RESEARCH PAPER

Epidemiological study of HCV genotypes circulated in different regions of Pakistan: a lab based study

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Abstract

The objective of the present study was to find out the prevalence of different genotype of Hepatitis C virus (HCV) in different area of Pakistan include, Khyber Pakhtunkhwa province, Punjab province, Sindh province and Islamabad Capital Territory. The data were collected from the different Labs and Hospitals. The present study was carried out during January 2011 to December 2012. The overall result shows that the female are more infected as compare to male 190 (50.5%) and 186 (49.5%) respectively. The high number of cases was reported from the Khyber Pakhtunkhwa 201 (53.4%) where the lowest number of cases was reported in Sindh 32 (8.5%). The overall result shows that the 3a is the most common genotype while the genotype 1b is reported in lowest number. The age wise distribution shows that the high number of cases 135 (35.9%) was reported in age group 31-40 years while the low number of cases 6 (1.6%) was occurred in age group >60 years. The statistical analysis of the data shows that the P-value is less than 0.05 were considered as significant. From this study it was concluded that the genotype 3a is the most common genotype in different region of Pakistan.

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Introduction

Hepatitis C virus (HCV) is rapidly emerging as a major challenge for the global health, especially in the developing countries includes Pakistan (Raza *et al.,* 2007, Khan *et al.,* 2008). In 1989; Hepatitis C virus was isolated first time. HCV is positive-sense single stranded RNA virus belongs to family Flaviviridae. Its genome is about 9.6 kb in length has having a 5' non-coding region (5'NCR), a long open reading frame (ORF) encoding a polyprotein precursor of 3,000 amino acids and 3'NCR. Functions of 5'NCR as an internal ribosomal entry site (IRES) is important for cap independent translation of the viral RNA (Bartenschlager *et al., 2004*). HCV is a mostly responsible for viral hepatitis, hepatocellular carcinoma (HCC) and liver cirrhosis (Rice, 1996, Giannini and Brechot, 2003, Shepard *et al.,* 2005). Approximately all over the world 200 million peoples are infected with HCV including 17 million in Pakistan (Narendra *et al.,* 2004; Idrees and Riazuddin, 2008). HCV targeted B lymphocytes and hepatocytes of human cells (Omran *et al., 2009*). About 10 trillion virus particles of HCV are produce per day, although in late phase of infection (Ohno *et al.,1997*).

Hepatitis C virus has six primary genotype and hundrad of subtypes. (Alter *et al., 2007*). Genotypes 1, 2, and 3 circulated worldwide while subtypes 1a and 1b are mostly found in USA (Zein *et al.,1996*). Subtypes 1a and 1b are also circulated in Europe. In Japan 1b is most common subtype and is counted for 73% of HCV infection (Takada *et al.,1993*). HCV subtype 2a and 2b are also circulating in Europe, North America and Japan while 2c is most frequent subtype in Italy. Genotype 4a is mostly circulating in Middle East and North Africa (Chamberlain *et al.,1997*). While HCV genotype 5 and 6 are common in Hong Kong and South Africa (Simmonds *et al.,1993*).

The distribution of these genotypes depends on the transmission and geographic area. In Pakistan the type-3 is the most prevalent genotype with a ratio (68%-87%) (Ahmad *et al.,* 2007, Ahmad *et al.,* 2003,

Waheed *et al.,* 2009, Turhan *et al.,* 2005). The occurrence of HCV is high in developing countries due to poor economic status and the patients are unable to afford the expensive therapy (Alter, 2007). The current investigation was carried out to find out the presence of different genotypes of HCV in different regions of Pakistan.

Materials and methods

Study areas

Pakistan is administratively divided into 4 provinces: Punjab, Khyber Paktunkhwa, Sindh and Baluchistan. For this study different regions i.e. Province Punjab, Province Khyber Paktunkhwa, Province Sindh and Capital of Pakistan Islamabad were selected.

Data collection

Sum of 376 HCV positive patients data were collected from different hospitals and laboratories of Pakistan from January 2011 to December 2012.

Study design

Very few numbers of such specific studies is available related to the distribution of genotypes of HCV circulating in Pakistan. Therefore, the current study was designed as non-probability-consecutive sampling was made from 376 patients for finding out different and the most prevailing genotypes among the population of Pakistan.

Statistical Analysis

Statistical analysis of the data was performed by using "STATISTIX", version 9.0, Korean made software. $P<0.05$ values were considered the significant.

Results

A total of 376 HCV positive patients included in the present study of age ranged below 10 years and above 60 years were with maximum viral loads and were thus appropriate for HCV genotyping.

