



## Estimation of Transfusion Transmissible Infections in Blood Donors of District Peshawar: A Cross-Sectional Study

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### ABSTRACT

**Background:** Blood transfusion is ascertained as a life-saving practice, globally saving millions of lives per year. Transfusion transmissible infection (TTI) is any infection transferred from donor to recipient by blood or blood product through parenteral administration.

Due to unsafe transfusion, acute clinical illness, asymptomatic infection or persistent carriers of any infection are the most commonly observed outcomes.

**Objective:** The current study aimed to estimate the seroprevalence of TTIs in blood donors.

**Methodology:** This was a cross-sectional study conducted at New Bangash Clinical Laboratory, Haideri Blood Bank & Welfare Hospital, in collaboration with the Microbiology department of Comwave Institute, Islamabad. Blood donors' serum samples were screened through an ICT kit, followed by confirmation by ELISA (enzyme-linked immunosorbent assay) for hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV).

**Results:** In the current study duration total of 380 donors were visited and tested for TTIs, among which 291/380 (77%) were males, and the remaining 89/380 (23%) were females belonging to various age groups. Among the total 50/380 (13.15%) donors were found positive for HbsAg, 20/380 (5.2%) were positive for Anti-HCV and 2/380 (0.53%) showed co-Infection upon ELISA screening. The current study also finds that the urban population was more infected by transfusion-transmitted infections than the rural population. Co-infection prevalence is also found to be high in the urban population.

**Conclusion:** Transfusion-transmitted infections are also highly prevalent in District Peshawar. Individual hepatitis B and C viral infection, as well as coinfection in a few samples, were also found in the current study.

## **Introduction**

Hepatitis represents liver disease or infection, which may be illustrated by the presence of infected cells in liver tissues.<sup>1</sup> The infection caused by hepatitis viruses includes Hepatitis (HAV),(HBV), (HCV), (HDV), (HEV) and (HGV).<sup>2</sup> Hepatitis risk factors include drinking alcohol, use of drugs in large amounts, autoimmune diseases and metabolic disorders.<sup>3,4</sup> Hepatitis B virus-based (HBV) infections have been demonstrated to be the major cause of morbidity worldwide, estimated as 350 million people worldwide.<sup>5</sup>, while worldwide morbidity related to HCV is estimated to be 71 million people worldwide.<sup>6</sup> Public health measures are focused on the prevention of infections and the reduction of hepatitis associated morbidity and mortality worldwide. Similarly, success has also been achieved in preventing the transmission of HBV in newborns by the administration of HBV vaccines and immunoglobulin to mothers, but this strategy still needs to be improved.<sup>7</sup>

According to some estimates, 3 billion people are exposed to HBV infection all over the world, while HBV induces a variety of hepatic diseases, from acute or long-standing hepatitis to hepatic cirrhosis and hepatoma.<sup>8,9</sup> Hepatitis management is easier in the earlier stages but becomes complicated over time, as the disease advances. Among the eight genotypes of hepatitis B virus (A–H) reported, not much is known about the incidence of HBV genotypes. The genotype B and genotype C are the most common in the biggest continent (Asia), but it has also been suggested that genotype D is the principal, and represents an important health problem in Pakistan.<sup>6,10</sup> According to the World Health Organisation, it estimates 118.5 million blood donations are collected worldwide, out of which 40% are collected from high-income countries. While in the lower-income countries, 54% of blood transfusions are used for children <5 years old.

The incidence of transfusion-transmitted infections (TTIs) has been expanding, as by the 1930s, syphilis was the only recognised TTI, and by 2009, it had expanded to 77 organisms. Thus, the risk of HIV as TTI can be reduced to <1:1,000,000 by rigorous testing and screening of donors.<sup>11</sup> Thus, it is imperative that prevention is the key and is only possible by the application of preventive public health measures. The use of highly sensitive and specific serological and/or molecular assays, bacterial culture and pathogen reduction techniques has been applied for decreasing the risk of TTIs.<sup>12</sup> Prevalence of TTIs has been variably reported in the literature, with an overall prevalence of 1.46%, including HBV (0.71%), syphilis (0.47%), HIV (0.19%), and HCV (0.08%) in 4630 studied samples.<sup>13</sup> Aim of the current study was to estimate the seroprevalence of transfusion-transmissible infections (TTIs) in blood donors of the district of Peshawar.

