

# Prevalence, Genotypic Distribution and the Associated Risk Factors of Hepatitis C Infection in Pakistan Pediatric Patients

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## Abstract

Hepatitis C virus (HCV) is an important contributor to chronic morbidity and mortality in developing countries. The study's objective was to determine the genotype distribution and risk factors associated with the transmission of HCV infections in pediatric patients. Rapid screening and confirmation by the enzyme-linked immunosorbent assay (ELISA) were used to analyze 585 pediatric blood specimens hospitalized and visited the outpatient department of the largest tertiary care hospital in Pakistan. Detection and genotyping of HCV RNA were performed using a real-time polymerase chain reaction (RT-PCR). Demographic data and a history of risk factors were gathered through a survey questionnaire. HCV RNA was detected in 323 (72.4%) cases which showed viral load ranging from  $\text{Log}_{10}$  IU/mL  $< 3$  to  $> 6$  IU/mL. HCV genotype 3a was detected in 256 (79.3%) cases while type 3b and 1a was observed in 36 (11.1%) and 31 (9.6%) patients, respectively. HCV positivity was significantly associated with the cases from rural areas [ $p = 0.005$ ; odds ratio (OR) 1.65; 95% CI 1.16-2.23] and also significantly associated with low-income group [ $p < 0.001$ ; OR 5.75; 95% CI 3.90-8.40]. The primary risk factors associated with HCV transmission in children were family history ( $p = 0.002$ ), blood transfusion ( $p = 0.03$ ), surgical procedures ( $p = 0.02$ ), and history of injections ( $p = 0.05$ ). HCV genotype 3a is the most common genotype in children. The main risk factors for HCV transmission in children are blood transfusion, surgical procedures, and injection practices by informal health care providers.

**Keywords:** Hepatitis C virus, Genotype 3, Risk factors, HCV transmission, Children

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## INTRODUCTION

Viral hepatitis is a global health issue that recently has acquired considerable recognition and significance, given the enactment of the World Health Organization (WHO) elimination strategies.<sup>1</sup> Hepatitis C virus (HCV) infection has emerged as a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. HCV was categorized as a distinct entity in 1989 and considered discrete hepatitis that gradually develops cirrhosis.<sup>2</sup> The virus is not only responsible for liver problems but also lymphadenopathies.<sup>3</sup> HCV is a blood-borne infection transmitted through reused needles, contaminated equipment, and blood transfusion, which chronically affects nearly 71 million humans worldwide.<sup>1</sup> Until 2010, 40% of blood transfusions had no adequate screening facility for infectious diseases in Pakistan.<sup>4</sup> The incidence of HCV infections is comparatively low in economically developed countries.<sup>5</sup> Inadequate health care facilities, unsuitable screening facilities, low literacy rate, low socioeconomic status, lack of awareness on prevention and control measures contribute to the higher incidence of HCV in developing countries.<sup>6</sup>

The prevalence of HCV in pediatric patients has been reported as 3.26 million. Pakistani, Chinese, Indian, and Nigerian children account for more than 50% of the world's HCV pediatric cases.<sup>7</sup> HCV infections are predominantly transmitted to children horizontally; however, the vertical transmission route cannot be excluded.<sup>8</sup> Unhygienic syringes, intravenous drug abuse, barbers, sharing of razors, skin piercing needles, and toothbrushes are the primary horizontal transmission routes in Pakistani adults.<sup>9</sup> Pediatric transmission is mostly associated with the infected mother and children with congenital disorders who receive blood transfusions.<sup>10</sup> The rate of HCV infections is tremendously higher in children suffering from thalassemia-major.<sup>11</sup> Breastfeeding has no significant role in the transmission of HCV infection unless the nipples of the infected mother bleed and the blood mix up directly to the infant's blood.<sup>12</sup>

HCV infections are prevalent worldwide, highest in Egypt and the WHO Eastern Mediterranean Region.<sup>1,13</sup> Developed countries such as Australia, America, and Europe are comparatively less affected due to better facilities

and public awareness of transmission sources.<sup>14</sup> Nevertheless, in many parts of the world, HCV testing rates and care are still low.<sup>15</sup> Pakistan is considered to be among the countries with a higher HCV infection rate. Notably, Northern areas have been reported with the highest HCV infection rate in the tested population.<sup>6</sup> At least six major genotypes have been identified worldwide; a remarkable HCV feature in diagnosis, disease complications, treatment plan, and vaccine manufacturing.<sup>3</sup>

There is no effective HCV vaccine, and only preventive measures could decrease disease incidence.<sup>16</sup> Evaluation of HCV infection involves diagnosing HCV in patients by using serological and molecular testing; furthermore, it also aids in evaluating the severity of hepatic diseases. Nowadays, real-time polymerase chain reaction (PCR) is the most widely used diagnostic marker for hepatitis infection with excellent sensitivity.<sup>17,18</sup> Limited data is available on the prevalence of HCV infections, genotypic distribution, and associated risk factors in pediatric patients emphasized to carry out the current study.

