INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 17, No. 3, p. 273-280, 2020

RESEARCH PAPER OPEN ACCESS

Epidemiological patterns of HCV genotypes in KPK region of Pakistan

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Key words: Hepatitis, Genotype, HCC, HMC

http://dx.doi.org/10.12692/ijb/17.3.273-280 Article published on September 30, 2020

Abstract

Hepatitis C virus is a significant mediator for development of hepatitis and related cirrhosis and hepatocellular carcinoma. HCV has several genotypes and subtypes. Epidemiology of HCV genotypes is crucial to understand as they play a key role in defining the outcomes of HCV infection. This study was conducted to analyze the epidemiology of HCV genotypes and HCV viral loads in Charsada, Peshawar, Kohat and Frontier Region Peshawar of KPK Pakistan. A total of 1305 HCV infected patients visiting HMC Peshawar were included in this study. Blood samples were collected and HCV RNA quantification and genotyping were carried out through RT-PCR. Viral loads and hameto-biochemical markers were also analyzed for the studied patients. Chi square analysis and odds ratios were used to compare the differences in HCV genotypes prevalence. In total of 1305 samples, 55.7% were males and 44.2% were females. The most prevalent genotype was genotype 3a (44.2%) was highest in both genders followed by genotype 2a (25.4%), 1a (6%) and Untypable genotypes (6%) ($p>0.05$). Demographic analysis showed that HCV genotype 3a was the dominant genotype in all four regions with the prevalence of 48.2% in FR region, 44% in Peshawar, 44.2% in Charsada and 41.4% in Kohat region of KPK. Medium viral load of 6×10^5-8×10^5 IU/ml was the most prevalent load in 41% of the patients. Highly significant association ($p<0.0001$) was found among the levels of clinical parameter prevalence in studied patients. This study concludes that HCV genotype 3a is the most prevalent genotype in Peshawar, Kohat, Charsada and FR Peshawar regions of KPK. HCV genotype 2a is an emerging genotype in the KPK population.

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Introduction

Hepatitis C virus (HCV) belongs to the family of *Flaviviridae* and is one of the major public health problem throughout the globe. Around 170 million people are already infected by HCV with East and Central Asia, North Africa and Middle East having the highest prevalence rate of 3.5%, along with the Europe having prevalence rate of 1.5-3.5% (Lavanchy, 2009: Mohd *et al.,* 2013). Chronic HCV infection is related with morbidity and mortality in the infected patients and progression towards liver related complications like cirrhosis and hepatocellular carcinoma (HCC) (Koike, 2014).

Progression towards cirrhosis and HCC is dependent on many factors like, Male gender, old age, host genetic factors, viral factors, co-infections with HIV/HBV and Alcohol use (Doyle *et al.,* 2012). Viral kinetics studies have shown that viral loads in chronically infected patients range from 10^3-10^7 genome/ml. Viral mathematical modelling indicates that about 10^{12} virions are produced in the chronically infected persons each day (Neumann *et al.,* 1998).

Therapy for HCV infection has evolved since its discovery. Until the development of Direct Antiviral Agents (DAA) regimens the standard care treatment for HCV infection was comprised of pegylated interferon-α (IFN-α), plus oral intake of ribavirin (RBV) for 24 to 48 weeks in developing countries like Pakistan (Arshad *et al.*, 2019). This therapy is promising in the treatment of patients infected with genotype 2 or 3 leading to the sustained virologic response (SVR). SVR is the absence of HCV RNA in patient blood after completion of 6 month of antiviral therapy and is attained in 80-90% of treated patients.

Patients infected with genotype 1 and 4 have the SVR rates of 50%, however HCV antiviral therapy is related with some serious side effects and can lead to the cessation of the therapy (Fried *et al.,* 2002: Manns *et al.,* 2001). In order to reduce the side effects and better response rates anti-HCV therapy is aided by first generation of new DAAs. Anti HCV therapy with response rates upto 90% was observed in Phase II and III trials using second generation DAA having minimal side effects and shortened course of therapy (Jacobson *et al.*, 2011: Lawitz *et al.,* 2013). HCV phylogenetic and sequencing analysis showed that HCV can be classified into 7 genotypes (Cooke *et al.*, 2013). HCV genotypes differ by 30- 35% of their genome from each other, while these genotypes are further classified into 67 confirmed and 20 provisional subtypes. HCV Strains that belong to the same subtype differ at <15% of nucleotide sites (Smith *et al.,* 2014).

