

Research article

Hepatitis B Surface Antigen and Hepatitis C Antibody Seroprevalence Among Male Blood Donors in Lahore, Pakistan: A Cross-Sectional Study at Four Blood Banks

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Abstract: Hepatitis B virus (HBV) and hepatitis C virus (HCV) pose important challenges to blood safety, highlighting the need for continued surveillance among blood donors. This cross-sectional study assessed the seroprevalence of hepatitis B surface antigen (HBsAg) and anti-HCV antibodies among male blood donors at four blood banks in Lahore, Pakistan, and examined their associations with age and ABO/Rh blood group. A total of 600 donors were included, with 150 recruited from each of General Hospital Lahore, Services Hospital Lahore, Noor Thalassemia Centre, and Zaib Ali Foundation. Serum samples were screened using immunochromatographic rapid tests. Seroprevalence estimates were calculated with 95% Wilson confidence intervals (CIs). Associations with blood group were assessed using chi-square tests, and age distributions were compared using the Mann–Whitney U test. Statistical significance was defined as a two-sided $p < 0.05$. HBsAg seroprevalence was 4.8% (29/600; 95% CI, 3.4–6.9%), and anti-HCV seroprevalence was 4.5% (27/600; 95% CI, 3.1–6.5%). B-positive was the most frequent blood group (33.0%). Neither HBsAg nor anti-HCV reactivity was significantly associated with ABO/Rh blood group ($\chi^2 = 10.17$, $p = 0.179$ and $\chi^2 = 4.16$, $p = 0.762$, respectively). Age distributions did not differ significantly between reactive and nonreactive donors for either marker ($p = 0.210$ and $p = 0.922$, respectively). No significant association was observed between HBsAg and anti-HCV reactivity ($p = 0.858$). Approximately one in twenty donors screened reactive for each marker, underscoring the importance of reliable donor screening, appropriate confirmatory testing, donor counselling and referral. These findings represent screening-marker seroprevalence rather than confirmed active infection and should be interpreted within the limitations of rapid testing and the sampled male donor population.

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Introduction

Blood transfusion is an indispensable, life-saving intervention in modern clinical medicine, yet it carries an inherent risk of transmitting infectious agents from

donor to recipient [1]. Among these transfusion-transmissible infections (TTIs), hepatitis B virus (HBV) and hepatitis C virus (HCV) are of paramount public-health importance because of their capacity to establish

chronic infection, progress silently to cirrhosis and hepatocellular carcinoma, and burden already-stretched health systems [2, 3].

The safety of the blood supply is therefore directly dependent on the infectious status of the donor pool, making periodic donor-based seroprevalence surveys a cornerstone of haemo-vigilance [4]. Pakistan is regarded as a country of intermediate-to-high endemicity for viral hepatitis [5]. National estimates suggest that several million people live with chronic HBV or HCV infection [6], driven by a combination of unsafe therapeutic injections, reuse of contaminated needles and syringes, unscreened or inadequately screened blood transfusions, poor sterilization of surgical and dental instruments, and unregulated informal healthcare practices [7]. In this context, the prospective blood donor represents both a sentinel of community transmission and a potential source of iatrogenic spread if screening is imperfect [8].

Immunochromatographic (ICT) rapid test kits are widely used for the initial screening of HBsAg and anti-HCV in resource-limited blood banks because they are inexpensive, rapid, require minimal equipment, and can be performed by staff with limited laboratory training [9]. Although enzyme immunoassays and nucleic-acid amplification testing offer higher sensitivity [10], ICT remains the practical frontline method across much of the region, and prevalence estimates derived from it continue to inform routine practice [11]. The relationship between ABO/Rh blood group and susceptibility to viral hepatitis has been debated in the literature, with some studies reporting weak associations and others finding none [12]. Similarly, donor age is frequently examined as a correlate of infection because it reflects cumulative exposure [13]. Clarifying these relationships within a defined local donor population helps determine whether infections are concentrated in identifiable subgroups who might be targeted for enhanced screening or distributed broadly across the community [14, 15].