## MATERIALS AND METHODS

### Specimen collection

The study was conducted on 585 pediatric patients admitted or visited the outpatient department of the largest tertiary care public sector hospital of Lahore, Pakistan, following ethics approval (Ref. No. 1040/1041). Well-trained nurses and phlebotomists collected 3-4 mL of the whole blood in a silica gel vacutainer and transported it to the laboratory for serum separation. Samples were collected consecutively from children under 15 years old.

### Data collection

The demographic information was collected as a part of confidential questionnaires following informed patients' consent. The socioeconomic data was scaled as high, middle, and low.<sup>19</sup> History of the potential HCV transmission risk factors recorded from the parents includes family history, invasive medical and dental procedures, blood transfusions, toothbrushes, circumcision, and ear piercing.

### Serological diagnosis

A rapid immunochromatography (ICT)

technique was used to detect HCV antibodies' presence using the single-stage HCV test device (AccuQuik™ Test Kit, Cheyenne USA) using the patient's serum. Screening positive samples were confirmed by the Enzyme-Linked Immunosorbent Assay (ELISA)<sup>20</sup> tests (Southern Biotech ELISA Kit, Birmingham USA). Only positive HCV antibody specimens were quantified using the ELISA technique included in the study.

**Real-time Polymerase Chain Reaction (RT-PCR)**

QIAamp DSP Virus Spin Kit (QIAGEN) has been used to extract the genome from HCV ELISA positive samples from silica-membrane technology. HCV RNA amplified and quantitated by the artus® HCV RG RT-PCR Kit (QIAGEN) on Rotor-Gene Q (QIAGEN). All the QIAGEN kits and instruments were purchased from Hilden, Germany. The quantification of HCV IU/mL RNA was evaluated using four standards, and fluorescence signals greater than the cut-off value were found to be positive (Fig. 1a). Internal control was amplified through testing, and each lot included positive and negative HCV RNA control. Genotyping

RT-PCR positive cases were processed to detect HCV genotype from the extract RNA using AmpliSens® HCV-genotype-FRT (Moscow, Russia). Internal control was used to amplify each sample, and positive and negative controls were included with each tested batch (Fig. 1b).

**Statistical Analysis**

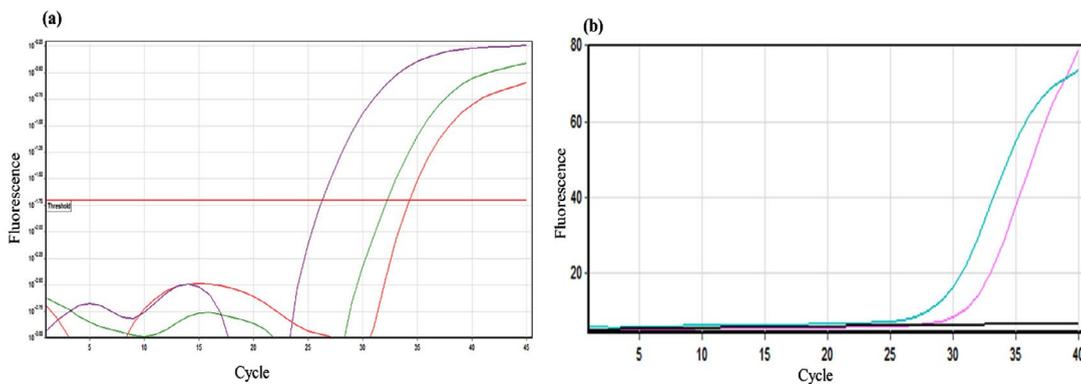
The data was analyzed (SPSS ver. 26.0) using binary logistical regression analysis, and

odds ratios (OR) with 95% confidence intervals (CI) were calculated. Variables with a p-value of < 0.10 were further analyzed by multivariate logistic regression analysis to predict independent variables associated with HCV infection. A p-value < 0.05 was considered statistically significant.

**RESULTS**

There were 585 HCV antibody-positive cases screened using ICT, of which antibodies were detected from 446 (76.2%) cases based on ELISA. Anti HCV ELISA positive cases were treated for final confirmation using RT-PCR, which detected HCV RNA in 323 (72.4%) cases.

