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Original Article

# Prevalence of active HCV infection and genotypic distribution among the general population of district Mardan, Pakistan

## Prevalência de infecção HCV ativa a distribuição genotípica entre a população geral do distrito de Mardan, Paquistão

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**Abstract**

Hepatitis C virus (HCV) is the serious global public health burden of liver disease. Approximately 170 million people in the world are infected with (HCV). In Pakistan, where the disease has high occurrence rate. The present study envisages an up-to-date prevalence of HCV and genotypic distribution in the general population of Mardan District, Khyber Pakhtunkhwa (KP), Pakistan. The blood samples from 6,538 individuals including 3,263 males and 3,275 females were analyzed for hepatitis C surface antigen by Immuno-chromatographic test (ICT), Enzyme-linked immunosorbent assay (ELISA), and reverse transcription-polymerase chain reaction (PCR). It was found that 396 (12.13%) out of 3263 individuals contained antibodies in their blood against HCV, while among the different age groups, the highest incidences of HCV antibodies were found in the 31-40 age group (11.01%). The ICT positive samples were further screened by nested PCR to determine the existence of active HCV-RNA. It was identified that 7.11% (3263) of the total population (6538) tested was positive, among which the 461 (14.07%) females possessed antibodies in their blood against HCV. Our data showed total HCV infection in the investigated population was 5.78%. Higher percentage of HCV prevalence was detected in males than females in the age group 31-40 and 41-50. To compare the prevalence of HCV genotypes age-wise in male and female genotype 3a was found most prevalent genotype followed by 1a, 2a and 3b, respectively.

**Keywords:** HCV, ELISA, PCR, prevalence, Mardan.

**Resumo**

O vírus da hepatite C (HCV) é o grave problema de saúde pública das doenças hepáticas. Aproximadamente 170 milhões de pessoas no mundo estão infectadas com HCV; no Paquistão, a doença tem alto índice de ocorrência. O presente estudo prevê uma prevalência atualizada do HCV e distribuição genotípica na população geral do distrito de Mardan, Khyber Pakhtunkhwa (KP), Paquistão. As amostras de sangue de 6.538 indivíduos, incluindo 3.263 homens e 3.275 mulheres, foram analisadas para o antígeno de superfície da hepatite C por teste imunocromatográfico (ICT), ensaio imunoenzimático (ELISA) e reação em cadeia da polimerase de transcrição reversa (PCR). Verificou-se que 396 (12,13%) de 3.263 indivíduos continham anticorpos no sangue contra o HCV, enquanto entre as diferentes faixas etárias as maiores incidências de anticorpos anti-HCV foram encontradas na faixa etária de 31 a 40 anos (11,01%). As amostras positivas para ICT foram posteriormente rastreadas por nested PCR para determinar a existência de HCV-RNA ativo. Identificou-se que 7,11% (3.263) do total da população (6.538) testada foram positivos, dentre os quais 461 (14,07%) mulheres possuíam anticorpos no sangue contra o HCV. Nossos dados mostraram que a infecção total pelo HCV na população investigada foi de 5,78%. Maior porcentagem de prevalência de HCV foi detectada em homens do que em mulheres nas faixas etárias de 31-40 e 41-50. Para comparar a prevalência de genótipos de HCV com relação à idade no genótipo masculino e feminino 3a foi encontrado o genótipo mais prevalente seguido por 1a, 2a e 3b, respectivamente.

**Palavras-chave:** HCV, ELISA, PCR, prevalência, Mardan.

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## 1. Introduction

HCV is a chronic infectious disease of the liver caused by a single strand RNA of Hepatitis C virus The virus belongs to the genus hepacivirus specifically affects the liver leading to morbidity and mortality (Bashir et al., 2017; Choo et al., 1989; Tariq et al., 2016). Which were learnt about 15 years later after the discovery of HCV. According to the World health organization (WHO), HCV has infected almost 200 million individuals worldwide and resulted in 350,000 deaths (Tariq et al., 2016). On comparison, the progression of HCV is more widespread in countries where HCV prevalence is low such as developing world of West of Europe, North America, and Australia, or intermediate in Japan, Italy, and Spain respectively (Afzal et al., 2016; Ahmed et al., 2006). The major mood of HCV transmission occurred from person to person by blood transfusion, dental and surgical instruments, reused razors, illegal drugs abuses, ear, and nose piercing and tattooing with unsterilized needles, unhygienic or poor sexual practices (Qureshi et al., 2013). Although, the signs and symptoms do not appear obviously. However, HCV infected individuals may suffer from lethal liver cancer/dysfunction and liver injury (Imran et al., 2013).

