ORIGINAL ARTICLE

PREVALENCE OF ACTIVE HEPATITIS C VIREMIA AND HCV GENOTYPES IN DIFFERENT REGIONS OF SINDH, PAKISTAN

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Background: Hepatitis C virus (HCV) infection is highly endemic in Pakistan with about 6% active HCV infection frequency. Sindh, the second largest populated province of Pakistan, is recognized with alarmingly high HCV seroprevalence in community. The present study estimates the frequency of active HCV infection with its genotypes and analyzes the associated determinants among the population in Sindh. **Methodology:** This was a cross-sectional study carried out in Asian Institute of Medical Sciences (AIMS) Hyderabad and University of Sindh, Jamshoro, Pakistan from Sep 2017 to Sep 2018. A total of 5,253 samples were confirmed anti-HCV antibody positive using third generation Elecsys Anti-HCV II assay. The samples were further processed for the Nucleic acid Amplification Test (NAT) and genotyping using Cobas® 4800 System. **Results:** HCV-RNA positivity was confirmed in 2,096 (39.9%) cases with statistically significant impact of gender on HCV-RNA positivity [OR=1.21, CI=1.08–1.35, $p<0.05$]. Pearson's correlation co-efficient between age and active HCV viremia suggested a weak negative correlation in general (r= -0.290) and male (r= -0.459) category but not female (r=0.255). Genotype 3 was found the most common (81.6%) followed by genotype 1b (3.7%) and genotype 2 (0.9%). Bivariate correlation analysis suggested a non-significant, but weak positive correlation between age and genotypes (r=0.261). **Conclusion:** Prevalence of HCV infection is about 40% with the predominant genotype 3 among HCV patients in Sindh Province, Pakistan.

Keywords: HCV genotype, Nucleic acid Amplification Test, active hepatitis C, HCV-RNA, Sindh

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INTRODUCTION

Hepatitis C infection is a major public health issue in Pakistan. Hepatitis C virus (HCV) is a blood-borne virus associated with severe health complications of Hepatocellular carcinoma (HCC) and liver cirrhosis in patients who develop chronic hepatitis.[1] The structure of HCV includes an outer envelope, multiple structural and non-structural proteins and a positive sense ssRNA genome of about 9.6 kb in size. Genomic heterogeneity is one of the predominant aspects in the life cycle of HCV strains and on the basis of that they are classified into 7 different genotypes if the nucleotide sequence variation is about 30–35% and several subtypes if the sequence difference is >15%.[2] Prevalence of HCV infection and its predominant genotype has distinct geographical variations influencing about 3% of the population (~180−200 million) around the globe.[3] Overall genotypes 1, 2, and 3 have the worldwide distribution, while genotype 4 is most frequently detected in Africa and the Middle East[4]; genotype 5 in South Africa; 6 in Southeast Asia and Hong Kong[5,6], and genotype 7 originates from Central Africa[7]. In Pakistan HCV viremia is known to infect more than 10 million people with the predominant genotype 3a followed by 2a, 1a, 1b, 3b, and 4.[8,9] The HCV seroprevalence has been extensively investigated across the different regions of Pakistan including Sindh.[10,11] Variation in HCV seroprevalence from 3 to 7% is observed in different regions of Pakistan.[12] For instance HCV infection has been observed alarmingly greater in frequency in rural *vs* urban population in Punjab and Sindh, the two larger provinces of Pakistan.[10,13] Literature has shown that 50–80% of patients with liver associated disorders are found to be anti-HCV antibody positive in Pakistan.[14,15]

Sindh is the second largest populated province of Pakistan and nearly half of its population constitutes the urban portion residing in Karachi, Hyderabad, Nawabshah, Sukkur, Larkana, Mirpurkhas, Ghotki and Umerkot districts. Though the seroprevalence of HCV infection is investigated for Karachi and few other cities in Sindh but frequency of active HCV infection is yet to be explored. In Hyderabad where population exceeds 5 million, HCV seroprevalence in pregnant women is 4.7%[16] and in healthy blood donors it is 12.5%[17]. The epidemiology of HCV infection in other different regions of Sindh is also largely investigated on the basis of anti-HCV prevalence[10,18,19] but the actual burden of HCV infection using Nucleic acid Amplification Test (NAT) in the regional population is unknown. Overall in Pakistan, very few studies have focused to estimate the active HCV infection in anti-HCV positive individuals.[20–22] Recent studies have shown ~ 6% active HCV frequency among healthy adult population of Pakistan.[9]

