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# PREVALENCE OF HEPATITIS C VIRUS AND ASSOCIATED RISK FACTORS: A STUDY FROM LOCAL POPULATION

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## ABSTRACT

**Background:** Current study was designed to find out the prevalence of active hepatitis C infections among general public in Lahore city. **Methods:** PCR reports for hepatitis C were collected from the different laboratories of the Lahore during the January 1, 2018 to January 1, 2019. Data from the reports were categorized into different age groups on the basis of record available. Data were analyzed statistically using Chi-Square test. **Results:** Out of the 4246 blood samples analyzed in this study, 210 were confirmed to be positive for active HCV infection. Gender-wise active HCV prevalence revealed no significant difference [OR = 1.10 CI = (0.83-1.46), p > 0.05]. However, among the age groups the highest prevalence was observed in the age groups 20–29 (7.7%) and 30–39 years (6.4%) with odds of prevalence of 14.8% (OR = 2.48, CI = (1.40-4.38), p < 0.05) and 10.3% (OR = 2.03, CI = (1.10-3.71), respectively. In age groups above 40 years (40–49, 50–59 and >59 years), a decrease in levels of active HCV prevalence was observed. **Conclusions:** Among tested samples, 4.9% of the subjects were confirmed to harbor

active HCV infections and the "middle aged" population in Lahore was found to be at a higher risk of the HCV ailments compared to both their younger and older peers.

**Keywords: Active HCV prevalence, Lahore, Pakistan, Nested PCR**

## INTRODUCTION

Hepatitis C is an infectious liver disease of humans and is mostly caused by the hepatitis C virus (HCV) [1]. In most of the cases, this infection remained asymptomatic especially in its early stages but once it developed, it has the potential to progress and causes liver fibrosis and ultimately cirrhosis. It may also contribute to the deadly liver pathologies such as liver failure and liver cancer [2]. In 2004, the World Health Organization (WHO) reported that annual deaths all over the world due to liver cancer and cirrhosis caused by HCV were about 308,000 and 785,000, respectively [3]; and about 200 million people, the 3.3% of the world's population, are infected with HCV [4]. Moreover, around 3 to 4 million individuals are diagnosed as new cases every year [5]. In Pakistan, an alarming rate of HCV outbreaks have been reported in many studies. The previous literature depicted that there are about 10 million people, infected with HCV in Pakistan [6-8]. Prevalence of HCV has been estimated to be variable in different regions of Lahore even in the same region time to time [9]. According to various studies, the presence of HCV infections among different categories (excluding chronic liver disease patients), was 5.31% in Islamabad [10], 0.4-

31.9% in various regions of Punjab province [6, 7, 11-13], 4-6% in Sindh province [6, 7, 13, 14], 1.1-9% in Khyber Pakhtunkhwa province [6, 7, 13, 15-17], 1.5% in Quetta region [12,13] and 25.7% in Gilgit Baltistan province [18, 19]. While in Lahore, the second largest city of Pakistan with a population of more than 7 million [20], HCV prevalence was estimated from 0.58-17.78% [6, 13, 19, 21-24]. The most accurate method to detect the active infection is by PCR. Other methods are used to check the presences of Hepatitis C virus are non-PCR immunoassays such as ELISA, RIBA, EIA, ICT etc., due to the presence of anti-HCV antibodies in their blood [7]. PCR has emerged as the most powerful diagnostic tool for the detection, quantification and genotyping of active HCV RNA in the blood. In the present study, over 2000 blood reports were randomly collected from individuals visited different clinical laboratories in Lahore, Pakistan. These samples were screened through confirmatory nested PCR qualitative assay to determine the percent prevalence of active HCV in various subjects. To the best of our knowledge, this is the first comprehensive report concerning the prevalence of active HCV in Pakistan's 2nd largest city and its surroundings, with a

statistically significant number of samples. The data was also correlated and categorized in terms of gender and age groups.