The general population of Pakistan are infected with HCV, indicating that with the second-highest HCV burden after Egypt (Averhoff et al., 2012; Wedemeyer et al., 2014). The chronic HCV infection depends on several risk factors, encompassing the ethnic group, gender type, host genetic factors, age at the time of infection, immune response, and development of jaundice and viral genotype as well subtypes (Afzal et al., 2014). Replication of Hepatitis C virus by mean of RNA-dependent RNA polymerase is error-prone resulting in the severe heterogenetic and different variant of HCV. As per estimation, 2-10 per nucleotide mutations occur per year (Attaullah et al., 2017; Riaz et al., 2016; Smith et al., 1997). Based on phylogenetic and sequencing analysis, the HCV genome is classified into 7 major and 67 known subtypes and further 20 provision subtypes. Among these genotypes 3 and 1 are world widely distributed (Messina et al., 2015). The identification of genotype is the fundamental measure for the sustainable virological response (SVR), for the selection of therapeutic stratagems and clinical treatment (Zein, 2000). On the ease of availability and low-cost income was an option for treatment of interferon (IFN), high SVR against specific genotypes in Pakistan. The pegylated IFN (PEG-IFN), ribavirin, or various treatment combinations were used (Afzal, 2017; Ahmed et al., 2006; Shah et al., 1997; Yu et al., 2007).

The initial diagnosis of HCV can be achieve by mean of anti-HCV antibodies test or Enzyme-Linked Immunosorbent Assay (ELISA) (Khan et al., 2013). But due to false accuracy results, PCR method holds an advantage over other techniques such as (ELISA), Recombinant Immunoblot assay (RIBA), Enzyme Immunoassay for the Qualitative Detection (EIA), ICT and so on, being a quick and reliable tool to diagnose, identify genotype, and quantify active HCV-RNA.

Previously, rare studies have been undertaken in different regions of Pakistan (Ahmad et al., 2007; Aslam and Aslam, 2001; Kumar et al., 2020, 2017). Thus, urging needs to study the relationship between HCV and population-based on their route of entry, age, and gender with their overall HCV genotypes in Pakistan. Therefore, Mardan is a congested city of Khyber Pakhtunkhwa, Pakistan (Figure 1), was selected due to lack of information and knowledge about the HCV infection, limited health facilities, and other unfavorable environmental conditions. A survey-based prevalence, epidemiology, and genotypic distribution of HCV age base infection in the district Mardan can provides a solid foundation ailment strategy conferring to viral genotype.

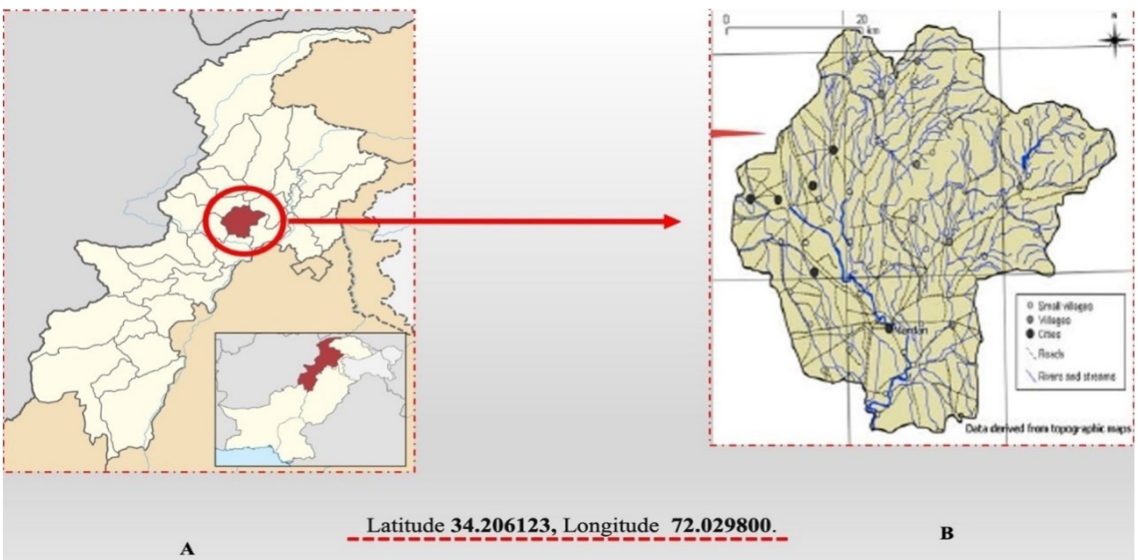


**Figure 1.** Map of the Khyber Pakhtunkhwa province Pakistan. The district Mardan in encircled, where the present study was conducted for determining the prevalence of active HCV infection and genotypic distribution (Khan et al., 2011): Genotypes of HCV.

