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EPIDEMIOLOGY AND RISK FACTORS OF HEPATITIS C INFECTION IN THE SLUM POPULATION OF ISLAMABAD, PAKISTAN

Amir Muhammad[1,a], Asad Zia[2,a], Ajmal Khan[3], Noman Asghar[4], Naveed Iqbal[*5]

[1]*Department of Paediatrics, Medical Teaching Institution, Lady Reading Hospital, Peshawar, Khyber Pakhtunkhwa, Pakistan.*

[2]*Public Health Reference Laboratory (PHRL-KP), Khyber Medical University, Peshawar, Khyber Pakhtunkhwa, Pakistan*

[3]*Institute of Biotechnology and Microbiology, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, 24420, Pakistan.*

[4]*Department of Pathology, Lady Reading Hospital, Medical Teaching Institution, Peshawar, Khyber Pakhtunkhwa, Pakistan.*

[*5]*Department of Medicine, Medical Teaching Institution, Lady Reading Hospital, Peshawar, Khyber Pakhtunkhwa, Pakistan*

[1]amir.muhammad@lrh.edu.pk, [2]asadzia96@gmail.com, [3]ajmalkhan@bkuc.edu.pk, [4]noman.asghar@lrh.edu.pk, [*5]naveed.iqbal@lrh.edu.pk

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***Corresponding Author:**
Dr. Naveed Iqbal, Assistant Professor, Department of Medicine, Medical Teaching Institution, Lady Reading Hospital, Peshawar.,

[a]The two authors contributed equally

Abstract

Background: *Hepatitis C virus (HCV) infection is the leading cause of chronic liver disease in Pakistan with about 10 million infected individuals. Therefore, we investigated the prevalence of HCV and the associated risk factors in the slum population of Islamabad Pakistan.*

Material and methods: *A total of 754 individuals were selected, consisting of 415 females and 339 males, between January 2024 and June 2025, using cluster random sampling method. Samples were initially screened for anti-HCV in serum by immunochromatography (ICT) and enzyme-linked immunosorbent assay (ELISA). Positive samples were further confirmed for HCV RNA by using polymerase chain reaction (PCR).*

Results: *The overall active prevalence of HCV in apparently healthy individuals was found 19.2% (145 individuals) in the slums of Islamabad, Pakistan. The prevalence in males and females was 49 (14.4%) and 96 (23.13%), respectively. Chi-square statistic showed that the prevalence of HCV in females was significantly higher than males with p = 0.0001. Previous history of cesarean section and piercing were significantly correlated with HCV in the females. Stepwise binary logistic regression showed that low literacy rates, gender, marital status, risk from HCV infected individual in the family, circumcision, previous history of dental surgery were significant with HCV infection.*

Conclusions: *High infection rates were reported in the females and illiterate population due to the lack of awareness of potential risk factors associated with HCV transmission. Therefore, we recommend the massive awareness campaign in the general population of slums about the HCV mode of transmission and its presentation.*

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1. Introduction

Hepatitis C virus is a well-known *Flaviviridae* that is considered as one of the main cause of inflammation of liver, cirrhosis and hepatocellular carcinoma [1]. HCV has the ability to persist in the infected human being resulting chronic infection, most often without any symptoms, until the appearance of long-term complications such as hepatic fibrosis, cirrhosis and hepatocellular carcinoma (HCC)[2]. Pakistan has about 10 million HCV infected individuals (about 7% of the total population)[3] ; range from 0.4% to 33% in different population groups[4]. A high prevalence of 14.63% HCV infection has been previously reported in apparently healthy population living in central regions of Pakistan [5].

Awareness about the routes of transmission of hepatitis C and hepatitis B virus in Pakistani population has been rated poor [6] especially in illiterate individuals [7]. However, increased awareness level about hepatitis has been reported in individuals having formal education [7] and access to internet [8]. Being an underdeveloped country, Pakistan is spending very little on education and health [9] that further worsen the situation.