## **Methods**

The current study was conducted at New Bangash Clinical Laboratory, Haideri Blood Bank & Welfare Hospital in collaboration with the Microbiology department of Comwave Institute, Islamabad, from January 2025 to June 2025. Ethical approval was obtained from the Institutional Ethical Review Board of Comwave institute Islamabad, the inclusion criteria were patients irrespective of gender with age >18 years old, who were visiting the above-mentioned study settings for blood donation. Patients had a prior history of HCV, HBV, Malaria, typhoid, or syphilis infections, with positive testing in the last month.

The required materials were Disposable blood Dropper/ Pipette, Jel Tube, Centrifuge, Timer, Buffer, ICT Strips, and Syringe (5CC). About 5cc of blood was collected through a disposable syringe and centrifuged at 4000rpm for serum separation and transferred to the laboratory for further processing. The Strip method for Hepatitis B Virus (HBV) was followed by taking about 2 CC of sample of the patient's blood sample and collected in a Jel (Serum) Tube. The blood was allowed to clot and centrifuged for 5 minutes to get the serum in a centrifuge machine. The

obtained serum in gel blood tubes 100 µl) was placed in the (S) region of the strip by a pipette or a dropper, and the Test Strip was placed on a clean and level surface at room temperature for 10 minutes. The strip contains 3 regions, S, C, and T (S, Sample, C. Control and T. Testing, respectively). Two one-on C and one on T lines appear on the kit. 1 line appeared on the control region (C), and another line appeared on the test region (T), which indicated that the result was positive. One line appeared in the control region (C). No clear pink line appeared in the test region (T), which indicated that the result was negative. The Control line failed to appear, which stated that the work was invalid. The ELISA method for HBV and Hepatitis C Virus (HCV) was carried out by the addition of 100µl buffer to each well of a microtiter plate, and coating was performed. The plates were incubated up to 120 minutes at 37°C or incubated all overnight at 4°C to eradicate the covering solution and wash the plate three times by filling the wells with 100µl (phosphate.

Buffered Saline) PBS-0.05% tween 20. After washing (cleaning) the wells, the remaining drops are removed by patting the plate on a paper towel. The remaining protein-strap sites were blocked in the coated wells by the addition of 100µl blockin buffer, 3% skim milk in Phosphate Buffered Saline / well. Incubated for 1 hour at RT with gentle shaking, and the plates were washed 3 times with 100ul PBS-0.05% tween 20. Further, the process was carried out by adding 50µl of diluted antibody to each well and incubating the plate at 37°C for an hour with gentle shaking. Added 50µl of the conjugated secondary antibody, diluted at the optimal concentration (according to the manufacturer) in blocking buffer immediately before use. Incubate at 37°C for an hour. Thus, the substrate solution was prepared by mixing acetic acid, TMB, and 0.03% H<sub>2</sub>O<sub>2</sub> with a volume ratio of 4:1:5. The absorbance (optical density at 450nm) of each well with a plate reader was recorded. Collected data were noted in Microsoft Excel, and descriptive statistics were carried out accordingly. Data has been presented in tables and graphs below.

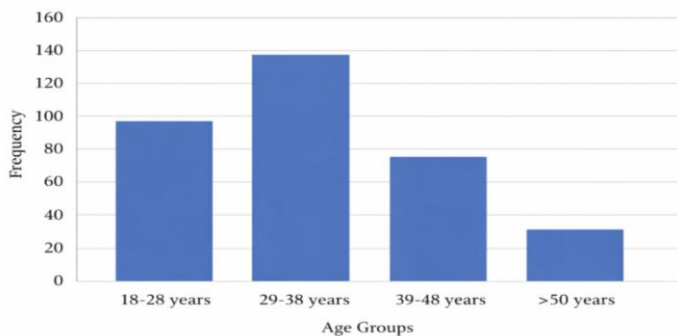
**Results**

Of the studied participants, 77% (289/380) were male, and 23% (89/380) were female participants, as reported in Table 1.