Geographical analysis of the prevalent HCV genotypes showed that genotypes 1, 2, and 3 are distributed worldwide. In USA, china and Europe the most prevalent genotype is 1a and 1b. HCV subtypes such as 2a and 2b are prevalent in North America, Japan, Europe, while genotype 2c is most prevalent in Italy (Webster *et al.*, 2015: Umer and Iqbal, 2016).

In North Africa and Middle East HCV genotype 4 is most prevalent genotype along with genotypes 5,6 prevalent in South Africa and Hong Kong. HCV genotype 3a is prevalent in Pakistan, India and Europe (Khan *et al.,* 2014: Umer and Iqbal, 2016: Thrift *et al.,* 2017). In Pakistan there is a lot of data available on the prevalence of HCV and its various genotypes across the country (Attaullah *et al.*, 2011: Khan *et al.,* 2014), however there is a limited or no data available regarding the prevalence of all HCV genotypes in Charsada, Kohat, Peshawar and Frontier Region Peshawar. The study area is located in the northwest region of KPK province. The latitudes and longitudes of the study area are Peshawar and FR region 34.0150˚N, 71.5805˚E, Charsada 34.1494˚N, 71.7428˚E, and Kohat 33.5834˚, 71.4332˚E respectively. This study focuses on the prevalence of HCV genotypes along with patient HCV viral loads and biochemical parameters in Charsada, Kohat, Peshawar and Frontier Region Peshawar regions of Khyber Pakhtunkhwa province Pakistan (Fig. 1). Chi square distributions and odds ratios along with confidence intervals were used to assess the association of prevalent genotype and patients. To the best of our knowledge this is the first study, which focuses on the detailed analysis of all HCV genotypes in the above mentioned regions of KPK, Pakistan.

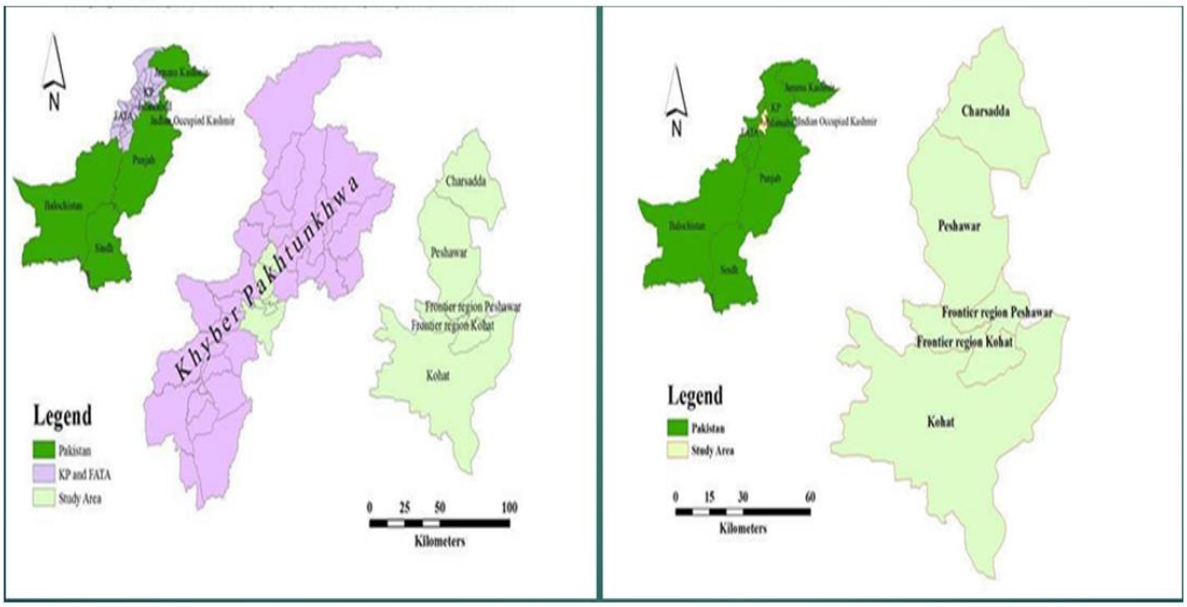

Fig. 1. Location map of the study area.
Map representing the location of the studied population in the KPK province of Pakistan.