WHO estimates show that diseases associated with poverty account for 45% of the disease burden in the poorest communities[10]. Pakistan has considerably higher rate of illnesses (including infections) in areas with low income (sometimes <5 dollars/month per capita), poor sanitation and unhygienic living conditions. Such underprivileged population of Islamabad capital territory which are less explored for disease studies. Further, there is no study available which describes the prevalence of HCV in these underprivileged areas in Pakistan. We conducted a study to estimate the HCV prevalence and associated risk factors in such areas.

2. Material and Methods

2.1 About the city

Islamabad is a modern city and was declared as capital of the republic of Pakistan in 1960[11]. As soon as the area was declared as capital territory, developmental projects were immediately initiated to build a modern city near the Margalla hills. The total population of the federal capital is about 2 million. An estimated 80,000 people live in the underprivileged localities of the capital city (see Figure 1 for map of the city). Some have legal status (including French Colony, Paris colony, 100 quarters, 66 quarters, Hansa Colony, Charles Colony, Said Pur Model Village and I-9 Christine colony) while the remaining are illegal (including those situated at G-7, G-8, F-6, F-7, I-11 and Banni Gala area) mostly consisted of migrated people or the local residents before the area was declared as capital [12].

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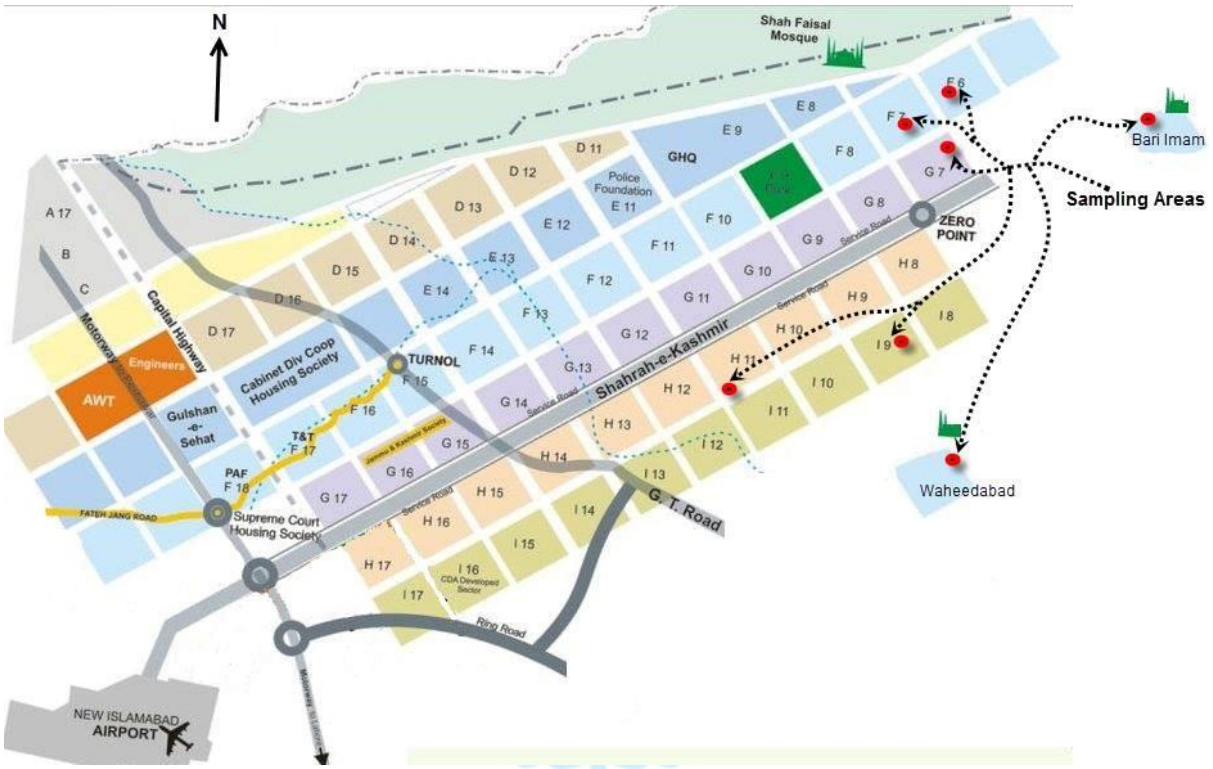