**Table 1: Description of gender distribution of the Study Participants**

<b>Sex</b>	<b>Frequency</b>	<b>Percentage</b>
Male	290	77%
Female	89	23%
Total	380	100%

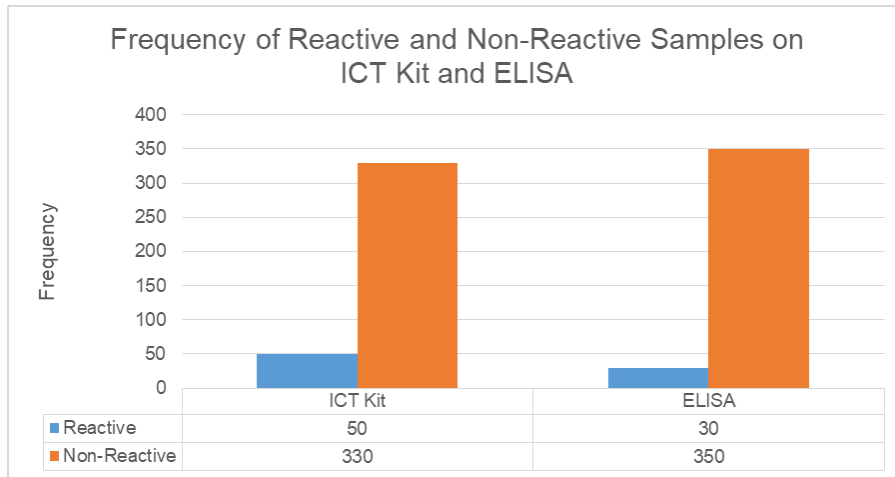
The mean age of the study participants was 35.25 years, with stratification of the study participants in the age groups below as illustrated in Figure 1. Most of the participants were in the group of 29-38 years, including 150 of the study participants.



**Figure 1: Age groups of the Study Participants**

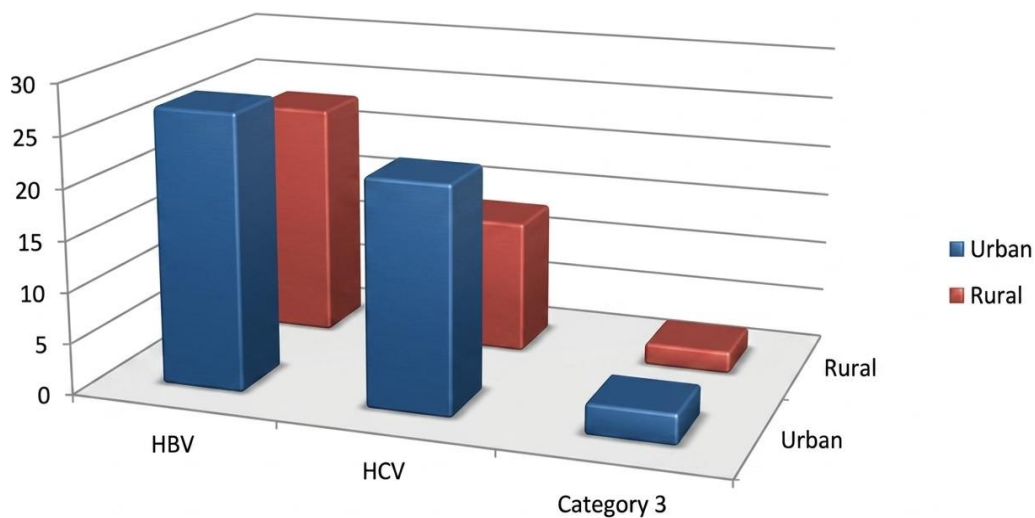
**Evaluation of seroprevalence through ELISA:** Among the total 380 volunteers, 30 (7.8%) were found positive for Hbs Ag, 20 (5.2%) for Anti-HCV and 02 Co-Infection were found upon ELISA analysis.

**Evaluation of seroprevalence through the ICT kit:** Among the total 380 volunteers, 50 (13.1%) were found positive for Hbs Ag, 35(9.2%) for Anti-HCV and Co-infection, 05 through the ICT kit.



**Figure 2: Illustration of the ICT KIT and ELISA Samples**

A total of 380 samples were analysed with the ICT kit and the ELISA. The ICT Kit identified a higher number of reactive samples (50) compared to ELISA, which identified only 30 reactive samples. This suggests a discrepancy of 20 samples where the ICT Kit showed reactivity that was not mirrored by ELISA. Among the total 380 volunteers, 30 (7.8%) were found positive for HbsAg, 20 (5.2%) for AntiHCV and 02 Co-Infections were found upon ELISA analysis. Among the total 380 volunteers, 50 (13.1%) were found positive for HbsAg, 35(9.2%) for AntiHCV and Co-infection05 through the ICT kit. A total of 380 of 210 were from urban 170 were from rural areas. Among the Urban population, 27 were found sero-positive upon ICT for HBV, and the rural population 23 positive for HBV upon the ICT kit method. Among the Urban population, 22 were found sero-positive upon ICT for HCV, and among the rural population, 13 were positive for HCV upon the ICT kit method, as reported in Figure 3.