Materials and methods

Sample Collection

Blood samples were collected from HCV positive patients visiting the outdoor patient department (OPD) Hayat Abad Medical Complex (HMC) Peshawar, which is a tertiary care hospital in the city of Peshawar Khyber Pakhtunkhwa (KPK). A total of 1305 HCV positive samples were collected from the patients of four different Districts of KPK including Peshawar, Charsada, Frontier Region and Kohat.

Patient consent was obtained from each patient by signing a consent form, while the study was ethically approved by the ethical committee of the HMC. Patients below the age of 10 years or HIV co-infected were excluded from the study. Serum was separated from the collected blood samples by centrifugation at 5000 rpm for 5 min. Serum was stored at -20˚C.

Biochemical and Serological Analysis

Hameto-Biochemical analysis like hemoglobin, platelets, TLC, total bilirubin, ALT, ALP, Total proteins, albumin and globulin were performed by Sysmex Hematology Analyzer KX-21 (Japan). Serological analysis like anti-HCV antibody was confirmed using second generation ELISA kit MBS (Italy) according to manufacturer protocols.

Quantitative and Genotypic Analysis

HCV quantitative and genotype analysis were performed for all patients following the methods and procedures by Idrees, 2008. (Idrees and Riazudin, 2008).

Statistical Analysis

Frequencies for the genotypes and patients were compared. Chi square test was implemented for the comparison of genotypes and corresponding category. The level of significance was $p<0.05$. The magnitude of effect was determined by calculating odds ratios and 95% confidence intervals. Demographic data were independent variables. Statistical analysis for the concerned data was carried out using Graph pad prism V. 5.00.

Results

Overall and Gender based distribution of HCV genotypes in studied patients

Of the total 1305 samples collected, 728 (55.7%) were male, whereas 577(44.3%) were female. A total of 11 HCV genotypes and subtypes were observed in studied patients. Prevalence of HCV genotypes in studied patients showed that the most prevalent genotype was 3a with rates of 44.2% (n=577), followed by genotype 2a, 25.4% (n=332) respectively (Table 1).

Similarly gender based distribution of HCV genotypes showed that HCV genotype 3a was the most prevalent genotype in both male and female patients, 43.4 (n=316) vs 45.4% (n=261) (OR: 1.36; 95% CI: 0.83-2.23) of the patients. HCV genotype 2a was the second most prevalent genotype in male 26% (n=190), and female patients, 24.8% (n=142) (OR: 1.23; 95% CI: 0.74-2.06). Chi square analysis revealed no significant association among gender and HCV genotype prevalence (p>0.05).

Data for genotype 1a, 1b, 1c, 2b, 3b, 4, 5a, 6a and untypable genotypes is shown in Table 1.

Table 1. Gender based genotypic distribution in studied patients.

HCV genotype	Male n (%)	Female n (%)	Total n (%)	OR	95% CI	*p* value
1a	48(6.7)	29(5)	77(6)	Ref	-	0.499
1b	25(3.6)	18(3.1)	43(3.3)	1.19	0.55-2.55	
1c	11(1.5)	4(0.6)	15(1.2)	0.60	0.17-2.06	
2a	190(26)	142(24.8)	332(25.4)	1.23	0.74-2.06	
2b	28(3.8)	31(5.3)	59(4.5)	1.83	0.92-3.60	
3a	316(43.4)	261(45.4)	577(44.2)	1.36	0.83-2.23	
3b	29(3.9)	29(5)	58(4.4)	1.65	0.82-3.30	
4	10(1.4)	14(2.4)	24(1.8)	2.31	0.91-5.89	
5a	12(1.6)	11(2)	23(1.7)	1.51	0.59-3.88	
6a	12(1.6)	8(1.3)	20(1.5)	1.10	0.40-3.01	
Untypable	47(6.4)	30(5.1)	77(6)	1.05	0.55-2.02	
Total	728(55.7)	577(44.2)	1305(100)			

p<0.05 was considered significant

District based prevalence of HCV genotypes in analyzed patients

Demographic distribution of HCV genotypes revealed that district Kohat and Peshawar were the most infected districts with rates of 28.1% (n=367) and 28% (n=365) respectively. District Charsada and Frontier Region of Peshawar (FR Peshawar) district were found to be 24% (n=312) and 20% (n=261) for HCV. HCV genotype 3a was the most prevalent genotype in all the studied locations with the highest prevalence in FR Peshawar 48.2% (n=126), followed by Charsada and district Peshawar with rates of 44.2% (n=138) and 44% (n=161) respectively.