Hyderabad is a big city of Sindh, and people from adjoining or distant places frequently visit the city hospitals and the diagnostic centres for their health related issues. The data about the prevalence of active HCV infection and genotype distribution in Hyderabad and other districts of Sindh is scarce[9,22,23] and requires to

document the statistical analysis of HCV affected population. In the current study we investigated more than 5,000 blood samples from the symptomatic hepatitis patients visiting the diagnostic centre in Hyderabad. All anti-HCV antibody positive samples were analyzed for active HCV infection using RT-PCR assay. Moreover, 212 samples were investigated for the prevalent HCV genotypes and the data obtained was analyzed to see its specific association to a particular region, gender and age of patients. The present study attempted to find out the prevalence of active HCV infection and the most common genotypes in hepatitis patients of Hyderabad and other districts of Sindh.

METHODOLOGY

The study was approved by advanced studies and research board of University of Sindh, Jamshoro. Patient's age and gender information was registered at the time of their blood collection and processing of specimen. The information about their location was collected after verbal consent from patients if genotyping was performed.

Laboratory data of 5,253 HCV patients was obtained from Asian Institute of Medical Sciences (AIMS), Hyderabad. All included cases were confirmed anti-HCV-antibody positive based on the third generation Elecsys Anti-HCV II assay (Cobas®), while the cases that were positive for Hepatitis B virus were excluded from this study. All seropositive cases were subjected to NAT to detect HCV-RNA using automated real-time PCR during the period from Sep 2017 to Sep 2018. PCR assay amplify three genomic regions in virus including non-structural protein 5B [NS5B], core, and the 5′ untranslated region [UTR] followed by identification with specific probes. A total of 212 HCV-RNA positive cases were further scrutinized for HCV genotype detection by Cobas® HCV test kit as per the standard protocol using Cobas® 4800 system. The automated system can perform the qualitative detection of HCV genotypes 1-6 and genotype 1 subtypes a and b. Samples were included from both genders of varying age groups. HCV genotypes were investigated in a total of 212 samples categorically processed from 19 different regions of Sindh including Hyderabad, Nawabshah, Ghotki, Mirpurkhas, Dadu, Badin, Umerkot, Matiari, Tando Allahyar Matli, Tando M. Khan, Meerpur Mathelo, Sukkur, Sanghar, Naushahro Feroze, Jacobabad, Jamshoro, Khairpur Mirus and Thatta.

The results for positive and negative cases are expressed in both absolute and relative values (percentages). The statistical analysis of variables (i.e., age, gender, genotype and location) was performed using SPSS-20, Microsoft Excel 2010, online statistics calculators [Select Statistical Services UK and Graph-pad Prism] or manually where applicable. The impact of gender on active HCV infectivity and/or genotypes was assessed using Fisher's exact test. Regression analysis was performed to probe the correlation between age and active HCV infectivity and/or genotypes, and $p \leq 0.05$ was considered significant.

RESULTS

A total of 5,253 anti-HCV antibody positive samples based on Electro-Chemi-Luminescence Immunoassay 'ECLIA' were included in this study. The gender-wise composition of the samples was 2,673 (50.9%) from male and 2,580 (49.1%) from female patients. The PCR results show that 2,096 (39.9%) patients had an active HCV infection. The overall active HCV prevalence in males was found to be 42.1% (n=1110) and in female cases 37.6% (n=986). This data is shown in Table-1. The statistical values for the measurement of differences between gender and the impact of gender on HCV RNA positivity along with level of significance were calculated to be: OR=1.21 [CI (95%) = 1.08 – 1.35, *p*-value= .0009] These values indicate that active HCV infection is more common in males as compared to the females and that the gender plays a significant role in HCV infection in this region. The data was also analyzed for the determination of correlation between HCV-RNA positivity and the age of patients. A continuous series of the data based on various age groups was formed. The magnitude of age group was randomly chosen to be 10. Highest percentage of HCV-RNA positive cases was seen in the age-group of 51–60. The Pearson's correlation co-efficient was calculated to be r= -0.290, with *p*-value= .448, suggesting an insignificant however, a weakly negative correlation between age and active HCV cases (Table-1). The data were also processed to determine the correlation of HCV RNA positive cases with the age for both male and female categories. A weak negative correlation for male category and a weak positive correlation for female category was observed (Table-1).