This study aimed to estimate HBV and HCV seroprevalence among male blood donors attending four blood banks in Lahore and to characterize their distribution by blood group and age. By sampling equally across governmental hospitals and non-governmental/charitable blood-collection facilities, the study aims to provide a representative picture of the contemporary donor pool in a major Pakistani metropolis. The specific objectives of the study were: (i) to determine the overall seroprevalence of HBsAg and anti-HCV among 600 male blood donors in Lahore; (ii) to describe the frequency distribution of ABO/Rh blood groups in the donor pool; (iii) to examine the

distribution of HBV and HCV seropositivity across blood groups and age; and (iv) to test for statistical associations between viral serostatus and both blood group and age.

Materials and Methods

Study design and setting

We conducted a descriptive, facility-based cross-sectional study in Lahore, Punjab, Pakistan. Four blood-collection facilities were selected to reflect the range of institutions that serve the city: General Hospital Lahore and Services Hospital Lahore (large tertiary-care government hospitals), the Noor Thalassemia Center (a specialized transfusion center), and the Zaib Ali Foundation (a charitable/non-governmental blood bank) [1].

Sample size and sampling

A total of 600 blood donors were enrolled, with an equal quota of 150 donors drawn from each of the four facilities. All donors in the study were male [1]. Sampling was consecutive, enrolling eligible donors who presented for donation during the study period until each facility quota was met.

Data and specimen collection

For each donor, demographic information (age) and ABO/Rh blood group were recorded. A blood specimen was obtained and the serum tested for two viral markers: hepatitis B surface antigen (HBsAg) as a marker of HBV infection, and antibodies to hepatitis C virus (anti-HCV). ABO/Rh grouping was performed by standard antisera agglutination [16, 17].

Serological screening

Screening for HBsAg and anti-HCV was performed using commercial immunochromatographic (ICT) rapid test kits according to the manufacturer's instructions. Results were read visually within the specified time window; the appearance of both the test and control lines was interpreted as reactive (positive), while a control line alone was interpreted as non-reactive (negative). Donors reactive for either marker were classified as seropositive for that infection [18].

Statistical analysis

Data were entered and analyzed using standard statistical software. Categorical variables (blood group, Rh type, serostatus) were summarised as frequencies and percentages. Overall and age-specific seroprevalences were reported with 95% confidence

intervals calculated by the Wilson score method [19]. Associations between viral serostatus and categorical predictors (blood group, age group) were tested using the Pearson chi-square test of independence [20]. Because donor age was not normally distributed, age was compared between seropositive and seronegative groups using the non-parametric Mann-Whitney U test [21]. A two-sided $p < 0.05$ was considered statistically significant.

Ethical considerations

The study used routine donor-screening data collected as part of standard blood-bank practice. Donor confidentiality was maintained throughout, and no personally identifying information is reported. Reactive donors were managed and counseled in accordance with the routine policy of each facility.

Results

Blood-group distribution of the donor pool

A total of 600 male blood donors were screened. The distribution of ABO/Rh blood groups is shown in Table 1 and Figure 1. B-positive was the most frequent group, accounting for 198 donors (33.0%), followed by O-positive (148; 24.7%) and A-positive (126; 21.0%). AB-negative was the rarest group, present in only 9 donors (1.5%). Rh-positive donors predominated overwhelmingly across all ABO categories, consistent with the known Rh distribution of the South Asian population.

Table 1. Frequency distribution of ABO/Rh blood groups among 600 blood donors.

Blood group	Frequency (n)	Percent (%)	Cumulative (%)
A+	126	21.0	21.0
A-	10	1.7	22.7
B+	198	33.0	55.7
B-	16	2.7	58.3
AB+	78	13.0	71.3
AB-	9	1.5	72.8
O+	148	24.7	97.5
O-	15	2.5	100.0
Total	600	100.0	—

Overall seroprevalence of HBV and HCV

Of the 600 donors, 29 were reactive for HBsAg, giving an HBV seroprevalence of 4.8% (95% CI 3.4–6.9), and 27

were reactive for anti-HCV, giving an HCV seroprevalence of 4.5% (95% CI 3.1–6.5) (Tables 2 and 3; Figure 5). HBV prevalence was marginally higher than HCV, although their confidence intervals overlapped substantially, indicating no significant difference between the two markers in this population.