Demography of the HCV patients

A total of 376 samples of HCV infected patients from different provinces of Pakistan (Khyber pakhtunkhwa, Panjab, sindh, capital Islamabad) were

included for genotyping. Details about the demographic distribution of the patients enrolled were shown in figure1. Out of 376 patients 49.5% (n=186) were males and 50.5% (n=190) were females of age ranged below 10 and above 60 years. In total of 376 successfully genotyped patients 201 belonged to Province Khyber Pakhtunkhwa, 98 from Province Punjab, 32 from Province Sindh, 45 from Capital Islamabad. All the samples included in the present study were found HCV-RNA positive with enough viral load for further analysis.

Table 1. Distribution of HCV genotypes in patients according to gender wise.

Genotype/subtype	Male (%)	Female (%)	Total	P-Value
1a	09 (4.8%)	14 (7.3%)	23(6.1%)	
1b	02 (1.0%)	0 (0%)	02(0.5%)	
3a	121 (65.1%)	120 (63.2%)	241(64%)	0.0625
3b	01 (0.5%)	02 (1.1%)	03 (0.7%)	Non-significant
Mixed genotypes	04 (2.1%)	03 (1.6%)	07(1.8%)	
Untypeable	49 (26.3%)	51 (26.8%)	100(26.5%)	
Total	186 (49.5%)	190 (50.5%)	376	

Distribution pattern of HCV genotypes in studied population

Out of 376 diseased subjects, prevalence rate was more in female 50.5% (n=190) patients than in male patients 49.5% (n=186). Out of the total 376 processed serum samples, genotype specific PCR bands were found in 276 (73.40%) patients. The results of the current study show that the 3a was seen to be most prevalent genotype. The 3a genotype was detected in 241 (64.1%) patients followed by 1a in 23 (6.1%) patients, 3b in 03 (0.8%), 1b in 02 (0.53%) patients. Seven (1.9%) patients were found with mixed genotypes and 100 (26.6%) with undetermined genotypes. All the HCV genotypes were distributed in male and female patients following the same pattern as shown in table 1.

Table 2. Age wise distribution of the patients HCV genotypes.

Age group (years)	3a	1a	1b	3b	Untypeable	Mixed	Total	Percentage (%)
10-20	04	01	0	0	04	0	09	2.3
21-30	49	05	0	0	16	0	70	18.6
31-40	83	13	01	0	36	02	135	35.9
41-50	78	02	01	02	32	02	117	31.1
51-60	24	02	0	01	10	02	39	10.3
>60	03	0	0	0	02	01	06	1.6
Total	241	23	02	03	100	07	376	100
P-value	0.9599 Non- significant							

Age wise distribution of HCV genotypes in infected subjects

In the table 2 the prevalence of genotype of HCV in various age groups subjects is presented. The most frequent cases of HCV 135 (35.9%) were seen in between age group of 31-40 as compared to younger and old age groups.

Distribution HCV genotypes in different area of Pakistan

Table 3 shows the distribution of HCV genotypes that were documented from HCV patients belonged to different area of Pakistan. Out of 376 genotypes 201 (53.45%) were belonged to Khyber Pakhtunkhwa. Amongst 201 (53.45%) genotypes from Khyber pakhtunkhwa, 114 (57%) were 3a, 1a were 10 (5%), 1b were 02 (1%), 3b were 3 (1.5%), mixed genotype was found in 01 (0.5%) and the remaining 71 (35.5%) patients were of untypeable genotype. Out of 376 genotypes 98 (26.06%) were belonged to Panjab. Amongst 98 (26.06%) genotypes from Punjab, 81

(82.06%) were 3a, 1a were 05 (10%), 1b were 0 (0%), 3b were (0%), mixed genotype was found in 03 (3.1%) and the remaining 09 (9.2%) patients were of untypeable genotype. Out of 376 genotypes 32 (8.51%) belonged to Sindh. Amongst 32 (8.51%) genotypes from Sindh, 23 (72%) were 3a, 1a were 03 (9%), 1b were 0 (0%), 3b (0%), mixed genotype was found in 0 (0%) and the remaining 06 (19%) patients were of untypeable genotype. Out of 376 genotypes 45 (11.96%) belonged to Capital Islamabad. Amongst 45 (11.96%) genotypes from Islamabad, 23 (51%) were 3a, 1a were 05 (11%), 1b were 0 (0%), 3b were (0%), mixed genotype was found in 03 (7%) and the remaining 14 (31%) patients were of untypeable genotype.