Figure 1: Map of the capital territory of Islamabad, where the study samples were collected

2.2 Sample size

The sample size was calculated using a method described [13], given in appendix-II previously reported the prevalence of HCV in Pakistan 7% of the population and considering 1.85% precision and 95% confidence interval. The estimated population in underprivileged localities of Islamabad are around 80,000 [14].We estimated the sample size to 731 using the formula in Appendix-II. For the safe hand, we collected 800 samples from the population. However, after clearing the data, we had 754 samples.

2.3 Study design, sampling and data collection

We used cluster random sampling method to select the individuals from underprivileged localities in Islamabad capital territory of Pakistan. The capital city has a total of 34 underprivileged localities. Each locality was considered as single cluster. Initially, seven localities were selected by simple random sampling out of 34 underprivileged localities, including: Muslim colony, regularized localities at sector G-7/2, hundred quarters at F-6/2, France colony at F-7/4, I9/4, Rohail colony at H-11/4 and Waheedabad for our study (Figure 1 for details). In the second stage, a total of 754 individuals (males and females) were selected through simple random sampling procedure from each selected cluster with equal proportion. We selected three settled underprivileged localities including that of G-7/2-regularized, hundred quarters and France colony; and four illegal (Muslim colony, I-9/4, Rohail colony and Waheed Abad. The study was carried out in 2015 (January 01 to December 14) with the aim to estimate the prevalence of active HCV infection in the underprivileged population with lowest income (<5 dollars/month per person) in the underprivileged localities of Islamabad and its possible modes of transmission. Written and informed consent were obtained from the volunteers participating in the study. A printed questionnaire was completed by each of the participant before blood sample collection. Blood samples were collected (5 ml) from both male and female volunteers, and were transported to the laboratory in cold chain. Serum was separated and stored in three separated aliquots at −20 °C until further process. The study was approved by the

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departmental ethical committee of the Quaid-i-Azam University, Islamabad.

A structured questionnaire was used to collect information on risk factors associated with the HCV infection in the studied population. The questionnaire with 20 different questions with multiple choice responses related to the following components:

1) General demographics such as age, gender, education level, marital status; 2) Opinion on HCV routes of transmission and methods of diagnosis; 3) history of transfusion, tattooing, surgery (cosmetic surgery, dental and medical surgery or any minor surgeries), acupuncture, and stitches; 4) history of hepatitis C in the family; 5) self-medication; 6) drug abuse and injection drug users (IDUs).

For convenience the questionnaire was translated to Urdu language using standard translation tools and the participating individuals were briefed about the contents.

2.4 Immunochromatographic (ICT) assay and Enzyme linked immunosorbent assay (ELISA) for the detection of HCV infection

All samples were initially subjected simultaneously to two different ICT procedures (obtained from Accurate (specificity 99% and sensitivity 100%), USA; and Acon, USA (sensitivity more than 99.9% and specificity 99.8%) for detection of anti-HCV antibodies, 180 patients were positives by these ICTs. To avoid experimental error, tests were performed in duplicate.

Serum samples found positive for Anti-HCV antibodies by ICTs were further processed for the detection of anti-HCV antibodies by using ELISA kit obtained from BIOKIT, S.A, Barcelona-Spain (Bioelisa HCV 4.0 specificity of the kit is 99.63%, Sensitivity 100%) according to the manufacturer's protocol. 175 patients were found positive by ELISA.

