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EPIDEMIOLOGICAL INVESTIGATION OF HEPATITIS C IN EDUCATIONAL INSTITUTES OF QUETTA CITY, PAKISTAN

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Abstract

Hepatitis C virus (HCV) infection remains a significant global public health issue, with approximately 157 million cases reported worldwide, including 71 million chronic infections, according to the World Health Organization. This study investigates the prevalence of HCV among students in various educational institutions in Quetta, Balochistan. A total of 400 serum samples were initially screened using immunochromatographic test (ICT) kits, and positive cases were further confirmed through quantitative PCR (qPCR). The study analyzed a range of demographic variables, including age, gender, educational level, and cultural background, and assessed risk factors such as prior surgical procedures and blood transfusions. Results showed that 7 out of 400 samples (1.75%) tested positive via ICT, while 5 (1.25%) were confirmed by qPCR. Most participants (75%) were aged 15–25 years, with a higher proportion of females (250/400; 63%) compared to males (150/400; 37%). These findings emphasize the need for targeted awareness campaigns in educational institutions to promote hygiene and reduce the risk of HCV transmission.

Key Words: *Educational institutes, Hepatitis C, Prevalence, Quetta*

INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded, positive-sense RNA virus that causes chronic liver disease, cirrhosis, and hepatocellular carcinoma (liver cancer). It belongs to the Flaviviridae family and the Hepacivirus genus. Hepatocytes and B lymphocytes are the primary targets of HCV infection (1). The virus is transmitted through blood transfusions, injectable drug use, hemodialysis, organ transplantation, and sexual contact (2). HCV has six major genotypes, with genotype 1 being the most prevalent in America and genotype 3a increasingly reported in Europe. Given the wide geographic distribution, variable disease severity, and multiple transmission routes, comprehensive preventive and control measures are crucial to reduce its prevalence (3).

HCV contributes significantly to global morbidity and mortality, as over 90% of infected individuals are unaware of their condition and do not seek treatment (4). Many people remain uninformed about the increasing burden of infectious diseases, whereas individuals with higher education levels tend to be more aware. The high prevalence of HCV can be attributed to insufficient healthcare facilities, low socioeconomic status, and limited public knowledge regarding the transmission of major infectious diseases (5).

Globally, HCV affects approximately 2.8% of the population. The highest prevalence rates are observed in Asia, Europe, and Russia (6%–7%). In Africa, Nigeria reports infection rates between 3% and 9%, while Northern Europe shows rates below 1%, and Northern Africa exceeds 2.9%. The UK and Scandinavia report very low prevalence (0.1%), whereas Egypt has the highest rate globally, ranging from 15% to 20%. Despite being discovered in 1989, HCV remains a major global public health challenge (6).

In Pakistan, more than 10 million individuals are infected with HCV, contributing to high morbidity and mortality rates. The prevalence is estimated at 4.95% in adults, 1.72% in children, and 3.64% in youth. Among high-risk groups, 57% of injectable drug users and 48.67% of multi-transfused individuals are infected. Pakistan ranks 134th out of 174 countries in HCV prevalence, with particularly high rates in Sindh and Punjab, and about 2.5% in Balochistan. Rural areas are especially affected due to unsafe medical procedures and unregulated blood transfusions. In Sindh, the situation is alarming due to widespread use of unsterilized medical equipment and the reuse of syringes (7).

This study was designed to evaluate the seroprevalence of hepatitis C infection among school-going children in Quetta, Pakistan.

MATERIALS AND METHODS

This study was conducted to evaluate the prevalence of Hepatitis C virus (HCV) in selected educational institutions of Quetta, Balochistan, from August to November 2024. A total of 400 serum samples were initially screened using Immuno-Chromatographic Test (ICT) kits. Samples testing positive were subsequently confirmed by quantitative Polymerase Chain Reaction (qPCR).

STUDY POPULATION

The target population included a diverse group of individuals aged 15 to 60 years from both genders. Participants were recruited from five educational institutions, including three colleges—Government Girls College Quetta Cantt, Jinnah Town College Quetta, and Kechibagh College Quetta—and two private academies—Nawaar Institute of Public Services and Students Academy, Quetta. The study population consisted of students, faculty members, and administrative staff.