A region-wise analysis of the data demonstrated that ~73% cases (n=155 out of 212 samples) were from five different regions, namely; Ghotki, Hyderabad, Nawabshah, Matiari and Tando Allahyar districts of Sindh. The relative frequencies, in terms of percentage, of different genotypes for these regions is displayed in Table-2. The overall frequency of HCV genotypes detected in various samples was 86.3% (n=183). In general, the genotype 3 was found the most prevalent genotype in Ghotki, Hyderabad, and Nawabshah, Matiari, Tando Allahyar, and other regions with the overall frequency of 81.6% (n=173). The genotype 2 was detected only in Tando Allahyar and Matiari regions with the overall prevalence of 0.9 % (n=2). The genotype 1b was most prevalent in Nawabshah (4.8%) with an overall percentage of 3.7. The frequency of genotype 1b was observed 5.2% in other regions.

The HCV genotype analysis was performed in 212 non-duplicate HCV RNA positive samples including 116 samples (54.7%) from male and 96 samples (45.3%) from female patients. The overall percentage of HCV genotypes for male and female was calculated to be 85.3% and 87.5%, respectively. The OR with 95% of CI and *p*-value using Fisher's Exact test employing 2×2 contingency table were determined to be [OR= 0.83, CI= (0.38–1.84), *p*-value= 0.692], suggesting an insignificant impact of gender on HCV RNA positivity. Among various genotypes the most commonly detected genotype was genotype 3 (81.6 %) followed by genotype 1b (3.7 %) and then genotype 2 (1%). The relative values of each genotype in both the genders were also calculated and compared. Statistically insignificant differences in the percentages of various genotypes between each gender were seen. Genotype 3 distribution among males and females was observed to be 81.3% (n=94) and 82.2% (n=79), respectively (Table-3). These analyses suggest an impartial impact of gender on all genotypes detected here.

The data was analyzed for any correlation between various genotypes of HCV and the patients' age. The mean age of the patients was 41.5±13.2 years. The genotype data were continuously categorized into various age groups, with randomly selected magnitude of 10 years of age. The Pearson's correlation co-efficient between age and HCV genotype was calculated to be r=0.275, suggesting a non-significant (p=0.509), weak positive correlation. The data for each of the detected genotypes were also processed for the assessment of their correlation with age. The calculated Pearson's correlation co-efficient are given in Table-4. The bivariate correlation analysis suggested a non-significant, however a weak positive correlation between age and genotype 3 (r=0.261), genotype 2 (r=0.436) and genotype 1b (r=0.198).

Table-1: The absolute and relative values of HCV-RNA positive cases in anti-HCV positive patients

Anti-HCV Ab. Positive		Age groups (Years) 1-10 (n=8)	11-20 (n=160)	21-30 (n=935)	31-40 (n=1458)	41-50 (n=1467)	51-60 (n=820)	61-70 (n=298)	71-80 (n=89)	81-90 (n=18)	Total (n=5253)	*r*	*p*
HCV-NA Positive	Count	3	65	358	577	559	371	128	29	6	**2096**	-0.290	0.448
	Percentage	37.5	40.6	38.2	39.5	38.1	45.2	42.9	32.5	33.3	**39.9**		
Male	Positive	3	32	181	288	313	199	73	18	3	**1110**	-0.459	0.213
	Total	3	80	422	707	781	434	150	51	6	**2634**		
	Percentage	100	40	42.8	40.7	40	45.9	48.6	35.3	50	**42.1**		
Female	Positive	0	33	177	289	246	172	55	11	3	**986**	0.255	0.507
	Total	5	80	513	751	686	386	148	38	12	**2619**		
	Percentage	0	41.2	34.5	38.5	35.8	44.5	37.1	28.9	25	**37.6**		
	OR CI (95%)	NA	0.95 0.51−1.78	1.43 1.09−1.86	1.1 0.89−1.36	1.2 0.97−1.48	1.05 0.8−1.39	1.6 1.01−2.55	1.38 0.54−3.31	3 .38−23.68	**1.21** **1.08−1.35**		
	p	NA	1.000	0.010	0.391	0.106	0.1514	0.0476	0.648	0.344	**0.0009**		

Note: The Odd Ratio (OR), Confidence-Interval (CI), along with *p*-values are calculated for both genders in total and in each age group, while correlation coefficients (*r*) along with *p*-values are also expressed for specific gender verses age of the patients. A significant impact of gender on HCV positivity is shown while in specific age groups the same is impartially affected by gender. Weak negative correlation is seen for male and while positive correlation is seen for female category.