2.5 Detection of HCV RNA in the serum samples

All anti-HCV antibodies positive serum samples were investigated for the presence of HCV RNA. For this purpose, RNA was extracted from the serum samples using Virus RNA isolation Kit (INSTANT, AJ Roboscreen, GmbH Germany). Qualitative HCV detection was based on amplification of viral 5'non-coding region (5'NCR) using reverse transcriptase (RT) PCR as described previously by[15]. Briefly, viral RNA was reversed transcribed into complimentary DNA (cDNA) using 100 units of M-MLV reversed transcriptase (Invitrogen), with 5 pM of outer anti-sense primer 5′-GGCGACACTCCACCATAGAT-3′. Two rounds of PCR amplifications were performed. The first round PCR were performed using outer sense 5′-GGCGACACTCCACCATAGAT-3′ and outer anti-sense 5′-GGCGACACTCCACCATAGAT-3′ primer pair, for second round inner sense 5′-GGAACTACTGTCTTCACGCAG-3′ and inner anti-sense 5′-TCGCAAGCACCCTATCAGGCA-3′ were used. The PCR products were run on 2% agarose gel contained ethidium bromide as DNA stain. The specific HCV PCR bands were visualized under UV trans illuminator. The reported sensitivity of this assay is 10 IU/mL[16]. All those samples that were positive by both serological tests (ICTs and ELISA) and PCR were considered as HCV positive. A total of 145 samples were found HCV positive by PCR. The criteria for HCV screening are shown in Figure 2.

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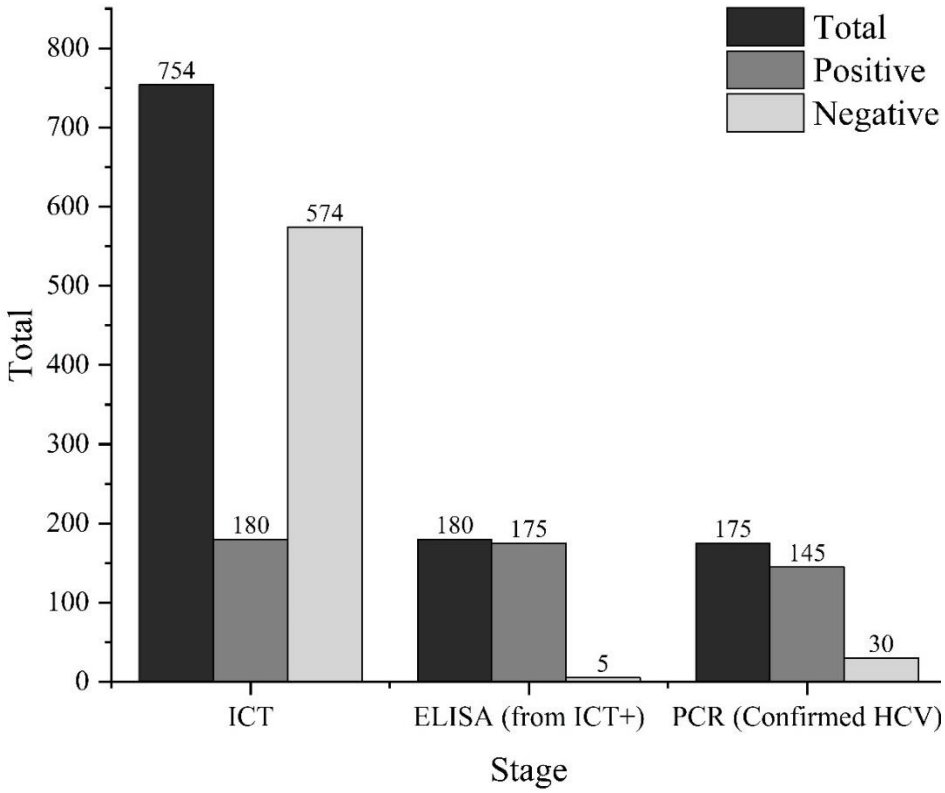

Figure 2: Flow of HCV Screening and Diagnostic Confirmation.

A total of 754 samples were screened using ICT, followed by ELISA confirmation, and PCR testing to identify active HCV infection cases.