QUESTIONNAIRE ADMINISTRATION

A structured questionnaire was employed to collect demographic data and evaluate potential risk factors contributing to HCV infection. Information was gathered on variables such as age, gender, education, language, medical history, geographical background, chronic illnesses, and residential setting.

PROCEDURE FOR IMMUNO-CHROMATOGRAPHY TEST (ICT)

The ICT method is a rapid and user-friendly test providing results within 20 minutes. After sterilizing the fingertip, blood was collected using a lancet; the first drop was discarded, and two drops were applied to the ICT cassette. Results were read after 15–20 minutes based on visible lines at the 'T' (test) and 'C' (control) regions (8).

TEST PROCEDURE FOR PCR

SERUM EXTRACTION

Blood samples were centrifuged at 3000 rpm for 3 minutes to extract serum, which was transferred into 1.5 mL Eppendorf tubes under a Class II biosafety cabinet. The serum was stored at –20 °C for further analysis.

NUCLEIC ACID EXTRACTION

All components of the extraction kit were mixed by inverting a 96-well plate and allowing it to stand for 3 minutes. To each designated well (AI-HI and A7-H7), 15 µL of proteinase K and 10 µL of internal control (IC) were added. Then, 200 µL of each serum sample, along with positive and negative controls and four standards, were added in a specific order. The plate was placed into a Zbio-B-200 nucleic acid isolation system for 9–10 minutes. Afterward, nucleic acid extracts from A6-H6 and A12-H12 were transferred into 1.5 mL PCR tubes (9).

HEPATITIS C VIRUS qPCR TEST

Quantitative real-time PCR was conducted using an HCV RNA quantification kit. The assay is based on reverse transcription PCR combined with real-time fluorescence detection, targeting conserved

regions of the HCV genome. Fluorescent signals are generated upon separation of reporter and quencher dyes. An internal control (IC) was included to monitor reaction efficacy and rule out false-negative results. Fluorescein amidite (FAM) dye was used for HCV RNA detection, while HEX (hexachlorofluorescein) dye detected the IC. Standards were processed concurrently with samples to quantify viral RNA (10).