Chi square distribution showed that there is no significant association (p>0.05) among the prevalent genotype and demography of the studied patients. Data for the rest of districts is shown in Table 2.

Table 2. Demographic distribution of different genotypes in studied patients.

HCV genotypes	Demography					
	Charsada n (%)	Peshawar n (%)	FR Peshawar n (%)	Kohat n (%)	Total n (%)	*p* value
1a	23(7.4)	16(4.4)	17(6.6)	21(5.7)	77(6)	0.939
1b	13(4.3)	14(3.8)	7(2.7)	9(2.4)	43(3.3)	
1c	4(1)	5(1.4)	2(0.8)	5(1.3)	15(1.2)	
2a	78(25)	99(27)	59(22.7)	96(26.2)	332(25.4)	
2b	11(3.5)	21(5.7)	9(3.4)	18(5)	59(4.5)	
3a	138(44.2)	161(44)	126(48.2)	152(41.4)	577(44.1)	
3b	13(4.5)	15(4)	9(3.4)	21(5.7)	58(4.4)	
4	4(1.4)	7(2)	6(2.2)	7(2)	24(1.8)	
5a	5(1.6)	7(2)	4(1.5)	7(2)	23(1.7)	
6a	4(1.2)	4(1)	7(2.6)	5(1.3)	21(1.5)	
Untypable	19(6)	17(4.6)	15(5.7)	26(7)	77(6)	
Total	312(24)	365(28)	261(20)	367(28.)	1305(100)	

Age based categorization of HCV genotypes in infected patients

The most infected age group in these patients was age group 31-40 with prevalence rate of 31.6% (n=410), followed by age group 21-30 and 41-50 , having rates of 26.5% (n=347) and 25% (n=326) respectively. Age group 10-20 had prevalence rate of 8.7% (n=114), whereas age groups 51-60 and above 60 had rates of 7% (n=92) and 1.2% (n=16). The most prevalent genotype in this study was genotype 3a, 44.2% (n=577), likewise this genotype was most prevalent in age group 21-30 with rates of 52.7% (n=183), followed by age group 10-20, 44.7% (n=51), and age group 31-40, 43.1% (n=133). Data for age groups is shown in Table 3.

Table 3. Age based distribution of genotypes in patients.

Genotypes	Age group						
	10-20 n (%)	21-30 n (%)	31-40 n (%)	41-50 n (%)	51-60 n (%)	Above 60 n (%)	Total n (%)
1a	5(4.3)	19(5.4)	23(5.6)	26(7.9)	3(3.2)	1(6.2)	77(6)
1b	4(3.5)	13(3.7)	17(4.1)	9(2.7)	0(0)	0(0)	43(3.3)
1c	2(1.7)	4(1.1)	6(1.4)	3(1)	0(0)	0(0)	15(1.2)
2a	27(23.8)	79(22.7)	103(25.3)	87(26.6)	31(33.6)	5(31.4)	332(25.4)
2b	7(6.3)	9(2.7)	21(5.1)	17(5.2)	3(3.2)	2(12.6)	59(4.5)
3a	51(44.7)	183(52.7)	177(43.1)	133(40.7)	30(32.6)	3(18.7)	577(44.2)
3b	7(6.3)	18(5.1)	21(5.1)	7(2.1)	5(5.6)	0(0)	58(4.4)
4	5(4.3)	3(0.86)	4(1)	9(2.7)	2(2.4)	1(6.2)	24(1.8)
5a	3(2.6)	4(1.3)	7(1.7)	5(1.5)	3(3.2)	1(6.2)	23(1.7)
6a	1(0.87)	6(1.7)	8(2)	3(1)	2(2.1)	0(0)	20(1.5)
Untypable	2(1.7)	9(2.7)	23(5.6)	27(8.6)	13(14.1)	3(18.7)	77(6)
Total	114(8.7)	347(26.5)	410(31.6)	326(25)	92(7)	16(1.2)	1305(100)