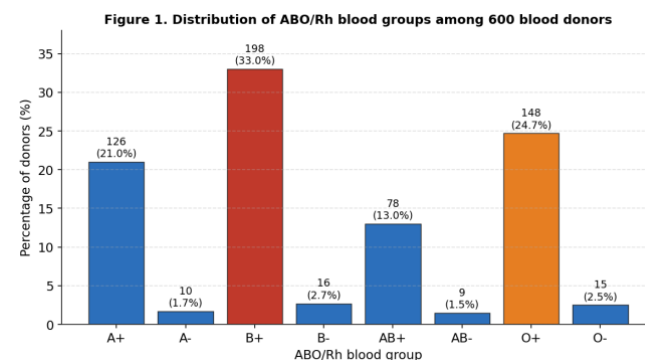


Figure 1. Distribution of ABO/Rh blood groups. B-positive is the most common group (33.0%); AB-negative is the least common (1.5%).

Table 2. Overall frequency of HBsAg (HBV).

HBsAg	Frequency (n)	Percent (%)
Negative	571	95.2
Positive	29	4.8
Total	600	100.0

Table 3. Overall frequency of anti-HCV (HCV).

Anti-HCV	Frequency (n)	Percent (%)
Negative	573	95.5
Positive	27	4.5
Total	600	100.0

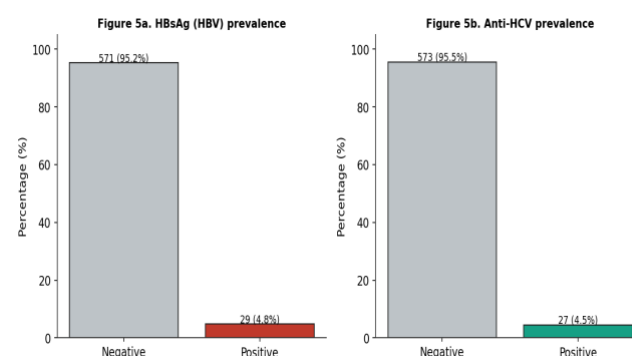


Figure 5. Overall seroprevalence of HBsAg (4.8%) and anti-HCV (4.5%) among 600 donors.

Distribution of infection by blood group

The number of seropositive donors within each blood

group is summarised in Table 4 and Figure 2. HBV seropositivity was highest in the A-positive, AB-positive and O-positive groups (7 reactive donors in each), while no HBV-reactive donor was found in the A-negative group. HCV seropositivity was highest among O-positive donors (9 reactive), with no HCV-reactive donor in the AB-negative group. Despite these apparent differences, the chi-square test showed no statistically significant association between blood group and either HBV ($\chi^2 = 10.17$, $df = 7$, $p = 0.179$) or HCV ($\chi^2 = 4.16$, $df = 7$, $p = 0.762$) seropositivity, indicating that the variation is compatible with chance and the differing group sizes.

Table 4. HBV and HCV seropositivity by blood group

Blood group	Donors (n)	HBV+ (n)	HCV+ (n)
A+	126	7	6
A–	10	0	1
B+	198	4	5
B–	16	1	1
AB+	78	7	4
AB–	9	1	0
O+	148	7	9
O–	15	2	1
Total	600	29	27

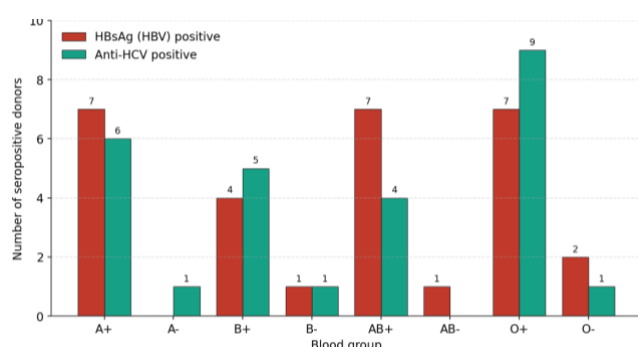


Figure 2. HBV and HCV seropositive counts by blood group. HBV peaks in A+, AB+ and O+ (7 each); HCV peaks in O+ (9).