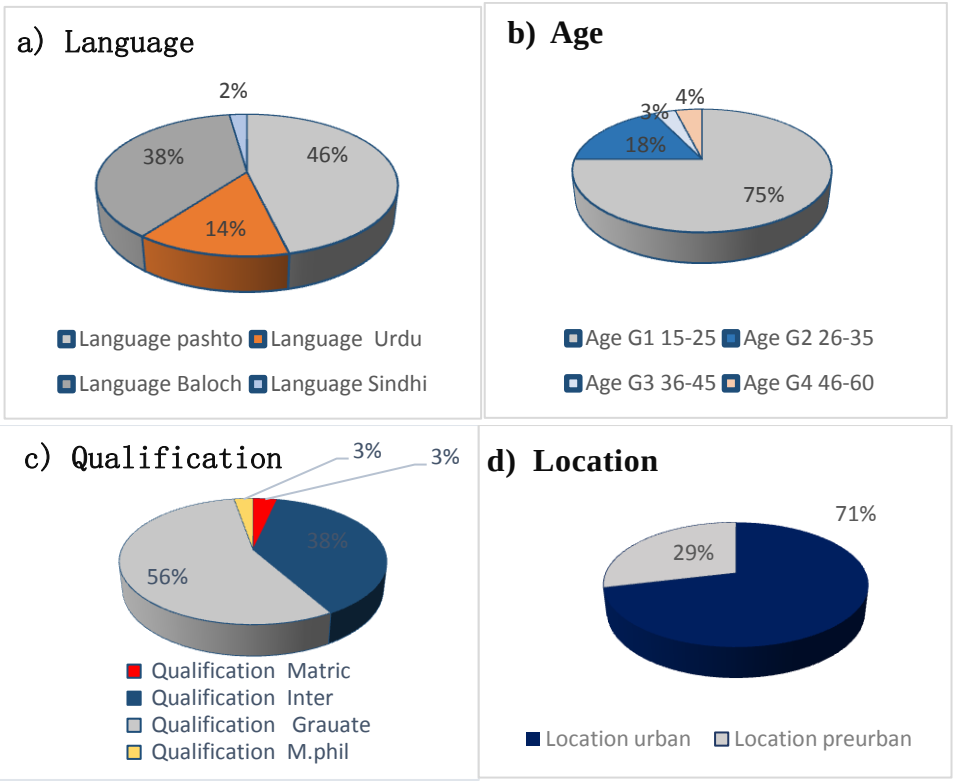
RESULTS

A total of 400 samples were collected from students and staff at various colleges in Quetta, representing diverse cultures and languages, 9 (2%) Sindhi, 185 (46%) Pashto, 56 (14%) Urdu speaking, and 150 (38%) were Balochi. Most participants 300 (75%) were aged 15-25 years, with 71 (18%) in the 26-35 Years range, 13 (3%) aged 36-45 years, and 16 (4%) aged 46-60 years. Educationally, 14 (3%) were in matric, 153 (38%) in intermediate, 222 (22%) were graduates, and 11 (3%) were postgraduates. The participants included 150 (37%) males and 250 (63%) females, with 285 (71%) from urban areas. Health issues noted included 06 participants with surgical treatment (2 of whom had blood transfusions), one undergoing dialysis, two with thyroid disorders, three with diabetes or high blood pressure, and one with allergies. Additionally, 1% reported with family history of HCV (Table I).

Table I. Demographic data of participants

Languages		**Age**		**Educational level**		**Location**	
Pashto	185	15-25	300	Matric	14	Urban	
Urdu	56	26-35	71	Inter	153	285	
Balochi	150	36-45	13	Graduate	222	Periurban	
Sindhi	9	46-60	16	Postgraduate	11	115	
Total	400	Total	400	Total	400	Total	400
Gender		**Blood transfusion**		**Chronic disease**		**Positive family members**	
Male	150	Male	1	Male	2	Male	0
Female	250	Female	7	Female	6	Female	4
Total	400	Total	8	Total	8	Total	4

Table I highlights a variety of demographics presenting, languages, age, educational attainment, location, gender, blood transfusion, health issues, and HCV positive family background. This encompasses people of all ages and educational backgrounds who speak Pashto, Urdu, Balochi, and Sindhi.



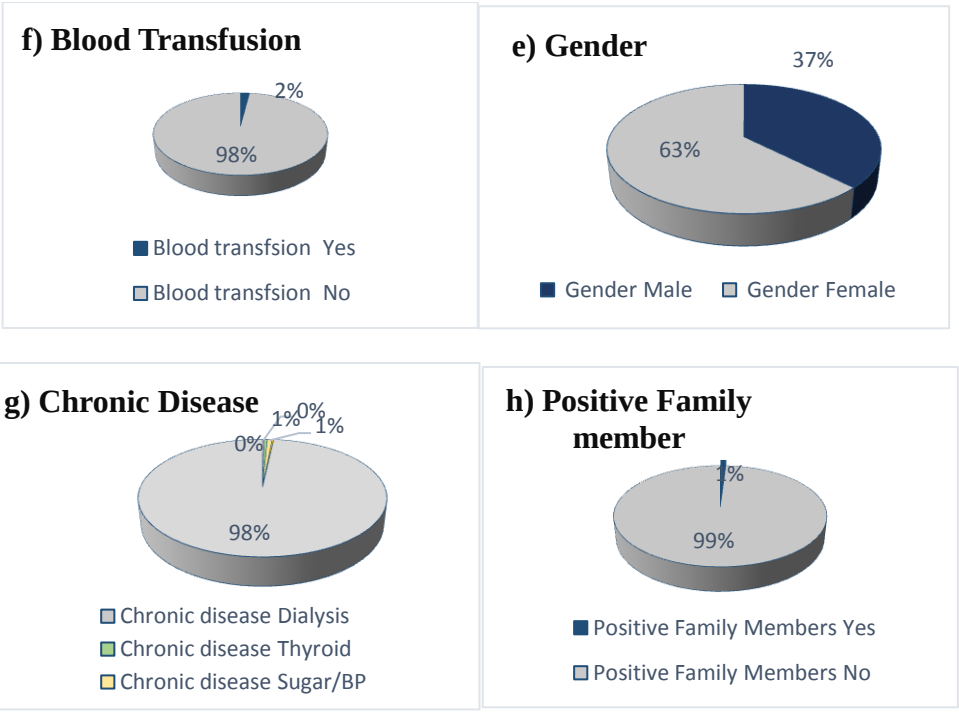


Fig.1. (a) Most participants spoke Pashto, Balochi was second. (b) 75% were aged 15-25 years, with fewer in older age groups. (c) Half held a Graduate degree, 38% were Intermediate, and 3% had postgaduate or Matric. (d) 29% lived in urban areas. (e) Nearly two-thirds were female. (f) 2% had received blood transfusions, (g) 2% had chronic illnesses, (h) and 1% tested HCV positive