## METHODOLOGY

In this study, 2123 blood reports were collected from different laboratories of the Lahore given sample for liver function tests and Hepatitis C status between 2018 to 2019. Reports included both males and females, having ages ranging from 10 to > 60 years. This study was approved by from institutional review board and ethical committee (IRBEC). History of individuals was recorded from the available reports. Protocol adopted to detect the Hepatitis C virus was as following, serum from each of these blood samples were separated by centrifugation at 4000 rpm for 5 min. Each sample was properly labeled and stored at −20°C until. Besides the gender groups, samples were also categorized into six age groups to determine the prevalence in each age group. In viral RNA extraction and cDNA formation, RNA was extracted from these samples using the viral RNA extraction kit according to manufacturer's instructions. Serum sample (140μl) was used to extract RNA that was eluted in 60μl elution buffer supplied with kit. RNA was used in making cDNA immediately after extracting RNA or stored at −80°C for further use. cDNA was prepared in a total reaction. Qualitative data was analyzed by using SPSS (version 22). Logistic regression was performed in order to examine the prevalence of HCV associated with sex and age. A *p*-value < 0.05 was defined as statistically significant.

## RESULTS

Out of 2123 blood samples, male to female ratio were the 958 and 1665 respectively. Moreover, data collected further stratified into six different age groups to assess the prevalence of HCV among age groups. The PCR results revealed that 104 (4.9%) individuals had active HCV infection **(Table 1)**. Gender-wise prevalence of active HCV infection was estimated to be 5.27% in male and 4.67% in female subjects. Although the probability trends were slightly higher among males of all age groups than females, statistically there was no significant difference in gender with OR = 1.10 CI = (0.83-1.46), p >0.05, as elaborated in **Table 1**. Substantial differences were observed considering the age group criterion. Out of the 229 individuals tested in ages ranging from10–19 years (both male and female), only 3.3% were HCV positive. The highest numbers of positive individuals with 7.7% prevalence were among the tested subjects in the age group 20– 29 years **(Table 1 and Figure 1)**. Statistically, the odd of prevalence of HCV in individuals in age group 20– 29 years was 14.8% higher than the individuals in age group 9–19 years (OR = 2.48, CI = (1.40-4.38), p < 0.05), as illustrated in **Table 1**. In the age group 30–39 years, HCV

prevalence, though slightly less than that found for the 20–29-year group, was still significantly higher (10.3%) as compared to age group 09–19 years (OR = 2.03, CI = (1.10-3.71). Subsequently, a decreasing trend of HCV prevalence was observed with increasing age of subjects above 40 years.

The lowest prevalence (3%) of active HCV was observed in the age group 59 years and above. Overall active HCV prevalence among all the tested samples was estimated to be 4.9% and the risk of HCV prevalence was significantly higher in the middle age groups (20–29 & 30–39 years).