## 2. Materials and Methods

### *2.1. Population sampling*

This study was conducted in district Mardan a north-west city of Khyber Pakhtunkhwa province of Pakistan. Blood samples of 6,538 (Males: 3,263 [49.90%], females: 3275 [50.0%]) individuals were taken and stored at 4 °C for serum separation. All the individuals were categorized into different age groups 11-20, 21-30, 31-40, 41-50, 51-60, and 61-70-or above respectively. The population was selected based on visiting the concerned laboratories for diagnosis. The participants younger than 9-10 years were excluded. Data from three years (2017 to 2020), of each patient were taken. All the patient information was exclusively used for research purposes only. The study was approved by the Ethical Committee and collections center or laboratories with the collaboration of the School of Life Sciences, Lanzhou University P.R China, and the Virtual University of Pakistan.

### *2.2. Sera-diagnosis via ICT and ELISA*

For anti-HCV antibodies test about 5-ml of the blood was collected in a disposable syringe from the radial vein of patients and stored in sterilized gel tubes. The serum was collected by coagulation of blood and stored at -20 °C until next use. Then sera were screened for the presence of anti HCV antibodies using a strip-based technology ICT commercially available from (ACON®, ACON Laboratories Inc., San Diego, CA 92121, USA) and ELISA from S.A. Barcelona Spain as recommended by the manufacturer instructions. The positive samples were further processed for molecular detection and genotypes. The Prevalence rate was determined as frequency based with the help of following Formula 1:

$$\text{Prevalence}(\text{as }\%) = 100 \times \frac{\text{Number of Positive Samples}}{\text{Number of Total Samples Measured}} \quad (1)$$

### *2.3. HCV confirmation by PCR and genotyping*

From anti-HCV antibodies positive samples, total RNA was isolated followed by (Kessler et al., 2002), then cDNA was synthesized using gene-specific reverse primers by reverse transcription, using Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase at 37°C for 50 mins. The viral RNA was confirmed through PCR primers (Table 1). For the genotypic investigation, total RNA from the positive sample was run by (Ohno et al., 1997). Briefly, reverse transcription PCR (RT-PCR) was performed for viral cDNA synthesis using core-gene specific reverse primers, and multiplex-PCR was performed. Nested PCR based two reaction mixtures containing two sets of primers (Table 2), were employed. The PCR-thermal cycler conditions were set 94 °C for 5 mins for pre-denaturation, followed by denaturation at 94 °C for the 30s (30 cycles). The primer annealing temperature was adjusted at 64 °C for the 30s; and with an extension of 72 °C for 30s, the final cooling temperature of 4 °C for 10 mins. The same conditions were applied for the nested PCR. Conversely, only primer annealing was carried out at 53 °C. At last, the amplified products of PCR were visualized on the gel documentation system after subjecting to electrophoresis on 2% agarose gel using with the DNA marker.

## 3. Results

### *3.1. Age-specific HCV frequency*

A total of 6,538 participants including 3,263 males and 3,275 female blood samples were collected. All blood samples were tested for the presence of anti-HCV antibodies by using ICT and ELISA. Overall, tested samples were categorized into age groups that fall into seven different age categories 11 to 70 years or above. At first all the male samples were positive by ICT 15.96% and were further assessed by ELISA. The ELISA results revealed that out of the total number 12.13% were positive (Table 1). The nested PCR assay of the samples positive by either ICT or ELISA revealed that 232 of 7.11% males are with active HCV RNA in their blood (Table 1). To calculate the age-wise prevalence in males, our results shown that all the age groups were affected while the HCV prevalence fluctuates in different age groups. To find highest incidence rate of 11.01% was recorded from the age group of 31-40 years, followed by 7.58% in the age group of 41-50. However, the lower HCV incidence of 1.04% was perceived in the age group of 60-70 years. Contrariwise, no HCV incidence was noted from the age group of 70 or above years (Table 1).