2.6 Statistical analysis

The prevalence of HCV was measured as percentage. The Chi- square (χ^2) statistics was used to determine the association among different variables and factors. Multivariable analysis was used to determine the impact of different risk factors on the prevalence of HCV simultaneously in the studied population. For this purpose, binary logistic regression model was used instead of general or classical linear regression model. This is because of that the response variable is categorical in nature. To select the significant variable, we used black ward stepwise binary logistic procedure (details are presented in **Appendix-I**). The outcome (response) variable was considered in to two categories: HCV negative and HCV positive. On the other hand, independent variables were: gender, previous history of cesarean section and piercing in the females, literacy rates, marital status, risk from HCV infected individual in the family, injection drug use, shaving at barber shop, circumcision in males and previous history of dental surgery.

3. Results

3.1 Demography of the studied population

We examined the prevalence of HCV in 754 individuals within the age range from 3 to 103 years, of which 339 (45%) were males and 415 (55%) females. The total prevalence of HCV (previously diagnosed and undiagnosed) was 14.45% and 23.13%, respectively. We found that the prevalence of HCV was significantly higher in females as compared to males based on the p-value (p=0.0001). The Figure-1 indicates the age-specific prevalence of HCV in the studied population. Using Chi-square (χ^2), we found a significant difference between the prevalence of HCV in different age groups with $p < 0.001$ (for both gender) (Table 1 for details). We reported a high prevalence of 19.23% HCV infection in apparently healthy individuals living in the underprivileged localities of Islamabad Pakistan. The incidence of infection is higher in females (23.13%) as

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compared to the males (14.45%) (Figure 3 and Table 2).

Table 1: Fitted parameter for logistic regression and multivariate logistic regression model of HCV infection with associated risk factors in the studied population

Predictor	Coefficient	Coefficient SE	Z-value	P-value	Odds Ratio	95% CI	
						Lower Limit	Upper Limit
Constant	-8.120	1.412	-5.75	0.001			
Age	0.017	0.008	2.14	0.032	1.02	1	1.03
Gender	2.053	0.562	3.65	0.000	7.79	2.59	23.45
Education	-0.904	0.314	-2.88	0.004	0.4	0.22	0.75
Marital status	0.924	0.378	2.45	0.014	2.52	1.2	5.28
Role of dental surgery	0.414	0.210	1.97	0.049	1.51	1	2.28
History of HCV infected patient in family	0.747	0.222	3.36	0.001	2.11	1.37	3.26
Circumcision	1.126	0.284	3.97	0.000	3.08	1.77	5.38

Table 2: HCV associated risk factors involving social and educational background of the target population

Factors		HCV positive	HCV Negative	Odd ratio	95% CI	
					Lower Limit	Upper Limit
Risks associated with Social and educational background						
Gender	Male	49 (14.45%)	390	0.5615	0.3843	0.8203
	Female	96 (23.13%)	319			
Educational level	Educated	15 (6.02%)	234	0.1849	0.1057	0.3233
	Uneducated	130 (25.74%)	375			
Marital status	Married	131 (26.04%)	372	1.4107	0.7351	2.7072
	Unmarried	14 (5.57%)	237			
Health Care worker	Yes	0	2	0	0	0
	No	145 (19.28%)	609			
Shaving at barber shop	Yes	48 (15.09%)	270	0.6213	0.4244	0.9095
	No	97 (22.24%)	339			
Piercing of nose and ears	Yes	9 (22.8%)	314	3.647	1.501	8.8611
	No	52 (14.98%)	295			
Tattooing	Yes	4 (11.11%)	32	0.5115	0.178	1.47
	No	141 (19.63%)	577			
Risks associated with health practices						
Surgery	Yes	31 (23.13%)	101	1.3677	0.8714	2.1467
	No	114 (18.32%)	508			
Hospitalized	Yes	38 (20.43%)	148	1.1062	0.7313	1.6733
	No	107 (18.83%)	461			
Blood Donation	Yes	22 (24.17%)	69	1.3998	0.8336	2.3505
	No	123 (18.55%)	540			
Surgical stitches	Yes	51 (21.25%)	189	1.2057	0.8231	1.766
	No	94 (18.28%)	420			
Drug Abuse	Yes	1 (9%)	10	0.416	0.0528	3.2758
	No	144 (19.38%)	599			
Dental Surgery	Yes	61 (24.69%)	186	1.6515	1.1384	2.3959