Table 3. Distribution HCV genotypes in different areas of Pakistan.

| Genotype/subtype	Khyber Pakhtunkhwa	Punjab	Islamabad	Sindh
1a	10 (5%)	05 (10%)	05 (11%)	03 (9%)
1b	02 (1%)	0 (0%)	0 (0%)	0 (0%)
3a	114 (57%)	81 (82.6%)	23 (51%)	23 (72%)
3b	03 (1.5%)	0 (0%)	0 (%)	0 (0%)
Mixed	01 (0.5%)	03 (3.1%)	03 (7%)	0 (0%)
Untypeable	71 (35.5%)	09 (9.2%)	14 (31%)	06 (19%)
Total	201	98	45	32
P-value	0.0000 Significant			

Discussions

Several epidemiological studies have been conducted to observe the incidence of different HCV genotypes in different parts of the globe. These studies have helped to resolve the epidemiological issues and also useful in the therapeutic assessments when the therapy of the HCV is prescribing, so it has been investigated that the severity of disease, response to treatment and prognosis depends on different factors out of which genotype is principal factor. Globally socio-economic consignment of HCV infectivity is increasing very rapidly. For the development of HCV therapeutic strategies better and reliable information about viral pathogenesis is need. In Pakistan, a considerable local difference was identified in the prevalence of HCV genotypes. For the predictive implications in chronic HCV patients the information about determination of different HCV genotypes in Pakistan is very significant.

In many regions of the world the HCV has come to the top of virus-induced liver diseases and has gained endemic proportions in our population. In Pakistan for the evaluation of genotypes of HCV infection no national data collection system is available. Pakistan is situated in the western part of the Indian subcontinent, with India on the east, Afghanistan and Iran on the west and Arabian Sea on the south. This geographical situation of Pakistan affected the epidemiology of HCV, mass immigration and illegal drug traffic from Afghanistan, absence of good preventive measures in the past e.g. fragile health structure, extensive reuse of non-sterilized syringes, use of contaminated razor by barber, unscreened blood transfusion, poor education and general poverty. Pakistan consists of four provinces; Balochistan, Punjab, Sindh, Khyber Pakhtunkhwa. The immigration of populations from countries of high endemic of the infection seems to contribute significantly to the rapid modification of epidemiological data, especially in areas bearing the high burden of immigrants. The present study emphasizes on the gender wise and region wise prevalence of HCV in Pakistan. Samples included in this study were brought to Genome Center for Molecular Based Diagnostic and Research, Lahore from various collections centers of Khyber Pakhtunkhwa, Sindh, Punjab and Islamabad Capital Territory. The rate of HCV infection is significantly high in Khyber Pakhtunkhwa 201 (53.45%), Punjab 98 (26.6%) and the lowest rate is observed in Sindh 32 (8.5%).

This study shows that the prevalence of Hepatitis C

virus is comparatively same both in males and females with 186 (49.5%) and 190 (50.5%) respectively. When age wise distribution is analyzed it was observed that patients between the age group of 31-40 years were found to be in higher number 135 (35.9%) as compared to other age groups and patients above 60 years were found to be least effected 6 (1.6%). It was found that the most prevalent genotype of HCV was 3a 241 (64.1%), where the lowest number of cases was reported of the genotype 1b 2 (0.53%). Out of 376 positive samples 100 (26.6%) were untypable. The high number of untypable genotypes is due to mutations and emergence of new strains and during therapy due to drug pressure it emerged. During replication cycle of HCV it mutates very rapidly and permits for the emergence of drug resistance mutations that is very common. In the population where the viruses are already present the rapid emergence of new resistance strains occurs. For the genotyping of newly emerged strains the clinical laboratories may require to update their genotyping assays.
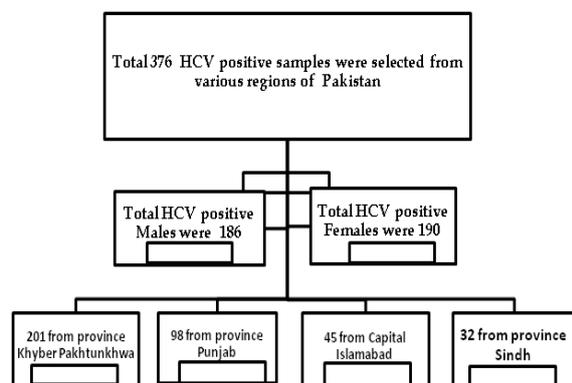
Fig. 1. shows demographic characteristics of the total enrolled patients. Sum of 376 HCV RNA positive patients were subjected for HCV genotyping. Of these 376 patients 186 were male and 190were female. All the patients belong to various regions of Pakistan.

Conclusions

From the present study it was concluded that genotype 3a is the most prevalent HCV genotype in Khyber Pakhtunkhwa, Punjab, Sindh and Islamabad. In the treated patients a high prevalence rate of untypable genotypes is present that need further

investigation for elaborating the complete profile of the disease. For successful genotyping of the untypable genotypes, developing new assays or using viral sequencing method could be followed. The HCV infect the most productive and economically age group 31-40 years. Awareness regarding the disease, case detection, treatment and proper management should need to control the rate of HCV infection in the country.