Following the same approach, the age-wise prevalence in female gender was also assessed (Table 2). To identify highest incidence rate 7.10% was observed in the age group

**Table 1.** Prevalence of active HCV among the different age groups of males as revealed by ICT, ELISA, and PCR tests.

| Serial No | Age categories (years) | No. of samples | ICT (Positive) | ELISA (Positive) | Positive PCR (Positive) | Prevalence (%) |
|---|---|---|---|---|---|---|
| 1 | 11-20 | 435 | 53 | 29 | 13 | 2.98 |
| 2 | 21-30 | 492 | 46 | 34 | 26 | 5.28 |
| 3 | 31-40 | 899 | 155 | 123 | 99 | 11.01 |
| 4 | 41-50 | 699 | 123 | 114 | 53 | 7.58 |
| 5 | 51-60 | 623 | 115 | 81 | 40 | 6.42 |
| 6 | 61-70 | 96 | 25 | 12 | 1 | 1.04 |
| 7 | 70-or above | 19 | 4 | 3 | 0 | 0 |
| | **Total** | 3263 | 521(15.96%) | 396(12.13%) | 232(7.11%) | 34.32 |

[1]Prevalence percentage of positive cases in males: PCR = (7.11%), ELISA: (12.13%), and ICT: (15.96%).

**Table 2.** Prevalence of active HCV among the different age groups of females as revealed by ICT, ELISA, and PCR tests.

| Serial No | Age categories (years) | No. of samples | ICT (Positive) | ELISA (Positive) | Positive PCR (Positive) | Prevalence (%) |
|---|---|---|---|---|---|---|
| 1 | 10-20 | 398 | 29 | 27 | 13 | 3.26 |
| 2 | 21-30 | 513 | 46 | 44 | 21 | 4.09 |
| 3 | 31-40 | 767 | 98 | 67 | 32 | 4.17 |
| 4 | 41-50 | 859 | 184 | 149 | 61 | 7.10 |
| 5 | 51-60 | 584 | 75 | 46 | 17 | 2.91 |
| 6 | 61-70 | 129 | 25 | 15 | 2 | 1.55 |
| 7 | 70-or above | 25 | 4 | 1 | 0 | 0 |
| | **Total** | 3275 | 461(14.07%) | 349(10.65%) | 146(4.45%) | 23.09 |

[1]Prevalence percentage of positive cases in females: PCR: (4.45%), ELISA: (10.65%), and ICT: (14.07%).

of 41-50 years, followed by 4.17% in the age group of 31-40. Similarly to detect lower incidence 1.55% was observed in the age group of 61-70 years (Table 2). These results revealed that different six age groups were affected significantly. Despite the fact, there were no PCR positive samples were detected in the age groups of 70 or above. Interestingly, it was observed that the age groups, the 41-50 year the HCV prevalence was higher in the all adults age group that is 21-40 years. However surprisingly, prevalence was highest in males from 31-40 and in females from 41-50 (Tables 1 and 2). We investigated that 11.01% of 99 males showed the higher positive PCR results in 31-40 age group for HCV (Table 1), while the prevalence in females was 7.10% (61) in the 41-50 age group (Table 2).

Taken together 6,538 contributors' blood was tested for liver function through ICT and ELISA. For HCV antibodies ICT 6,538 results of 15.01% were positive. The serum samples which were assured affirmative using ICT were furthermore investigated by ELISA. Of which 11.39% obtained positive. The ELISA positive samples were furthermore sorted for RT-PCR analysis in which 5.78% samples were confirmed to be active infected of HCV and harbor HCV-RNA (Table 1). The overall data shows the highest prevalence of anti-HCV antibodies tested by ICT, ELISA, and HCV-RNA among the general population of Mardan, KPK Pakistan in males than females (Table 3). The typical gel photograph is presented (Figure 2).

### *3.2. Genotypes frequency between male and female*

The prevalence of different genotypes from total HCV-RNA positive samples was further analyzed in the general population. Different HCV genotypes were detected by using allele-specific primers (Ohno et al., 1997). In order, to compare the prevalence of HCV genotypes according to age in male patients were categorized into 7 age groups following the prevalence from (Table 1). HCV genotype 3a was detected 31.94% of 74 with anti-HCV positive individuals. Moreover, genotype 1a was observed 17.24% of 40, followed by genotype 2a of 9.48%, and genotype 3b was 9.05%, a typical identified genotypes gel photograph (Figure 3). Furthermore, it was observed 17.24% of 40 tested samples where no genotype PCR band was observed and affirmed as untypable by the present method employed.