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	No	84 (16.56%)	423			
Circumcision	Yes	40 (31.25%)	88	10.202	4.7463	21.9288
	No	9 (4.25%)	202			
Any chronic disease	Yes	4 (30.76%)	9	1.8913	0.5742	6.2292
	No	141 (19%)	600			
Life threatening disease	Yes	2 (66.6%)	1	9.0597	0.8155	100.6465

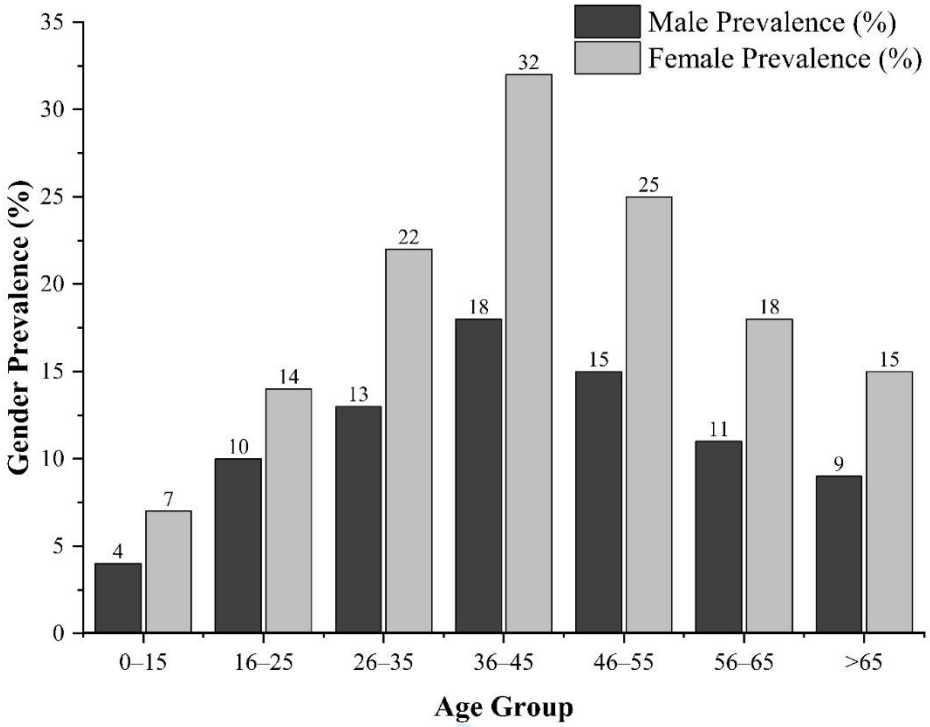

Figure 3. Comparison of HCV Prevalence Between Male and Female Participants

3.2 Caesarean section and HCV infection in the female population

Using Pearson Chi-Square statistics, we found that previous history of cesarean section was significantly associated with HCV with p<0.001 in females (with n=72, 17.3%). A total of 46 females had previous history of cesarean section during pregnancy; with 13 (28.26%) HCV infected individuals.

3.3 Piercing of body parts

In Indo-Pakistan, piercing of nose and ears is the traditional way for elaborate decoration of women and sometimes men as well. We found that piercing was a significant risk factor associated with HCV infection ($\chi^2 = 6.084$ with p=0.014). A total of 314 individuals has previous history of piercing of nose or ears (or both). Among them 9(22.8%) were found positive for HCV infection (Table 2).

3.4 Social factors associated with HCV infection

Stepwise binary logistic regression results (Table 2) indicated that marital status, education, positive family history of HCV infection, gender (male/female) and circumcision were found significantly associated with HCV infection. Surprisingly, we found an increased HCV prevalence of 26.04% (a total of 131 infected individuals out of 503) in married population while 5.57% (14 infected individuals out of 251) were unmarried. Reasons could be unprotected sex with the infected partner or possible house hold contact.