Competing interest

The author declares that they have no competing interest.

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References

Ahmad M, Bukhari A, Ghanni MHU, Khan A, Malik JI, Shah AH. 2003. Prevalence of hepatitis C virus and its serotypes in Bahawalpur Division. Biomedica **19,** 18-22.

Ahmad N, Asgher M, Shafique M, Qureshi JA. 2007. An evidence of high prevalence of Hepatitis C virus in Faisalabad, Pakistan. Saudi Medical Journal **28,** 390-395.

Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE. 1992 . The natural history of community-acquired hepatitis C in the United States. The new England journal of Medicine **327(27),** 1899-905.
http://dx.doi.org/10.1056/NEJM199212313272702

Alter MJ. 2007. Epidemiology of hepatitis C virus infection. World Journal of Gastroenterology **13,** 2436-2441.

Bartenschlager R, Frese M, Pietschmann T. 2004. Novel insights into hepatitis C virus replication

and persistence. Advances in Virus Research **63,** 71-180.

Chamberlain RW, Adams N, Saeed AA, Simmonds P, Elliot RM. 1997. Complete nucleotide sequence of a type 4 hepatitis C virus variant, the predominantgenotype in the Middle East. Journal of General Virology **78,** 1341-1347.

Giannini C, Brechot C. 2003. Hepatitis C virus biology Cell Death and Differentiation. Cell death and differentiation **10(1),** 27-38.
http://dx.doi.org/10.1038/sj.cdd.4401121

Idrees M, Riazuddin S. 2008. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. *BMC Infectious Diseases* 8-69.
http://dx.doi.org/10.1186/1471-2334-8-69

Khan S, Rai MA, Khan A, Farooq A, Kazmi SU, Ali SH. 2008. Prevalence of HCV and HIV infections in 2005-Earthquak areas of Pakistan. *BMC Infectious Diseases* 8-147.
http://dx.doi.org/10.1186/1471-2334-8-147

Narendra M, Dixit Jennifer E, Layden-Almer, Layden TJ, Perelson AS. 2004. Modelling how ribavirin improves interferon response rates in hepatitis C virus infection. Nature; **432,** 922-924.
http://dx.doi.org/10.1038/nature03153

Ohno T, Mizokami M, Wu R, Saleh MG, Ohba K, Orito E, Mukaide M, Williams R, Lau JYN. 1997. New hepatitis C virus (hcv) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. Journal of Clinical Microbiology **35,** 201-207.

Omran HMoataza, Youssef SSamar, El-Garf TWael, Tabll AAshraf, Bader- Eldin GNoha, Atef K, Nabil W, El-Awady KMostafa. 2009. Phylogenetic and Genotyping of Hepatitis C Virus in Egypt. Australian Journal of Basic and Applied Sciences **3(1),** 1-8.

Raza SA, Clifford GM, Franceschi B. 2007. Worldwide variation in the relative importance of hepatitis B and hepatitis C viruses in hepatocellular carcinoma: a systematic review. British Journal of Cancer **96(7),** 1127 –1134.
http://dx.doi.org/10.1038/sj.bjc.6603649

Rice CM . 1996. Flaviviridae: the viruses and their replication, In Fields BN, Knipe DM, Howley PM (ed.), Fields virology. Lippincott-Raven Publishers, Philadelphia, Pa. 931-960 p.

Shepard CW, Finelli L, Alter MJ (2005). Global epidemiology of hepatitis C virus infection. The Lancet Infectious Disease **5,** 558 – 567.
http://dx.doi.org/10.1016/S1473-3099(05)70216-4

Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J, Urdea MS. 1993. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. Journal of General Virology **74,** 2391-9.
http://dx.doi.org/10.1099/0022-1317-74-11-2391

Takada NS, Takase S, Takada A, Date T. 1993. Differences in the hepatitis C virus genotypes in different countries. Journal of Hepatology **17,** 277-283.

Turhan V, Ardic N, Eyigun CP. 2005. Investigation of genotype distribution of hepatitis C virus among Turkish population in Turkey and various European countries. Chinese Medical Journal **118,** 1392-1394.

Waheed Y, Shafi T, Safi SZ, Qadri I. 2009. Hepatitis C virus in Pakistan: A systematic review of prevalence, genotypes and risk factors. World Journal of Gasteroenterology **15,** 5647-5653.
http://dx.doi.org/10.3748/wjg.15.5647

Zein NN, Rakela J, Krawitt EL, Reddy KR, Tominaga T, Persing DH. 1996. **Hepatitis C virus genotypes in the United States**. epidemiology, pathogenicity, and response to interferon therapy. Annals of Internal Medicine **125(8),** 634-639.
http://dx.doi.org/10.7326/0003-4819-125-8-199610150-00002