Table-2: The absolute count and relative percentages of HCV genotypes

Genotypes	Absolute count and relative percentages	Ghotki (n=44)	Hyderabad (n=46)	Nawabshah (n=41)	Matiari (n=12)	Tando Allahyar (n=12)	Other regions (n=57)	Total (n=212)
Typable	Count	30	36	38	12	12	55	183
	Percentage	68	78.2	92.6	100	100	96.4	86.3
Untypable	Count	14	10	3	0	0	2	29
	Percentage	32	21.8	7.4	0	0	3.6	13.7
3	Count	29	34	36	11	11	52	173
	Percentage	65.9	73.9	87.8	91.6	91.6	91.2	81.6
2	Count	0	0	0	1	1	0	2
	Percentage	0	0	0	8.4	8.4	0	0.9
1b	Count	1	2	2	0	0	3	8
	Percentage	2	4.3	4.8	0	0	5.2	3.7

Note: The details of five main districts of Sindh representing the majority of the sample size are given. The other regions include Mirpurkhas, Dadu, Badin, Umerkot, Matli, Tando M. Khan, Meerpur Mathelo, Sukkur, Sanghar, Naushahro Feroze, Jacobabad, Jamshoro, Khairpur Mirus and Thatta.

Table-3: The absolute counts and relative percentages of various genotypes for male and female categories

Genotypes	Absolute count and relative percentages	Male (n=116)	Female (n=96)	Total (n=212)	OR [CI (95%)]	*p*-value (α=0.05)
Typable	Count	99	84	183		
	Percentage	85.3	87.5	86.3	0.83 [0.38−1.84]	0.692
Untypable	Count	17	12	29		
	Percentage	14.7	12.5	13.7		
3	Count	94	79	173	1.08 [0.44−2.68]	1

	Percentage	81.3	82.2	81.6		
2	Count	1	1	2	0.85 [0.05−13.75]	1
	Percentage	0.8	1.2	1		
1b	Count	4	4	8	0.84 [0.2−3.48]	1
	Percentage	3.4	4.1	3.7		

Note: The calculated OR and p-values for various genotypes suggest impartial effect of gender on the distribution of genotype. Key: OR = Odds Ratio, CI = Confidence Interval.

Table-4: Absolute and relative prevalence of HCV genotypes in different age groups

Genotype	Absolute count and relative percentages	1-10 (n=1)	11-20 (n=10)	21-30 (n=38)	31-40 (n=58)	41-50 (n=62)	51-60 (n=24)	61-70 (n=16)	71-80 (n=3)	Total (n=212)	*r*	*p*
Typable	Count	1	7	33	45	57	21	16	3	183	**0.275**	**0.509**
	Percentage	100	70	86.8	77.5	92	87.5	100	100	86.3		
Untypable	Count	0	3	5	13	5	3	0	0	29	**-0.439**	**0.275**
	Percentage	0	30	13.2	22.5	8	12.5	0	0	13.7		
3	Count	1	7	32	43	54	18	15	3	173	**0.261**	**0.532**
	Percentage	100	70	84	74	87	75	94	100	81.6		
2	Count	0	0	0	0	1	0	1	0	2	**0.436**	**0.279**
	Percentage	0	0	0	0	2	0	6	0	1		
1b	Count	0	0	1	2	2	3	0	0	8	**0.198**	**0.636**
	Percentage	0	0	3	3	3	13	0	0	3.7		

Note: The calculated Pearson's Correlation Coefficient and p-values for various genotypes suggest impartial effect of age of the patient on the distribution of genotype. Key: *r*=Pearson's Correlation Coefficient