Categorization of HCV viral load in genotypes, gender and patient demography

Viral load quantification was carried out through RT-PCR. Patients viral load was divided into three categories, lower levels having viral load of $<6\times10^5$ IU/ml, intermediate, 6×10^5- 8×10^5 IU/ml and high levels $>8\times10^5$ IU/ml. Viral load data analysis showed that intermediate levels were most prevalent with rates of 41% (n=535), followed by low levels with prevalence rates of 31% (n=404). Patients having high viral load above 8×10^5 IU/ml were 28% (n=367). Data shown in Fig. 02. Viral load was analyzed in prevalent genotypes along with gender and demography of the studied patients. As the most prevalent genotype in this study is 3a, so genotype 3a was compared with all other genotypes for HCV viral load. It was noted that high viral load levels was the most prevalent in other genotypes, 52.4% (n=192) followed by intermediate levels, 51.3% (n=274) and 51% (n=206) for lower levels. Similarly in genotype 3a infected patients, intermediate levels were prevalent in 48.7% (n=261) of the patients followed by 49% (n=198) and 47.6% (n=175) for low levels and high viral load levels respectively No significant association was found among the viral load and corresponding category analyzed. Data shown in Table 4.

Table 4. HCV viral load in patients from District Charsada, Peshawar, FR Peshawar and Kohat.

Genotype/Subtype	Viral load in IU/ml			*p* value
	$<6\times10^5$ n (%)	$<6\times10^5$- 8×10^5n (%)	$>8\times10^5$ n (%)	
Genotype 3	198 (49)	261 (48.7)	175 (47.6)	0.924
Other genotypes	206 (51)	274 (51.3)	191 (52.4)	
Male	301 (59)	204 (46)	223 (63)	0.476
Female	209 (41)	238 (54)	131 (37)	
Charsada				
Genotype 3	62 (54)	38 (38.7)	51 (51.5)	0.066
Other genotypes	53 (46)	60 (61.3)	48 (48.5)	
Peshawar				
Genotype 3	47 (45)	75 (54.7)	54 (43.2)	0.136
Other genotypes	57 (55)	62 (45.3)	71 (56.8)	
FR Peshawar				
Genotype 3	51 (60.7)	38 (47.5)	46 (47.4)	0.134
Other genotypes	33 (39.3)	42 (52.5)	51 (52.6)	
Kohat				
Genotype 3	61 (55)	58 (43.2)	54 (44.2)	0.140
Other genotypes	50 (45)	76 (56.8)	68 (57.8)	

Viral load levels below <12 IU/ml were considered negative

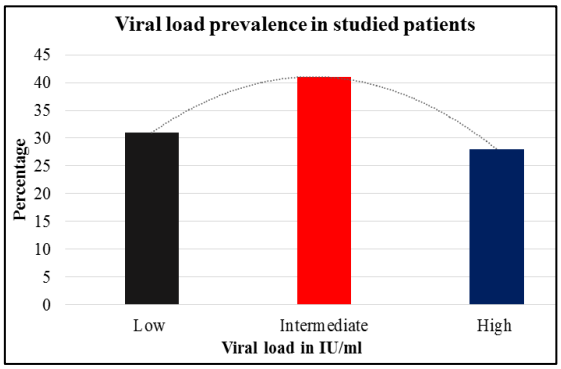

Fig. 2. Viral load levels in HCV patients.

Biochemical analysis of infected HCV patients

Table 5 shows the clinical profile of the infected HCV patients. Different biochemical parameters such as ALT, ALP, hemoglobin, platelets count were observed for the studied patients.

Levels of these biochemical parameters were elevated in most of the infected patients. Analysis revealed that TLC levels along with total bilirubin, ALT and ALP levels were elevated in high number of patients.

Chi square distribution revealed that the clinical parameters were highly significantly distributed (p<0.0001) in studies patients (Table 5).

Table 5. Levels of clinical parameters in studied patients.

Clinical Parameters	Normal n (%)	Raised n (%)	*p* value
Hemoglobin Level (g/dl)	731 (56)	574 (44)	<0.0001*
TLC Level	457 (35)	848 (65)	
Platelet Count	679 (52)	626 (48)	
Total Bilirubin (mg/dl)	418 (32)	887 (68)	
ALT/ ALP	299 (23)	1005 (77)	
Total Protein (g/dl)	587 (45)	718 (55)	
Albumin (g/dl)	600 (46)	705 (54)	
Globulin (g/dl)	535 (41)	770 (59)	
Albumin Globulin Ratio	626 (48)	679 (52)	

Discussion

The present study focuses on the genotypic prevalence of HCV in the different districts including Peshawar, Charsada, Kohat and Frontier Region of Khyber-Pakhtunkhwa (KPK) Pakistan. Performing investigations regarding HCV genotype prevalence in these regions is crucial for implementation of preventive strategies and treatment specifically in these regions of KPK as these regions are affected by terrorism and war. In this study the prevalence of HCV in males and females were 728 (55.78%) and 577(44.21%) respectively. The higher percentage of male subjects shows availability of male subjects for testing in these specified regions. Our results are coinciding with Inamullah *et al.* and Ali *et al.* (Inamullah *et al.,* 2011: Ali *et al.,* 2011).