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3.5 Illiteracy and HCV infection

Education level of the individuals was found significantly associated with HCV in the studied population ($\chi^2 = 0.901$ with p=0.004) (Table 1). HCV prevalence of 25% was observed in illiterate individuals (Table 2) of the studied population as compared to 6% HCV infection rated in educated individuals. Out of 754 individuals, 249 were literate and 505 were illiterate. We reported a low literacy (33.02%) in underprivileged localities that could be associated with the lack of awareness about infectious diseases including HCV that results in the increased incidents of infectious diseases. We observed negligible literacy rate of 20.48% (85/415) among female population of the studied localities. A total of 28.18% (93 out of 330) illiterate females were positive for HCV infection. However, the HCV prevalence in educated females of the underprivileged population was low (3.52%; 3 females out of 85). These results indicated that literacy rate is one of the most important risk factors in spread of HCV in the underprivileged population.

3.6 Dental surgery is associated with HCV infection

Previous history of dental surgery and hepatitis (inflammation of liver) were significantly correlated with HCV infection. In the studied population, individuals with previous history of dental surgery have higher HCV infection of 24.69% as compared to 16.56% HCV in those people that did not go through dental surgery.

4. Discussion

The present study investigates HCV prevalence in most underprivileged population of Islamabad; capital of Pakistan.

Moreover, the results of our study demonstrated high HCV prevalence of 28.18% (93 out of 330) in illiterate females as compared to literate females (3.52%; 3 females out of 85). Most of these females were either house-wives or maids in the surrounding settled areas (Table 2). The inability to read and write (illiteracy) has been found associated with HCV infection as well as with associated risk factors like injection drug use, shaving at barber shop, age and gender of the individual. All of these factors are significantly associated with HCV prevalence in the apparently healthy individuals in the selected population.

Yousfani and colleagues [17] have reported a high HCV infection of 16.50% in pregnant women in Hyderabad region of the Pakistan. However, relatively lower rates of 3.27-4.80% HCV infection has been reported in pregnant women of Islamabad [18]. Ashraf and colleagues [13] recommended the use of disposable needles for piercing of nose and ears to avoid HCV and HBV infection. Contrasting results has been reported by Idrees and colleagues[5], reporting increased HCV prevalence in males (15.09%) as compared to females (12.3%) in apparently healthy population of the central Pakistan. Naoman and colleagues also reported a higher prevalence of HCV in male population of Peshawar, the capital city of Khyber Pakhtunkhwa province [19]. Generally males have higher response rates to anti-viral therapy (interferon plus ribavirin) as compared to females HCV infected patients [20] which can further affect the gender based variation in the HCV prevalence. We reported a high HCV prevalence in married people (26.04%) as compared to unmarried individuals (5.5%) (Table 2). A relatively low inter spousal transmission of 4.30% and 4.40% has been reported in Islamabad [21]. However, a relatively high incidence of 18% to 38% was observed in the couples living in Karachi [22]. The difference in the inter-spousal HCV transmission could be attributed to difference in socio-economic conditions and life-style of the concerned population. A study conducted in 2010 in the low socioeconomic regions of Islamabad reported relatively low prevalence of HCV [23]. In another study, Khan and co-workers reported 9% HCV prevalence and different risk factors in a hospital based study in Mardan, a city in the north of Pakistan [24]. We report a high prevalence of 19.23% infection (indicated by detection of serum HCV RNA) in the people living in the underprivileged localities of Islamabad. Previously, a low level of 4.34% HCV was reported in Mardan [25], 9% in Sindh province of Pakistan [26] and 5.31% in Islamabad [27]. Variable percent HCV prevalence has been reported in different districts of Punjab province of Pakistan i.e. Lahore (31.85%),