The HCV RT-PCR positive patients' demographic information showed that 175 (54.1%) males and 148 (45.8%) females were infected. However, no significant association of HCV incidence with the gender of the patients was observed (p = 0.22). Majority of the cases (205, 63.5%) were detected in children > 10 years of age which was statistically insignificant (p = 0.07; OR=1.33 and 95% CI 0.97-1.83). A statistically significant association (p = 0.005) was observed in rural patients compared to urban areas (58.8% vs. 41.1%) with OR 1.65 and 95% CI 1.16-2.23. HCV positive cases were also significantly associated (p < 0.001) with the low-income group with OR 5.75 and 95% CI 3.90-8.40. Results of multivariate analysis also showed significant association of low-income group (p = 0.008; OR=1.41 and 95% CI 1.15-2.72) and rural area (p = 0.02; OR=1.78 and 95% CI 1.09-2.93) with HCV infection (Table 1). The highest



**Fig. 1.** (a) Negative control and patients' samples (below threshold). Positive control and HCV RNA positive cases (above threshold) are shown in a higher degree of fluorescence. (b) Fluorescence peak of a positive HCV genotype 1a in a patient sample.

**Table 1.** Demographic characteristics of HCV patients enrolled in the study (n = 585)

Demographics	HCV Positive (n = 323)	HCV Negative (n = 262)	Binary logistic analysis		Multivariate logistic analysis	
			p-value	OR (95% CI)	p-value	OR (95% CI)
<b>Sex</b>						
Male	175 (54.1%)	156 (59.5%)	0.22	1.24 (0.90-1.3)	0.57	0.97 (0.60-1.34)
Female	148 (45.8%)	106 (40.4%)				
<b>Age groups (Years)</b>						
<10 years	118 (36.5%)	109 (41.60%)	0.07	1.33 (0.97-1.83)	0.57	0.97 (0.60-1.34)
>10 years	205 (63.4%)	153 (57.2%)				
<b>Residence</b>						
Rural area	190 (58.8%)	184 (70.2%)	0.005	1.65 (1.16-2.23)	0.008	1.41 (1.15-2.72)
Urban area	133 (41.1%)	78 (29.7%)				
<b>Socioeconomic status</b>						
Low	186 (57.5%)	50 (19.0%)	< 0.001	5.75 (3.90-8.40)	0.02	1.78 (1.09-2.93)
Middle	95 (29.4%)	155 (59.2%)				
High	42 (13%)	57 (21.8%)				

**Table 2.** Risk factor associated with the transmission of HCV infection

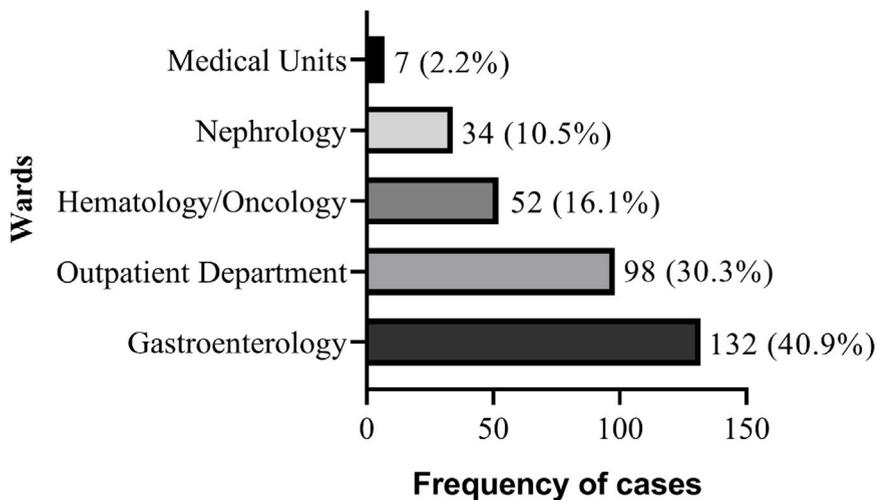
Risk Factors	HCV Positive (n = 323)	HCV Negative (n = 262)	Binary logistic analysis		Multivariate logistic analysis	
			p-value	OR (95% CI)	p-value	OR (95% CI)
<b>History of HCV infection in Family</b>						
Yes	25 (7.7%)	5 (1.9%)	0.002	4.31 (1.6-11.42)	0.01	3.75 (1.25-11.25)
No	298 (92.2%)	257 (98.9%)				
<b>Blood transfusion</b>						
Yes	57 (17.6%)	29 (11.0%)	0.03	1.24 (1.06-2.78)	0.93	1.02 (0.55-1.89)
No	266 (82.3%)	233 (88.9%)				
<b>Catheterization</b>						
Yes	30 (9.2%)	27 (10.3%)	0.77	0.91 (0.51-1.54)	0.15	1.39 (0.88-2.21)
No	293 (90.7%)	235 (89.6%)				
<b>Surgical procedures</b>						
Yes	217 (64.0%)	151 (57.6%)	0.02	1.50 (1.02-1.32)	0.15	1.39 (0.88-2.21)
No	106 (35.9%)	111 (42.3%)				
<b>Male circumcision</b>						
Yes	133 (41.1%)	90 (34.3%)	0.1	1.01 (0.95-1.80)	0.91	1.02 (0.60-1.57)
No	190 (58.8%)	172 (65.6%)				
<b>Female ear piercing</b>						
Yes	35 (10.8%)	41 (15.6%)	0.12	0.65 (0.40-1.06)	0.91	1.02 (0.60-1.57)
No	288 (89.1%)	221 (84.3%)				
<b>Injection's history</b>						
Both formal and informal healthcare provider	153 (44.2%)	103 (39.3%)	0.05	1.38 (1.01-1.45)	0.91	1.02 (0.60-1.57)
Formal health care provider	170 (55.7%)	159 (60.6%)				

