypes	Male	Female	Total	P-value
1	1a	39 (11.4%)	43 (14.3%)	82 (12.8%)	0.9928 Non-significant
	1b	8 (2.3%)	14 (4.6%)	22 (3.4%)	
3	3a	216 (63.5%)	170 (56.6%)	386 (60.3%)	
	3b	10 (2.9%)	05 (1.6%)	15 (2.3%)	
Mixed		08 (2.3%)	11 (1.7%)	19 (2.9%)	
Untypable		59 (17.3%)	57 (19%)	116 (18.1%)	

Table 3: Distribution of HCV mixed genotypes in both sexes of various area of KPK

District	3a+1a		3a+1b		3a+3b		P-value
	Male	Female	Male	Female	Male	Female	
Peshawar	0	01	01	0	0	01	0.6721 Non-significant
Mardan	02	02	03	0	0	01	
Swat	0	01	0	0	0	0	
Malakand	0	02	01	0	0	0	
Dir lower	01	01	0	0	01	0	
Abottabad	01	0	0	0	0	0	
Total	11		5		3		

undertaken in future to investigate the reasons behind the differences and similarities. This in turn can help in prevention and treatment. Also, in most of the earlier studies, women subjects were limitedly selected because of the social and religious taboos which prevent them from participation in such studies, and also because females are less likely to be exposed to risk factors like intravenous drug abuse and unsafe sexual activities. For instance, in study conducted in KPK in 2011 on prevalence of HCV genotypes, only two females were analyzed from the whole population for HCV infection.²⁹ However, in our study, the proportions of male and female subjects are almost equal. This can help in ruling out gender bias. Moreover, special stress has been given on determining the distribution of patients with mixed genotypes, as this greatly affects the treatment modality and prediction of outcome.

The results of our study are in accordance with the earlier studies which show that genotype 3a outnumbers all other genotypes irrespective of region or gender. Our study also showed an outstanding proportion of untypable genotype which is in conformity with the other studies conducted in Pakistan. This study suggests that our beautiful province of KPK has been engulfed by the rising flood of HCV infection, specifically the genotype 3a. Phenomenal steps need to be taken to combat this avalanche of spread especially of rare as well as untypable genotypes which are most probably mutants which can be resistant to conventional therapies. Sequencing of the untypable genotype is needed as this may open the door to the discovery of optimal therapy for such strains. Therefore, it is high time that updating of the current laboratory genotyping assays is done.

CONCLUSIONS

From the current study, it was concluded that the high prevalence rate of HCV is recorded in male population as compare to female population. It was observed that the HCV genotype 3a is the most common genotype in different areas of the Khyber Pakhtunkhwa, Pakistan.

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Authors Contribution:

HUR and MW – collected the data and design the study; MW, AUK, AAN, TA, NA, TS, F, NUA, MZ, EK and AS – help in literature search and gave a critical view of manuscript writing. All authors read and approved the final manuscript.

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