Out of 400 samples, Abbott yielded 3 positives and Cassette 4 positives, including 2 males and 5 females. Among them, 2 females had surgical treatment and blood transfusions, while 1 male and 1 female had a family history of HCV. Out of 07 ICT positives, 5 (1.25%) tested positive for HCV via qPCR. Two ICT (Cassette) kit samples were negative compared to qPCR results (Table II).

Table II. Clinical characteristics and demographic data of HCV positive participants

Gender			**Surgical Treatment**		**Blood Transfusion**		**Positive Family Members**	
	Positive	Negative	Yes	No	Yes	No	Yes	No
Male	2	148	0	2	0	2	1	1
Female	5	245	2	3	2	0	1	4
Total	7	393	2	5	2	2	2	5
Chronic Disease					**Location**		**ICT Test**	
Dialysis	Thyroid	Sugar/BP	Allergy	Nil	Urban	Periurban	Positive	Negative
1	2	3	1	393	3	4	7	393
qPCR Test								
Positive					Negative			
5					395			

Table II breaks down gender-specific information, surgical procedures, blood transfusions, and family medical history, also highlights health characteristics, along with geography distribution followed by ICT and qPCR.

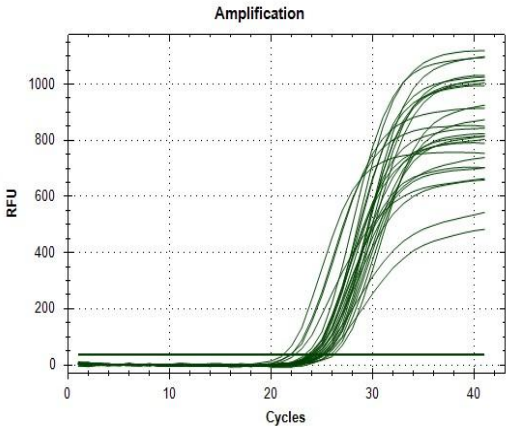


Fig. 2. Negative samples with Internal control

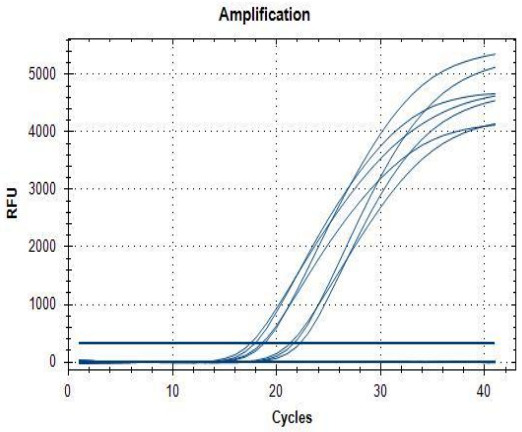


Fig. 3. Positive samples with positive controls

Fig. 2 shows all negative samples with IC, on y-axis RFU (relative fluorescence units) continuously monitors the amplification of HCV DNA. Fluorescence released with each DNA copy, so amount of fluorescence is directly proportional to quantity of DNA. Number of cycles are on x axis, curve produce at initial phase below the detection line which is called Ct value and Ct value of promoter kits was <30, while Fig. 3 shows positive samples with positive control.

Comparing the amounts of viral DNA by observing their Ct values. An elevated positive control in the cycle count shows intense fluorescence, indicating a high quantity of HCV DNA. Fluorescence exceeds a threshold at Ct value, essential for data assessment Fig. 2.

In Fig. 3 the amplification curves for five positive samples rise above the baseline, indicating effective detection of HCV DNA, and their Ct values reflect the amounts of viral DNA, early fluorescence signifying high quantities.