Age distribution of donors and infection

Donor age ranged from 22 to 35 years. The 22-year age group was the largest single cohort (98 donors, 16.3% of the sample), followed by 23-year-olds (70; 11.7%), while 33-year-olds formed the smallest group (17; 2.8%) (Figure 3). This young age profile is typical of a voluntary/replacement donor population in Pakistan.

The age-specific distribution of HBsAg and anti-HCV is presented in Tables 5 and 6 and Figure 4. The 22-year cohort carried the greatest absolute burden of infection, with 12 HBV-reactive and 7 HCV-reactive

donors—corresponding to age-specific prevalences of approximately 12.2% for HBV and 7.1% for HCV within that cohort. No HBV-reactive donors were detected among 30- and 31-year-olds, and no HCV-reactive donors among 27- and 31-year-olds.

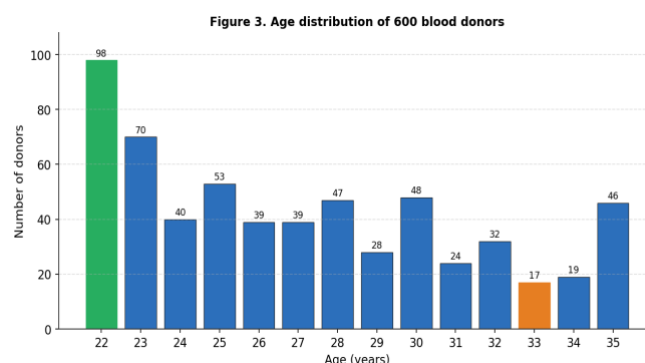


Figure 3. Age distribution of the 600 donors. The 22-year cohort is largest (green); the 33-year cohort is smallest (orange).

Table 5. Age-wise distribution of HBsAg (HBV).

Age (yr)	Negative	Positive	Total
22	86	12	98
23	69	1	70
24	39	1	40
25	52	1	53
26	37	2	39
27	37	2	39
28	46	1	47
29	27	1	28
30	48	0	48
31	24	0	24
32	31	1	32
33	16	1	17
34	18	1	19
35	41	5	46
Total	571	29	600

Age and serostatus: box-plot analysis

The distribution of donor age by serostatus is shown as combined box-and-violin plots in Figure 6. The median age of HBV-reactive donors (25 years) was slightly lower than that of non-reactive donors (27 years), but the Mann–Whitney U test showed this difference was not statistically significant ($p = 0.210$). For HCV, the age distributions of reactive and non-reactive donors were

essentially identical ($p = 0.922$). When age was collapsed into three groups (Figure 8), HBV prevalence was 5.7%, 3.0% and 5.8% in the 22–25, 26–30 and 31–

35 year groups respectively, and HCV prevalence was 4.6%, 4.5% and 4.3%, confirming a broadly flat age gradient.