**Table 3.** Overall distribution of the ICT, ELISA, and PCR based HCV results of males and females from Mardan, Khyber Pakhtunkhwa, Pakistan.

| Gender | Total Samples | ICT Result | ELISA prevalence | PCR |
|---|---|---|---|---|
| Male | 3263 | 15.96% | 12.13% | 7.11% |
| Female | 3275 | 14.07% | 10.65% | 4.45 |
| Total | 6538 | 15.01% | 11.39% | 5.78% |

Correspondingly, also mixed infection of HCV genotypes was detected 15.06% of 35 (Table 4).

In the same way, to compare the age-wise prevalence of HCV genotypes in female patients also categorized into 7 age groups (Table 5). These results showed that genotype 3a was detected in female patients 38.35% of 56. Thus, these results showed that genotype 3a was significantly more abundant in female patients than males. These shreds of evidence showed that genotype 3a was dominant genotype circulating in general population of Mardan. Furthermore, genotype 1a (16.43% of 24), and genotype 2a (9.58% of 14) and genotype 3b (8.2% of 12) were found in female. Additionally, mixed infection of HCV genotypes 15.08% of 22 and untypable genotype 12.32% of 18 was observed in female of Mardan population. Besides these genotypes 5a and 6a were not detected in both HCV-RNA positive patients of general population of Mardan KPK.

### *3.3. Online data retrieval and its comparison*

To elaborate our investigation a systematic review of the literature was conducted using online available tools, PubMed, PakMedinet, and World Wide Web portal (http://webfoundation.org/). Taken together 18 studies and average prevalence of HCV in terms of the individual province/ cities were compared with our present investigations (Table 6).

Moreover, aiding funds to facilitate the healthcare system and better treatment facilities. Other preventive measures such as public awareness and vaccination programs are extreme need to fortify. The government and health authorities such as national institute of health (NIH) need to re-enforce the management and control strategies.

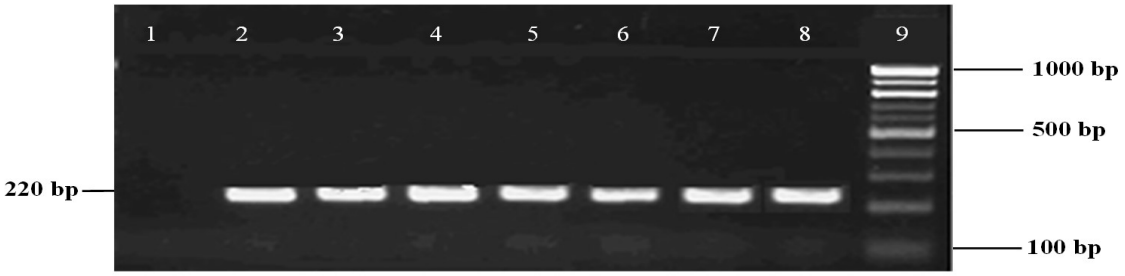


**Figure 2.** Representative gel photograph of HCV amplified products. Lane 1 represents negative control. HCV, Hepatitis C virus, lanes 2 to 8 are the typical positive samples for active HCV showing expecting 220 bp band of hepatitis C virus and lane, 8 is molecular marker.

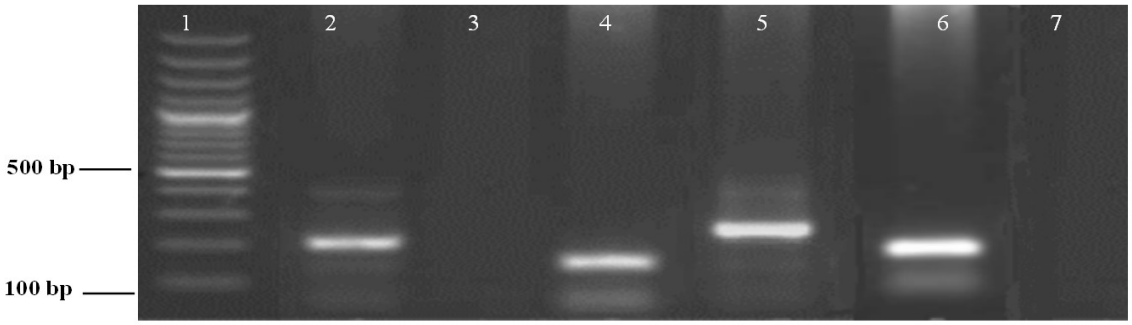


**Figure 3.** Typical Gels patterns of PCR products of different HCV genotypes identified in Mardan, KPK, Pakistan. Lane 1 = Molecular marker; Lane 2 = 208 bp HCV genotypes 1a; Lane 3 = untypable genotype; Lane 4 = 176 bp genotypes 3b; Lane 5 = 232 bp genotype 3a; Lane 6 = 190 bp genotype 2a; Lane 7 = negative control.