**Table 1: Prevelence of HCV in different age group**

| Age groups | Total samples | Male +/- | Female +/- | Probabilities | | p value | Odd ratio (95% CI) | Overall prevalence (%) |
|---|---|---|---|---|---|---|---|---|
| | | | | Male | Female | | | |
| *10-19* | 229 | 5/155 | 3/116 | 0.0343 | 0.0313 | – | – | 3.3 |
| *20-29* | 445 | 16/200 | 19/212 | 0.0810 | 0.0741 | 0.002 | 2.48 (1.40-4.38) | 7.7 |
| *30-39* | 313 | 12/177 | 8/164 | 0.0672 | 0.0614 | 0.023 | 2.03 (1.10-3.71) | 6.7 |
| *40-49* | 327 | 7/145 | 9/162 | 0.0529 | 0.0483 | 0.155 | 1.57 (0.84-2.93) | 5.1 |
| *50-59* | 365 | 8/144 | 8/203 | 0.0375 | 0.0342 | 0.779 | 1.10 (0.57-2.09) | 3.6 |
| *> 60* | 444 | 7/175 | 7/255 | 0.0321 | 0.0292 | 0.831 | 0.93 (0.49-1.77) | 3.0 |
| **Total** | 2123 | 55/906 | 54/1112 | 0.0508[b] | 0.0464[b] | – | – | 4.9 |
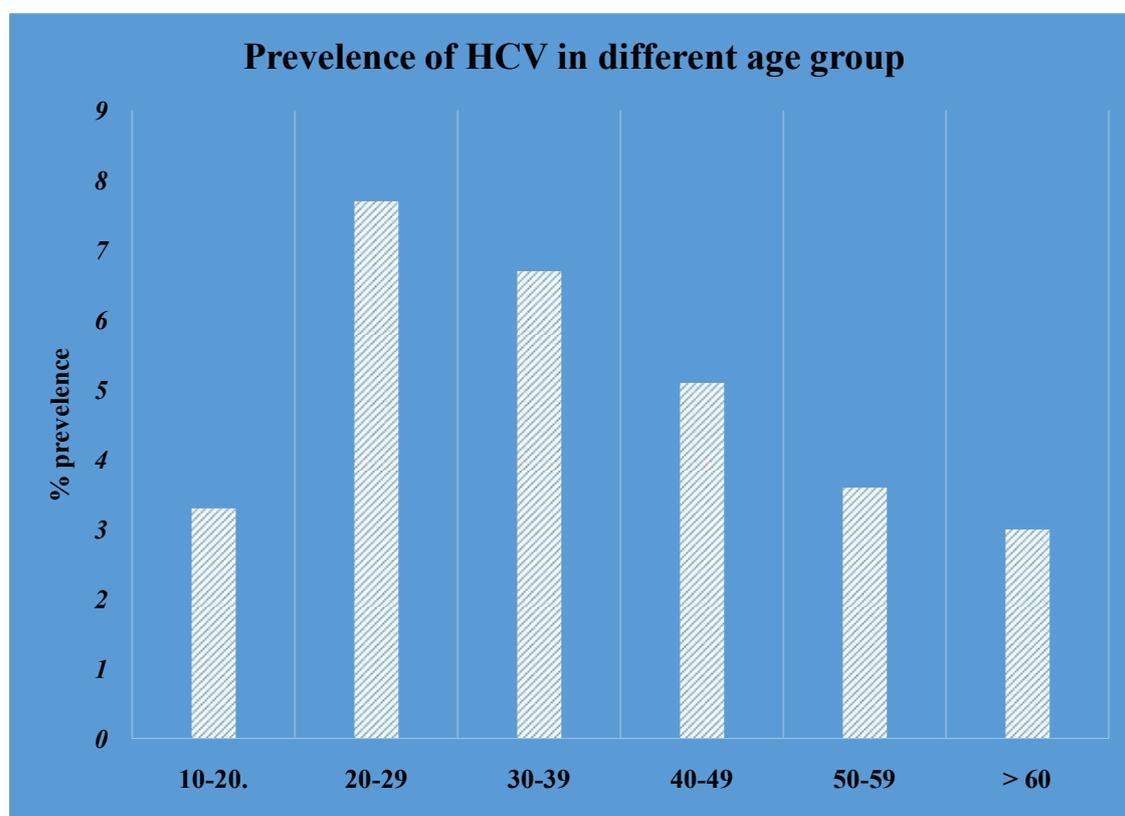