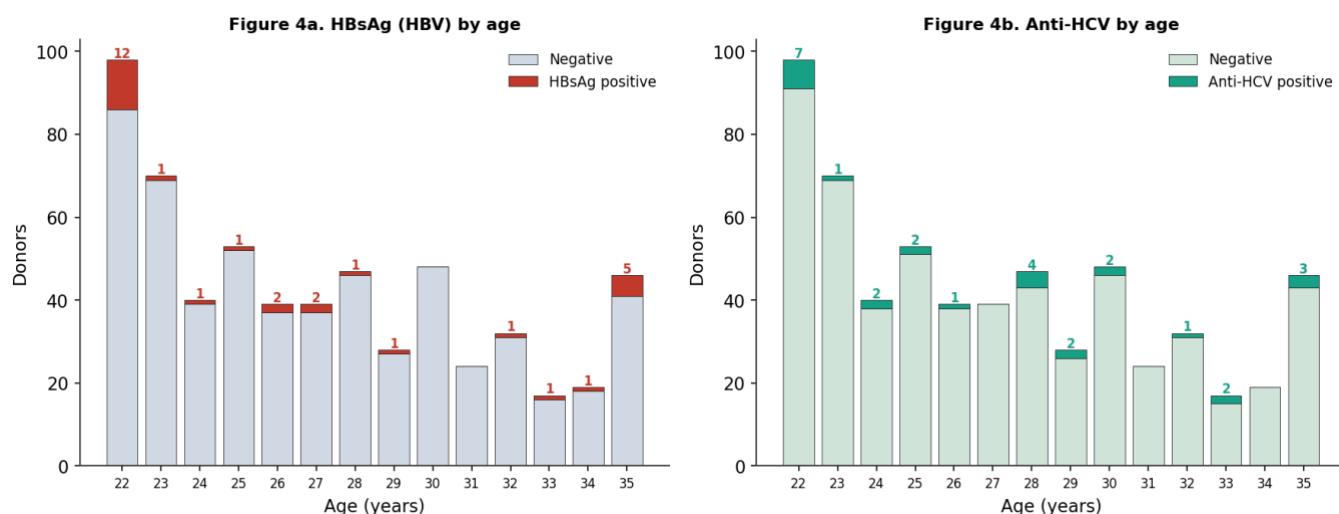


Figure 4. Stacked age-wise distribution of HBsAg (4a) and anti-HCV (4b). Reactive counts are labeled above each bar.

Table 6. Age-wise distribution of anti-HCV (HCV).

Age (yr)	Negative	Positive	Total
22	91	7	98
23	69	1	70
24	38	2	40
25	51	2	53
26	38	1	39
27	39	0	39
28	43	4	47
29	26	2	28
30	46	2	48
31	24	0	24
32	31	1	32
33	15	2	17
34	19	0	19
35	43	3	46
Total	573	27	600

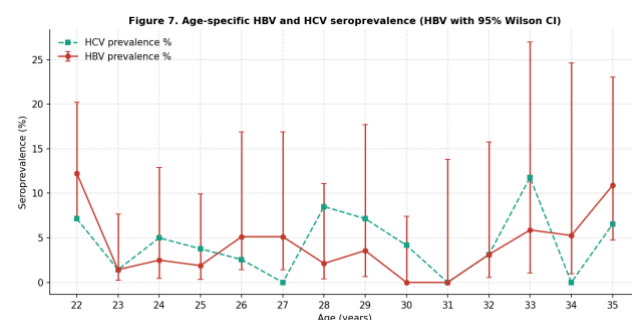


Figure 6. Age-specific seroprevalence of HBV (with 95% Wilson confidence intervals) and HCV. Wide intervals at individual ages reflect small cohort sizes.

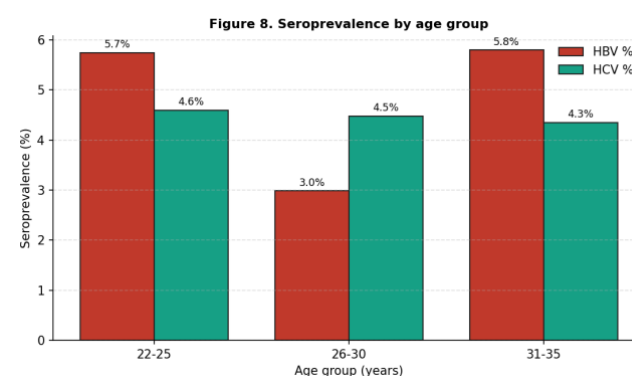


Figure 8. Seroprevalence of HBV and HCV by broad age group, showing a flat age gradient.