**Table 4.** Distribution of active HCV genotypes among the different age groups of males as revealed by PCR.

| Serial No | Age categories (years) | 3a | 2a** | 1a* | 3b* | Untypable* | Mixed* | Total* | % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 10-20 | 9 | 1 | 1 | 0 | 1 | 1 | 13 | 5.60 |
| 2 | 21-30 | 14 | 2 | 2 | 2 | 4 | 2 | 26 | 11.20 |
| 3 | 31-40 | 24 | 7 | 20 | 12 | 16 | 20 | 99 | 42.67 |
| 4 | 41-50 | 12 | 10 | 12 | 4 | 7 | 8 | 53 | 22.84 |
| 5 | 50-60 | 15 | 2 | 4 | 3 | 12 | 4 | 40 | 17.24 |
| 6 | 60-70 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0.431 |
| 7 | 71-or above | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Total | 74(31.94%) | 22(9.48%) | 40(17.24%) | 21(9.05%) | 40(17.24%) | 35(15.06%) | 232 | |

[a], [b]: **Genotypes of HCV, *Statistically significant: ($p$<0.05).

**Table 5.** Distribution of active HCV genotypes among the different age groups of females as revealed by PCR.

| S. No | Age categories (years) | 3a* | 2a* | 1a* | 3b* | Untypable* | Mixed* | Total* | % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 10-20 | 7 | 0 | 0 | 0 | 1 | 5 | 13 | 8.90 |
| 2 | 21-30 | 7 | 1 | 3 | 2 | 3 | 5 | 21 | 14.38 |
| 3 | 31-40 | 10 | 4 | 8 | 2 | 3 | 5 | 32 | 21.91 |
| 4 | 41-50 | 26 | 9 | 9 | 6 | 6 | 5 | 61 | 41.78 |
| 5 | 50-60 | 5 | 0 | 4 | 2 | 4 | 2 | 17 | 11.64 |
| 6 | 60-70 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 1.36 |
| 7 | 71-or above | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Total | 56(38.35%) | 14(9.58%) | 24(16.43%) | 12(8.21%) | 18(12.32%) | 22(15.08%) | 146 | |

*Statistically significant: ($p$<0.05).

**Table 6.** Reviewed of literature demonstrating the prevalence of hepatitis C across the different locations of Pakistan from 1999 till now. Each technique of study with its population size and type has been mentioned.

| S. No | Area Name | Population size | Population type | Prevalence % | Technique | Reference |
|---|---|---|---|---|---|---|
| 1 | Lahore | 538 | Pediatric population | 6.7% | ELISA | Parker et al. (1999) |
| 2 | Rawalpindi | 103858 | blood donors | 3.91% | ELISA | Khattak et al. (2002) |
| 3 | Quetta/Lahore | 351 | injection drug users | 88% | ELISA | Kuo et al. (2006) |
| 4 | Karachi | 4000 | healthy female | 4.5-14% | ELISA/PCR | Hakim et al. (2008) |
| 5 | Sindh | 5345 | blood donors | 7.5% | PCR | Mujeeb and Pearce (2008) |
| 6 | Lahore | 245 | Blood donors | 17.78% | PCR | Akhtar et al. (2013) |
| 7 | Peshawar | 982 | General population | 12.93% | CMIA | Ilyas and Ahmad (2014) |
| 8 | Islamabad | 215 | Hemophilic patients | 36% | ELISA | Asif et al. (2009) |
| 9 | Bahawalpur | 2239 | Blood donors | 6.7% | ELISA | Bhatti et al. (2017) |
| 10 | Hyderabad | 3078 | Pregnant women | 4.7% | PCR | Bibi et al. (2013) |
| 11 | Islamabad | 1977 | Hospital-based patients | 8.24% | ELISA | Rana et al. (2020) |
| 12 | Rawalpindi | 673 | Clinical visitor | 30.01% | Questionnaire data | Jamil et al. (2020) |
| 13 | Panjab | 8,353 | local community | 79.6% | PCR | Zafar et al. (2018) |
| 14 | Islamabad | 160,376 | General population | 3.26% | cross-sectional | Zaheer et al. (2014) |
| 15 | Peshawar | 1978 | General population | 6.21% | ICT/ PCR | Kumar et al. (2017) |
| 16 | Swat | 185 | infected patients | 62.16% | PCR | Inamullah et al. (2011) |
| 17 | Mardan | 1419 | Clinical/hospitalize | 8.52% | ICT/PCR | Ali et al. (2014) |
| 18 | Sindh | 31560 | The rural area of Sindh | 13.67% | ELISA/PCR | Bhatti and Manzoor (2016) |