DISCUSSION

Hepatitis C is highly endemic in Pakistan with the infectivity ratio of 1:20 individuals.[24] In developing countries, access to accurate diagnosis of HCV infection using molecular techniques is not only costly but unreachable particularly for people living in rural and remote areas. Currently the diagnosis of HCV infection relies mostly on serology and conventional evaluation of certain liver enzymes like Aspartate aminotransferase (AST). Estimation of an active hepatitis C infection is important to measure the actual burden of HCV infection within the population. Very few studies have been conducted in Pakistan to demonstrate the active hepatitis C infection prevalence. The studies that have taken place are largely concerned with the Punjab or Khyber Pakhtunkhwa provinces of Pakistan.[25,26] According to these studies, active HCV prevalence is 4.9% in general public of Lahore.[20,21] A large variation in the prevalence of HCV infection in different regions of the world is attributed to the demographics, prevalence of high risk factors such as ear and nose piercing, re-use of infected syringes and contaminated razors in barber shops and lack of access or availability to proper public health services specifically in rural areas.[10,19] In Pakistan, the Sindh province is mostly unexplored for the prevalence of active HCV infection despite a high seroprevalence of HCV in community (~28.6%) and hepatitis affected individuals (~58%).[10,14] To ascertain this a molecular surveillance is pursued in 5,253 anti-HCV seropositive samples of different areas within Sindh. HCV-RNA was detected in 40% (n=2,096) of anti-HCV antibody positive samples, which is comparatively lower than that observed in HCV seropositive patients (62.5%) in Khyber Pakhtunkhwa province.[27] A significant impact of gender was seen on HCV-RNA positive cases (p=0.0009) where male gender was observed more susceptible to HCV infection as compared to the females as observed by others.[13,21] A weak negative correlation was observed between age and HCV-RNA positive cases indicating an increased incidences of HCV infections at the middle age group which is similar to the findings reported by few others[20,27,28]

The frequency of HCV infection varies in different provinces of Pakistan and even in the different groups of same community.[15] For example, HCV seroprevalence is described 4.7% in pregnant women and 17.4% in the ocular patients in Hyderabad city in two different studies.[16,29] A variation has also been noticed in the prevalent HCV genotypes among different provinces.[28] In Pakistan HCV genotype 3 is detected as the most common genotype followed by genotype 1, 2, and 4.[9] The incidence of HCV infection with mixed genotype is 5.03%.[8] In the present study HCV genotypes were explored in 212 HCV-RNA positive samples from different regions of the Sindh. HCV genotypes were detected in 183 (86.3%) individuals while 29 (13.7%) were untypeable. More than 70% of the samples analyzed for HCV genotypes belonged collectively to Ghotki, Hyderabad, Nawabshah, Matiari, and Tando Allahyar districts of Sindh. In all these and other regions, genotype 3 is determined as the most predominant genotype with the overall prevalence of 81.6%. Previous studies conducted in Hyderabad and other rural areas of Sindh have also shown genotype 3 as the most prevalent (>70%).[13,30] In the present study, genotype 1b is observed as the second most prevalent genotype (3.7%) followed by genotype 2 (0.9%), indicating a similar pattern as reported by Riaz *et al*[30] in 2016 for Sindh. However, unlike our observation, the other studies described genotype 2 as the second most common genotype in Sindh.[9,13]

Genotype 2 is also described as the second predominant genotype in KPK, whereas in Punjab, genotype 1a is observed as the second common after genotype 3.[28] It is interesting to observe that in this study genotype 2 was not detected in Hyderabad, Nawabshah or Ghotki which represented majority of sample size (60%) indicating a differing pattern of HCV genotypes distribution of in this region. A substantial percentage of genotypes remained un-typed (13%) suggesting the prevalence of other subtypes in this region and emphasizing the development of efficient assays to recognize the type-specific genotypes.

CONCLUSION

The overall prevalence of active HCV infection is 40% in the anti-HCV positive patients with a comparatively significant influence on male gender. HCV genotypes were retrieved in 86.3% cases with the predominance of genotype 3 in different regions of Sindh. Genotype 1b was found as the second most prevalent genotype in Sindh whereas genotype 2 was found only in two districts adjacent to Hyderabad city. Middle aged people (31−50 years) are more susceptible to HCV infection.

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