Table III. Rate of HCV infection by age group using ICT and PCR

Age group	Sample size	Positive by ICT	Percentage	Positive by PCR	Percentage
15-25	300	4	1%	3	1%
26-35	71	1	1.40%	1	0.14%
36-45	13	0	0.00%	0	0%
46-60	16	1	6.25%	1	6.25%
Total	400	7	1.75%	5	1.25%

Table III shows the ICT and qPCR test positivity rates in 400 participants, displaying the various age ranges by quantity of positive instances with percentages that correlate with them

DISCUSSION

WHO classifies that nations with HCV prevalence >8% is considered high, 2–8% is considered intermediate, and less than 2% is considered low (12). Regions with high Prevalence include Rwanda 16% in 55 years old population (13), Nigeria overall seroprevalence was 4.67% higher rates in males (5.18%) and the 50 –57 age group in blood donors (14). China and India 1%-5% prevalence (15). Northern Africa 2.9%. Low prevalence regions Saudi Arabia 0.32% (16). Iraq 0.12%. United Kingdom 0.06%-0.1%. Brazil 0.28%-0.7%. Turkey 0.46%. In Ethiopia general population HCV ratio is 0.2%, but in blood donors 4.3% (17), Iran (0.7%), Afghanistan (0.82%) (18). The United Kingdom 0.06%, and Turkey 0.46% (19). In US between 2.7 and 3.9 percent of people are thought to have a persistent HCV infection (20).

Pakistan 4.8% national average, Punjab and Sindh have >5%, with Khyber Pakhtunkhwa at 5.9%. Balochistan 2.5%. Pediatric population 1.72%, adult Population 4.95%, youth 3.64%. Healthcare professionals and dialysis Patients 5%. Blood transfusion recipients 49.17%(21). Injecting drug users (IDUs) have highest prevalence 50%-60%. Rawalpindi in transgender population HCV prevalence was 34.61% (22). KPK province recorded 5.9%, which is much higher throughout country, while Peshawar district 2.22% (23). According BMC Hospital and SPC Hospital Quetta in Balochistan among pregnant women HCV ratio is 0.6% (24). While blood donors in Pakistan overall HCV prevalence was 2.71%. Regional variations were observed Punjab had highest prevalence (3.94%) and Balochistan the lowest (0.87%).

Compared to previous studies in Pakistan, particularly in Balochistan, HCV prevalence in educational institutions is relatively low at 1.25%. This may reflect differences between the general population and educational settings. Similarly, Khan et al. (2018) found a 1.8% HCV prevalence among college students in Peshawar, aligning more closely with these findings (25). WHO reports that students faced fewer HCV risk factors, such as intravenous drug use and risky medical procedures. Lower transmission rates may stem from enhanced health education initiatives in these settings. Among Punjab university students, HCV prevalence was 4.3%, over three times higher than in this study, which found a prevalence of 1.5%, consistent with Ahmed et al. (2017) using ELISA and PCR for blood donors (26). High prevalence 1.7% was reported in Quetta using ICT and PCR (27). kech 4.3%, (28). Between 2015 and 2022, approximate 25.7 million individuals were diagnosed with HCV infection, 12.5 million receiving direct antiviral treatment. Despite significant progress since 2016 towards eradicating hepatitis, urgent global

action is needed to improve access to hepatitis services, especially in high-prevalence countries, to meet the 2030 goals (29).

This study aims to determine the frequency of Hepatitis C Virus infection in Quetta's educational institutions and assess the performance of ICT kits against qPCR. Both methods were used, but ICT kit yielded two false positives, possibly due to other viral interactions, presence of antibodies, or issues with kit quality. ICT kits detect antibodies, which may not be present in early infections and cannot differentiate between acute and chronic phases it provides qualitative results qPCR can identify trace levels of viral RNA, making it more effective for early diagnosis, also measures viral load, which is crucial for monitoring disease progression and treatment efficacy. PCR is essential for confirming HCV infection (30).

CONCLUSION

In conclusion, this study highlights the seroprevalence of hepatitis C infection in educational institutes in Quetta, Pakistan. The infection recorded were more in female than male and more productive 15-25 years age group were more vulnerable. Other chronic diseases, surgical procedures, blood transfusion and rural/urban life style were the most common risk factors findings in positive patients. This emphasizes how common is the HCV infection in educational institutions of Quetta, Balochistan and need stern action to educate the society and adopt hygienic measures to ease the health care burden in the area.

Conflict of interest:

The authors declare no conflict of interest.

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