The most frequent genotype prevalent in both the genders was genotype 3a (44.2%). Our study confirmed that HCV genotype 3a is the most prevalent genotype in Pakistan as previously reported studies conducted in Pakistani population (Khan *et al.,* 2014: Idrees and Riazudin, 2008: Ali *et al*., 2016). The other genotypes which were detected in the studied population were genotype 2a (25.4%), genotype 1a (6%), genotype 4 (1.8%), genotype 5a (1.7%), genotype 6a (1.5%) and Untypable genotype (6%). This data shows prevalence of other genotypes as well in KPK population, which could be the worst scenario regarding the treatment of HCV infection because not all the HCV genotypes respond to the available interferon plus ribavirin therapy which is the standard of care for HCV infections (Arshad *et al*., 2019). The demographic data analysis shows variable prevalence of HCV genotypes in the studied districts and FR. A number of studies have already reported HCV genotype 3a as the major prevalent genotype in the districts of KPK (Ali *et al.,* 2011: Afridi *et al.,* 2013). There are very few studies which are carried out to determine the prevalence of HCV genotypes specifically in the terrorism affected districts such as Waziristan, Bannu, FR Peshawar and FR Kohat. Our study is the first of its kind which determines the HCV genotypic prevalence in the Kohat and FR Peshawar regions of KPK Pakistan. The second most prevalent genotype in the studied population is HCV genotype 2a and 1a which is the second most prevalent genotype in south Asian population (Wasitthankasem *et al*., 2015). This genetic heterogeneity of HCV in the studied population reveals lack of proper measures and guidelines to control HCV infection. Prevalence of HCV genotypes in age group was also determined and it was found that HCV genotype 3a was prevalent in all age groups followed by genotype 2a. The government and health sector should take solid steps in bringing awareness among the people of all ages about the HCV infection. A number of studies have reported varying age groups for HCV infected patients, which are in coherence with our study (Arshad *et al*., 2019: Ali *et al*., 2016: Ali *et al*., 2011: Afridi *et al*., 2013).

Viral load determination is one of the critical factor for deciding HCV therapy because patients having high viral loads are difficult to treat as compared to low viral loads, secondly patients having high viral loads have greater chances for the carrying HCV quasispecies. HCV quasispecies can result in lack of response to antiviral regimens, i.e. peg-interferon plus ribavirin. Now patients having high viral loads are more prone to development of chronic HCV infection and subsequently cirrhosis and HCC if they are not properly treated with the standard of care for HCV infection. Recent studies conducted by Dr. Rice and his colleagues reported 98-100% cure rates for HCV which could be a phenomenal invention in this century for the treatment of HCV infections (Vilarinho *et al.,* 2016). Biochemical analysis for the studied group of patients was also carried out.

In the studied patients the biochemical parameters were mostly elevated for the different parameters like ALT platelets, hemoglobin and ALP which is one of the predictor factor for the serological analysis of different hepatitis infections. Clinical parameters can elevate during HCV infection and different studies have reported elevated levels of the above mentioned parameters in studied patients, which coincides with our study (Pradat *et al.,* 2002).

Conclusion

This study reported that HCV genotype 3a is the most prevalent genotype in Peshawar, Kohat, Charsada and FR Peshawar region of KPK Pakistan. HCV genotypes 2a, 1a and untypable genotypes were also the emerging genotypes in the KPK population. Gender based differences showed that genotype 3a was more common in females. Viral loads differences showed that low and medium viral loads were more prevalent in studied patients.

Recommendations

Studies regarding the HCV genotype patterns in other districts of KPK should be carried out. Awareness about the HCV infection and prevalent genotypes in these districts is indispensable for the population. Education regarding the risk factors and transmission of HCV should also be provided to the patients in order to combat HCV infection.

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