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Faisalabad (7.03%), Gujranwala (12.96%), Gujrat (10.65%), Sialkot (9.44%), Sargodha (6.03%), Mandibaha-ud-din (13.56%) and Jhang (8.64%)[28]. Studies conducted in Pakistan have reported history of reused syringes, transfusion of blood products, dental procedure, surgical operations and tattooing [29] and sexual contact with an intravenous drug user to be associated with HCV infection [30]. Another important source for the HCV infection could be unsafe injection use. Ministry of Health in Pakistan reported that more than 72% therapeutic injections and 50% immunization injections in public health care facilities are unsafe and could be a source of HCV transmission [31]. Another source of infections could be inappropriate disposal of medical wastes especially used syringes; which are collected by the rage pickers and sold to recycle these products. These rage pickers are mostly children of vulnerable groups like refugees, residents of the underprivileged localities, and homeless people; that sell these to the local dealers (Kabaris) to recycle it to other useful plastic products [32].

The present study reports high incidence of HCV infection in age-groups 36 to 45 years (Figure 4). A study by Muhammad and Jan (in 2005) reported that middle age group (40 to 50 years of age) has high incident of HCV in Buner area in the north of Pakistan [29]. However, Butt and colleagues[33] performed a comprehensive study of HCV prevalence in Pakistan and reported the highest percentage of 31.5% (6371 out of 20,552) fall in the age group 31-40 years. The high prevalence in certain age groups could be because of the common risk factors [34].

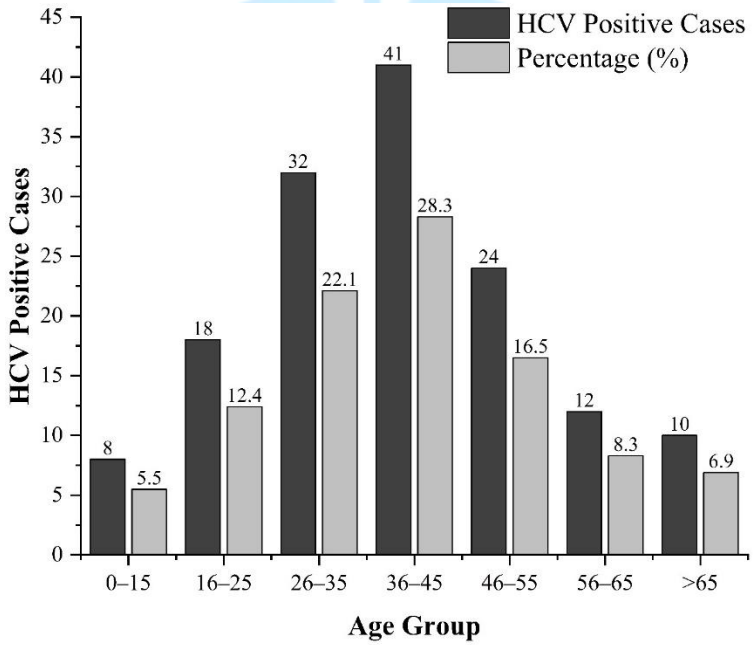

Figure 4. Age-specific Prevalence of HCV Infection.

HCV infection was most common in the 36–45 years age group, with comparatively lower prevalence observed in younger and older populations.

5. Conclusions

The current study is first of its kind to report prevalence of HCV in the underprivileged population of Islamabad. We reported high prevalence of HCV infection in age group of 36-45 years. Moreover, high incidence of HCV infections is reported in females of the underprivileged population of Islamabad. Awareness campaigns, early diagnosis and effective follow-ups are recommended with special emphasis on illiterate individuals of the underprivileged localities.

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6. Limitations of the study

The present study did not report information about viral loads and HCV genotyping. More detailed studies are recommended targeting underprivileged population of the capital city.

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Competing interests

The authors declare that they have no competing interests.

Ethical statement

The study has been approved by the departmental ethical committee of Qauid-i-Azam University Islamabad under the number *biotech-F-15-657*.

Authors' contribution

NI, AM and AZ conceived the study. AZ, NA, and AM collected samples and AZ performed the experimental work. AM, AK, and AZ analyzed the data and drafted the manuscript. NI and AM critically revised the manuscript. All the authors read, revised and approved the manuscript.

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