**Figure 1: Prevelence of HCV in different age group**

## DISCUSSION

In Pakistan, ~6% population is suspected to be infected with HCV **[6, 7, 25]** and HCV prevalence data published so far is highly variable. In most of the studies conducted so far, either the number of samples reported are too small to draw any solid conclusion or the methodological differences have made it impossible to conduct a formal meta-analysis to determine accurate prevalence estimates **[7]**. Among all the published reports, 99% of the data originated from erroneous non-PCR qualitative screening methodologies, mostly based on the detection of anti-HCV antibodies. Active HCV prevalence estimated during our research (4.9%) is lower than that reported by Aslam *et al*. [24] (6.7% based on 488 samples from the general population of Lahore). Similarly, the current estimate is much lower when compared with a nationwide data surveillance study conducted through an ELISA blood screen by Qureshi, *et al*. **[12]**, who reported 6.8% HCV prevalence in Lahore based general public. Our estimates were, however, much higher than those of another study in which 203 blood samples from staff and students of University of the Punjab, Lahore, were assayed through ELISA by Tanvir *et al*. in 2008 **[26]**. In yet another series of research conducted in pediatric population by Khan *et al*. **[22]**, Parker *et al*. [23] and Hyder *et al*. **[27]** HCV prevalence was reported as 4.09%,

1.3% and 0.58%, respectively, which is again lower as in comparison to our reported results **(Table 1)**. The only PCR-based HCV active prevalence study conducted in Lahore was reported by Zafar *et al*. **[28]** for a cohort of pregnant women. Out of 300 screened samples, 4% were found to be positive. Although this figure is relatively close to our figure (4.9%) the number of samples tested and the study subjects were less broad. Moreover, our active HCV prevalence estimate in general public of Lahore city is much lower than the values reported by Chaudhary *et al*. **[29]** and Akhtar *et al*. **[21]** among blood donors in Lahore, which revealed HCV prevalence as 6.06% and 17.78%, respectively; indicating highly variable results **(Table 2)**. Both studies were conducted using ELISA as a screening tool. HCV prevalence in IDUs **[30]** and hemophilia patients **[31]**, reported as 88% and 56% respectively, are much higher in comparison with our results. Gender-wise HCV prevalence revealed no significant difference [OR = 1.10 CI = (0.83-1.46), p > 0.05] in male and female populations, as detailed in **Table 1**. Our study is in agreement with the previous country-wide as well as Lahore based surveys **[12, 20, 32]**. It is, however, in contrast with a recent study showing female to male ratio of 1:16.5 (Table 2) presumably due to the subjects were restricted only to blood donors and ELISA

was used as a diagnostic tool [21]. Considering the age group criterion, significant differences were observed in the prevalence of HCV in both genders. The highest prevalence 7.7% and 6.4% were observed in age groups 20–29 and 30–39 years, respectively; with odds of prevalence of 14.8% (OR = 2.48, CI = (1.40-4.38), p < 0.05) and 10.3% (OR = 2.03, CI = (1.10-3.71), respectively. Both of these values are higher compared to the age group 9–19 years. In age groups above 40 years (40–49, 50–59 and >59 years), a decreasing trend of active HCV prevalence was observed **(Table 1)**. These results are in agreement with the previous studies particularly those conducted in Lahore city revealing the higher risk of HCV prevalence in middle aged groups (20–40 years) **[20, 24, 33-37]**. The high prevalence of HCV in middle aged groups can be correlated to more exposure of HCV infection and other risk factors such as non-blood transfusions, widespread reuse of syringes, and a range of other high-risk traditional practices.

## CONCLUSIONS

Using the PCR based diagnostic assay for 2123 blood samples, the overall prevalence of active HCV was estimated as 4.9% in general public of Lahore. No significant differences in male and female genders were observed. However, HCV prevalence varied in different age groups. It was least prevalent in age groups 9–19 and above 59 years. However, middle aged populations, especially 20–29 and 30–39 year individuals were observed at higher risk of hepatitis C ailments with 7.7% and 6.4% active HCV prevalence, respectively. This report will provide the active HCV prevalence data for further meta-analysis, which can be helpful to health policy makers to devise strategies for the control of hepatitis C disease in Lahore in particular and in Pakistan in general. From the results of the present study, future PCR-based studies will result in lowering the previously reported estimates (i.e. 6%) of prevalence of HCV in Pakistan.

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