This was an initial study and case-control investigation should be conducted in the future.

## 4. Discussion

The emerging infection and diseases pose a serious threat to human health. All these mostly occurred in underdeveloped countries like Pakistan due of limited resources. The new arising pathogens are characterized by a new model of pathogenicity (Khalil et al., 2017). Hepatitis viral rate is alarming in Pakistan (Al Kanaani et al., 2018). The increasing HCV infections precisely depict the prevalence of HCV, and the biogeographic distribution of its genotypes. In this study, 6,538 HCV-suspects were randomly collected from the congested city of Mardan.

The prevalence of HCV has been observed by ICT 15.01%, ELISA 11.39% and PCR 5.78%, which comparatively much varies than many cities of Pakistan (Table 1-6), by comparing to Rawalpindi 2.45% (Masood et al., 2007), Islamabad 5.31% (Idrees et al., 2008), Buner 5% (Muhammad and Jan, 2005), Multan 4.06% (Idrees et al., 2008), Northern areas of Pakistan 25.70% (Tariq et al., 1999), and Faisalabad 20.89% (Ahmad et al., 2007). We have also found that the overall percentage was significantly higher in men than women over the last 3 years. This higher prevalence of HCV in male might be due the higher exposure in environment, but HCV is more prevalent among male than females of 2013 and 2017 years (Kumar et al., 2017). Although, epidemiology of HCV exhibit variation from region to region or even within the same population (Wild and Hall, 2000). Such differences of the prevalence of HCV might be associated with different lifestyles, age factors, poor literacy rate and health facilities, low socio-economic status, and lack of awareness (Shah et al., 1997). Considering the age group distribution, the prevalence of HCV infection showed gradually increased among the middle age of 31-50 years. These may reflect that in 31–40 years age group, males have highest prevalence than females while in 41–50 years age group females were more affected than males. It will be interesting further to find out the factors associated in this gender specification.

Previously studies has shown that the severity, prognosis of disease and response to therapy may vary according to the genotypes (Antaki et al., 2013; Wyles and Gutierrez, 2014). Our data showed that HCV genotype 3a is the most prevalent in the Mardan population, which are corroborated with (Ahmad et al., 2010; Aziz et al., 2013; Khan et al., 2011b). The frequency of genotype 1a was observed in male and female (17.24% and 16.43%) respectively. However, the frequency of the patients infected the method employed with unidentifiable or mix genotype in both genders. In most of previous studies reveal the detection of an unknown/undetermined HCV genotype (Ali et al., 2014; Rauf et al., 2011). These studies specified that identification of these unknown genotype(s), and exploration of its/their possible role for clinical investigations, diagnosis, sequencing and development

of Hepatitis C, vaccine development and other prevention measures still remains challenging.

Genotype 4a is very rare in Pakistan and also absent in present study, nonetheless, a study on Lahore city in Pakistan identified 4a genotype (Ahmad et al., 2010), in the Middle East (Gower et al., 2014). Genotypes 5a and 6a were also missing in present observations. These studies highlight a important clinical parameters (Ahmad et al., 2010). Moreover, HCV has six different genotypes, which have further subtypes. These genotypes confirmed 30-35% nucleotide differences in HCV genome (Smith et al., 2014). However, it has been claimed that age is the main and important risk factor for HCV (Kumar et al., 2020, 2017; Mengal et al., 2012). Therefore, based our study different age groups containing HCV infection and even though comparing the different age groups, the adult male population was the most dominant in HCV prevalence at the age of 31-40 (11.01%) followed by females in the age group 41-50 (7.10%).The highest prevalence of HCV infection existed among middle age of male and old age female with 3a genotype. Therefore, most of the young age individuals remain ignorant about their status of HCV infection. Taken together age has been a main factor in HCV studies, with infection more predominant in older ages of population (Kumar et al., 2020, 2017; Mengal et al., 2012).