**Figure 3: Illustration of samples from the Rural and urban areas**

## **Discussion**

Blood donation can save millions of lives worldwide, but unsafe transfusion can further endanger the patient's life; thus, adoption of safety measures and preventive protocols is crucial. The current study focused on the diagnosis of TTIs through seroprevalence in blood donors, tested through the ICT kit and ELISA methods. In this study, seropositivity of HBV was found to be 13.5% among the studied population. Most of the studied participants were in the age group of 29-38 years, followed by 18-28 years and 39-48 years. On the ELISA analysis, 7.8% of participants were positive for Hbs Ag and 5.2% were tested positive for Anti-HCV, and 02 cases were reported with co-infection. While the seroprevalence through the ICT kit reported 13.1% of positive cases of Hbs Ag and 9.2% of Anti-HCV. Thus, a total of 50 cases were found reactive in the ICT kit, 13.15% positive, and 30 cases were positive reported on the ELISA, showing 7.89% of positivity.

Contrary to the current findings, a study conducted in Pakistan reported a lower prevalence of positive seroprevalence cases and reported 3.93% of cases positive for at least a single type of TTI. In the reference study, HCV was the common TTI reported in 1.58% of participants, HBV was prevalent in 1.38% and syphilis in 1.03% of the studied sample.<sup>14</sup>, while the current study did not report any case of syphilis. Although the prevalence of TTIs from Pakistan has been variedly reported and that is due to studies conducted within different regions of the country and with different sample sizes. As a study conducted in 308,767 donors reported 3.33% of TTIs, with HCV as the leading TTI, reporting a prevalence of 1.4%, followed by syphilis 0.9% and HBV, 0.68%, and HIV was common in 0.26% of the collected samples.<sup>15</sup>

Likewise, the prevalence of TTIs has been reported to be 3.58% in the studied 5000 samples of blood donors from Pakistan. The positive cases included HB (0.7%), HC (1.3%), Syphilis (1.3%), HIV (0.2%) and Malaria (0.1%) respectively.<sup>16</sup> The resulting prevalence reported in the current study, as 13%, is much higher when compared with earlier evidence from Pakistan, which leads to many questions. The prevalence of HB and HC may be higher in Peshawar and the surrounding areas, which is why samples taken from the general population may report a higher prevalence of HB and HC infections. Prevalence of HB and HC in the general population has been estimated to be 1.6% and 7.5%, with an estimated 12,000 deaths for HB and 19,000 deaths per year for HC, respectively. Thus, 545,000 new cases are reported annually, while 22% of all the cases are diagnosed, and 2% receives the treatment.<sup>17</sup> Estimation of incidence and prevalence of HB and HC has been a challenge due to a lack of a centralised health management information system, although incidence and prevalence have been variably reported in the published evidence. A systematic review from Pakistan reported the HBsAg ranging from 0.9% to 44.4% and anti-HCV from 0.3% to 62.6% in the Pakistani population from studies published in 2001 to 2022, thus concluding evidence from 22 years.<sup>18</sup>

The current study helped estimate the prevalence of TTIs in blood donors from the surrounding rural areas of Peshawar and the Peshawar city. The study had several limitations, including the sample size, as such studies need to be conducted in large samples to truly estimate the prevalence, although the sample size of n=380 participants was not sufficient, and the study findings cannot be generalised to wider populations. Furthermore, very little information was available about the past medical history of the blood donors, and the selection criteria lacked the rigorous sampling technique to exclude patients who may have remained positive to HB or HC or any other infections in the past. Future research studies should be conducted in wider population groups with inclusive and large samples, and selection criteria should ensure that blood donors being selected are included in the study with more details about their health conditions, comorbidities and past medical histories.

## **Conclusion**

Blood donation is an important part of the healthcare system, which ensures the safety of millions of lives each year. Although transfusion-transmissible infections pose a serious health threat to the patients and are critical in the spread of infections. The current study found 13.5% of samples positive for TTIs collected from surrounding rural areas from Peshawar and Peshawar city, which shows a higher prevalence of TTIs among the blood donors, as well as signifies the higher prevalence of hepatitis B and hepatitis C in the local population.

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