numbers of HCV RT-PCR positive cases were 132 (40.9%), isolated from the gastroenterology, and 98 (30.0%) from the outpatient department. Other HCV cases belonged to hematology/ oncology (52; 16.1%) and nephrology (34; 10.5%), while few cases (7; 2.2%) were detected from medical units (Fig. 2).

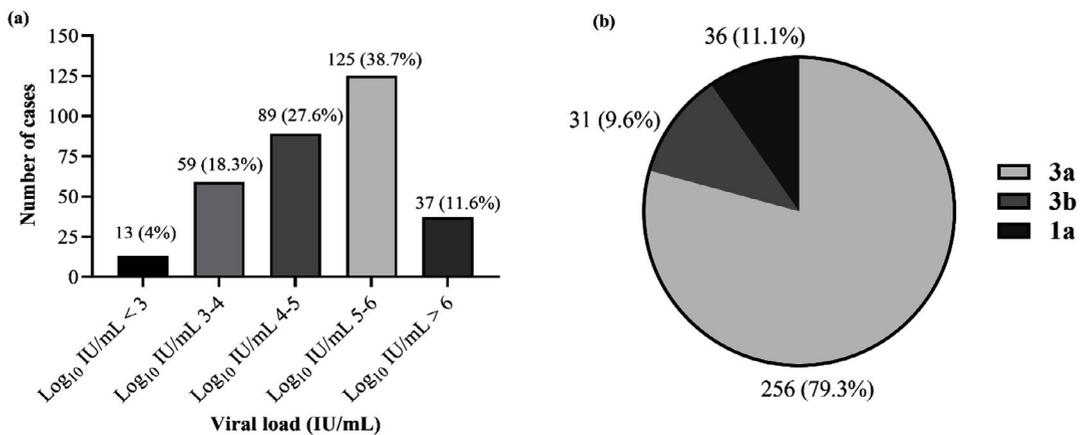
HCV viral load  $\text{Log}_{10}$  IU/mL > 5-6 was observed in 125 (38.7%) cases followed by  $\text{Log}_{10}$  IU/mL 4-5 in 89 (27.6%),  $\text{Log}_{10}$  IU/mL 3-4 in 59 (18.3%) and  $\text{Log}_{10}$  IU/mL < 3 in 13 (4%) patients (Fig. 3a). There were 37 (11.5%) patients who had  $\text{Log}_{10}$  IU/mL > 6 serum viral load. HCV genotype 3a was detected in 256 (79.3%) cases, 3b in 36 (11.1%)

patients while 31 (9.6%) cases had genotype 1a (Fig. 3b).

The analysis of various risk factors showed a significant association of HCV transmission with the family history ( $p = 0.002$ ), blood transfusion ( $p = 0.03$ ), surgical procedures (0.02), and history of injections (0.05). The other risk factors in our study include catheterization ( $p = 0.77$ ), male circumcision ( $p = 0.1$ ) and female ear piercing ( $p = 0.12$ ) had no significant association. Multivariate analysis revealed a significant association of family history of HCV infection with the disease ( $p = 0.01$ ). All other risk factors exhibited a non-significant association (Table 2).