The Mardan city was taken as a second model city after Peshawar the capital province of Khyber Pakhtunkhwa, as entire districts have a mixed population (Kumar et al., 2020). Among the Khyber Pakhtunkhwa districts Mardan has been facing basic health requisites problems and suffer from natural disasters including floods, population pressure and earthquakes. However, most of previous findings concern (Table 6), about ICT and ELISA or other non-PCR based (Ahmad et al., 2010). In our findings, RT-PCR depict the more exact picture of active HCV prevalence and genotyping distribution in different age groups of males and females.

Mardan is in the southwest of province Khyber Pakhtunkhwa, Pakistan. The proportion of the urban district is 20.2% whereas 79.8% is the rural proportion. A report conducted by Government of Pakistan, compared to urban areas HCV prevalence is more dominant in rural areas (Afridi et al., 2013). Based on our prevalence frequency of different HCV genotyped reported so far lower in females 4.45% in comparison with males 7.11%. Our investigations suggest that the males community is acquiring HCV infection more rapidly due to maximum exposure to the external environment. On the other hand, barber shaving, Homosexuality may refer to males who have sex with males (MSM), or females who have sex with female (FSF) and use of drugs are common practices in these areas. The arguments are in line for higher occurrence of HCV among males, however in contrast females living house life. Overall, our data support the hypothesis of a homogeneous distribution of HCV infection between the male and female related to various outdoor factors. Similarly, HCV higher prevalence in males followed by females was observed when risk factors regarding hepatitis B and C (Khan et al., 2011a). Furthermore, our finding indicates the dominant genotypes 3a and 1a, among the tested population. These results validate the findings of (Ahmad et al., 2010; Kumar et al., 2017), reported that 59.9% and 16.5% of individuals were infected with 3a and 1a genotypes, both these being higher percentages than the other genotypes detected. Genotype 3a of 34.1% as the most prevalent genotype in district Swat, followed by 2a (8.1%), 3b (7%), 1a (5.4%) and mixed genotype 7.6% (Inamullah et al., 2011). Another study of 49.5% prevalence genotype 3a described in district Swat (Ahmad et al., 2009). To mitigate the risks associated with HCV infection, it is important to understand the interactions of pathogen-host-environment and monitor the molecular evolution and genomic surveillance.

Based on our visual investigation, most of the patients associated with low economic status and visited by other local medical practitioners. Lack of emergency medical services and initial help to victims are commonly practiced by untrained worker might be another major cause hepatitis C (Bukhari, 2013). Use of no proper sterilization and contaminated equipment may represent another possible reason for high prevalence. Besides this sharing personal possession such as shaving razors, nail cutters, the needle used for ear and nose piercing, toothbrushes, and personal close or/and transgender sexual contact with an infected person (Qureshi et al., 2013).

The results of our study showed prevalence and distribution of HCV genotypes varies with age. For example, genotype 3a was more common in male the ages 31-40, while it was more prevalent in female between ages 41-50. To our knowledge, it is the comprehensive report to evaluates the distribution of genotypes of HCV in Mardan, but it has some limitations, such as subtype with a relatively small sample size and a selection bias is possible given the use of the data from a multiple laboratory. But our study may be helpful for understanding the genotype distribution of HCV in Mardan and can provide important information about HCV prevalence among different ages and sexes. However, Khyber Pakhtunkhwa may not have much strength or capacity to mitigate these problems itself immediately. By so far, great push up efforts are required by the national/international community to reduce the existing threat.

## 5. Conclusion

In conclusion, HCV prevalence and genotype distribution has a certain relationship with different age and gender. We found that HCV 3a is a predominant genotype followed by 1a, 2a, and 3b in Mardan. Further studies are needed to collect bigger samples to estimate the different epidemiology of the HCV genotypes. As males are more frequently prone to high-risk than female also indicating that the infection is more proliferated between the adult group either males or females. To control HCV disease, there is a dire need to design a treatment achieving maximum control with minimal hostile effects. Major awareness events should be launched by Government and Semi government authorities or non-government organizations (NGOs), ample use of vaccine and other preventive measures to cease the outspread.

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