**Fig. 2.** Distribution of HCV confirmed cases in different wards (n = 323).



**Fig. 3.** (a) HCV viral load (IU/mL) among the pediatric HCV confirmed cases (n = 323). (b) Prevalence of HCV genotypes among positive cases.

## DISCUSSION

HCV is a major global health concern, and its paradigm varies from region to region based on its historical and present risk factors. Chronic hepatitis C virus (HCV) infection affects more than 71 million people worldwide, mostly due to its predisposition to hepatitis C fibrosis, cirrhosis, and liver cancer. HCV prevalence is highest in Mongolian children (1.74%), followed by 1.02% HCV affected Pakistani children.<sup>7</sup> Pediatric HCV prevalence in Pakistani Punjab has been reported as 1.88%.<sup>21</sup> This study represents the prevalence of HCV infection among children, different genotypes, and associated risk factors. Among 323 HCV-positive children, the male gender has a higher which is in (54.1%) in comparison to females (45.9%), which is in accordance with some previously reported studies.<sup>6,22</sup> We found more number (63.4%) of HCV-positive pediatric cases in the age group of >10 years that is similar to a work performed in Brazil.<sup>23</sup> Age has been argued to be a major factor in HCV studies, with infection more predominant in older people.<sup>24</sup>

Our study reports 79.3% prevalence of genotype 3a, 11.1% 3b and 9.6% type 1a. HCV type 3a has been reported to be the most prevalent genotype.<sup>25,26</sup> Genotype 3 is also frequently reported in adult patients in Pakistan.<sup>27</sup> These findings are also supported by two other studies in which genotype 3 was reported as 72.9% and 89%.<sup>22,28</sup> However, HCV genotype 1b was reported in Mankidia and Juanga tribes in India.<sup>29</sup> We observed that most of the HCV-positive cases belong to rural areas. The contributing factors in HCV transmission were lack of awareness, low literacy rate, socioeconomic barriers, and improper screening facilities. The Pakistani community's limited knowledge about the sources responsible for HCV transmission is an important reported risk factor.

The primary contributing factor in HCV infection transmission among pediatric patients is blood products' transfusion.<sup>11</sup> Blood transfusion practices remain a challenge in developing countries due to the absence of standardized infrastructures, a permanent energy source, well trained and equipped workers. The crucial factor in implanting a healthy transfusion is to ensure the blood donor's accurate screening.<sup>9</sup> The most significant risk factors among HCV-positive children

observed in the current study were surgical intervention and blood transfusion.<sup>30,31</sup> This higher rate of HCV transmission is due to the lack of public awareness of the virus's spread and the use of non-sterilized syringes.<sup>9</sup> The use of invasive procedures and blood transfusion remained a substantial HCV transmission source in Cambodia.<sup>32</sup>

The logistic regression analysis showed a significant association among children with blood transfusion history who had HCV infection compared to the anti-HCV negative children ( $p = 0.03$ ). Two previous studies from Pakistan agree with the current findings, according to which blood transfusion, unsafe dental practices, and non-sterile procedures are the main factors of HCV acquisition.<sup>9,33</sup> More research is needed with more powered sample sizes, diverse ethnicities, and a high number of individuals infected with different HCV genotypes.<sup>34</sup> Our study highlights the dire need for intervention programs; awareness campaigns should be appropriately focused. By ensuring safe blood supplies and healthcare-related procedures should be the highest priority. The study's limitation includes considering cases admitted only in a tertiary care hospital, whereas chronic hepatitis patients are also brought to the district and sub-divisional hospitals. Moreover, this is a single-center study probably with biased selection and data confined to Pakistan and cannot be extrapolated to other countries.

## CONCLUSION

The study's findings could play a vital role in strengthening public health initiatives. HCV genotype 3a is predominant among pediatric HCV-infected patients. HCV viral load varies from  $\text{Log}_{10} < 3$  to  $> 6$  IU/mL among the infected children. Information on transmission, risk factors, and mortality from HCV infection is of particular importance for rural communities and populations with low socioeconomic status. The assessment of different risk factors, including blood transfusion, surgical procedures, and injection practice from informal healthcare providers, is the key contributing factor in disseminating HCV infections. It is a dire need to implement infection control preventive strategies and treat pediatric patients with child-specific regimens to minimize HCV cases.

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## CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

All authors have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## FUNDING

None .

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

The study was approved by the Ethics Committee of SAHS, The Children's Hospital, Lahore, Pakistan (Ref. No. 1040/1041).

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