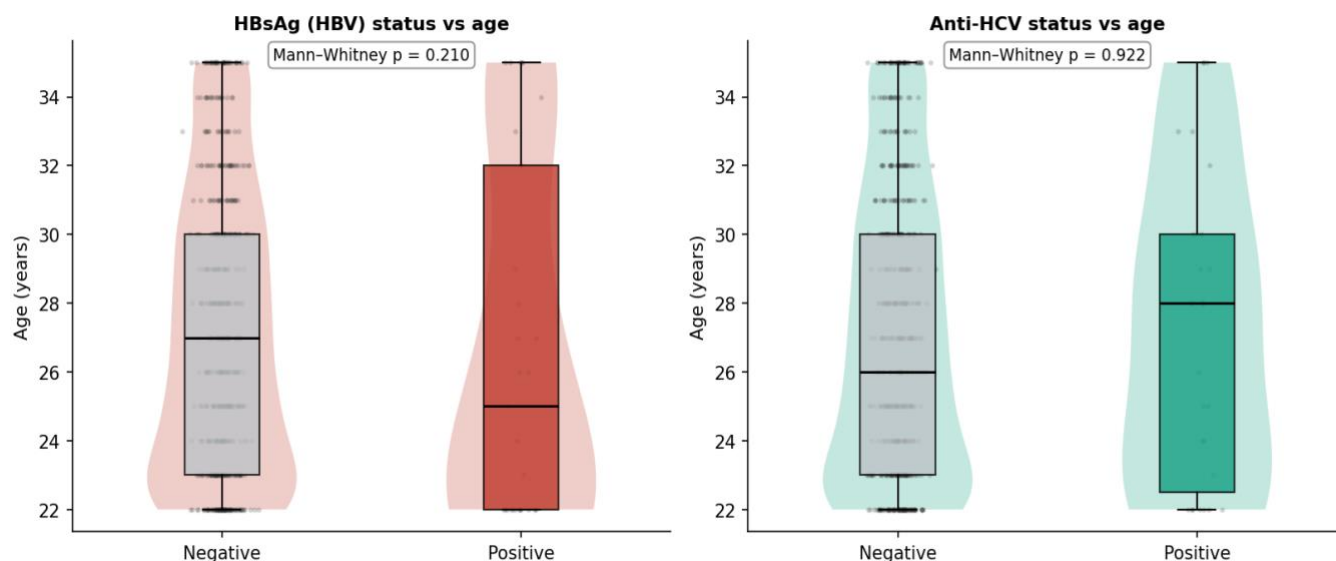


Figure 7. Box-and-violin plots of donor age by HBV (left) and HCV (right) serostatus. No significant age difference was observed for either marker.

Co-occurrence of HBV and HCV

Cross-tabulation of the two markers showed that two donors were reactive for both HBsAg and anti-HCV. The chi-square test showed no significant association between HBV and HCV serostatus ($\chi^2 = 0.03$, $p = 0.858$), indicating that co-infection occurred at approximately the frequency expected by chance given the two marginal prevalences.

Discussion

This cross-sectional study of 600 male blood donors sampled equally across four blood banks in Lahore found an HBsAg seroprevalence of 4.8% and an anti-HCV seroprevalence of 4.5%. Both figures sit within the range reported by other Pakistani donor studies, which have variously reported HBV between roughly 2% and 6% and HCV between roughly 3% and 8% [22], and both are substantially higher than the sub-1% figures typical of donor pools in high-income settings with universal vaccination and nucleic-acid screening [23]. The near-parity of HBV and HCV in this sample is a notable feature and underlines that both viruses continue to circulate actively in the community from which these donors were drawn. The predominance of the B-positive blood group (33.0%), followed by O-positive and A-positive, is consistent with the well-documented ABO distribution of the Punjabi and wider South Asian population [24]. Although HBV seropositivity appeared concentrated in the A-positive, AB-positive and O-positive groups and HCV in the O-positive group, none of these apparent differences reached statistical

significance [25]. The high positive counts in the larger groups are largely a reflection of group size rather than of biological susceptibility [26]. Our finding of no significant blood-group association is in agreement with the majority of published work, which has generally failed to establish a robust, reproducible link between ABO/Rh phenotype and hepatitis susceptibility [27]. The donor pool was young, dominated by donors in their early twenties, with the single largest burden of infection falling on 22-year-olds. However, the box-plot and Mann–Whitney analyses demonstrated that age did not differ significantly between reactive and non-reactive donors for either virus, and the grouped analysis revealed a flat age gradient [28]. The apparent concentration of cases in the youngest cohort is therefore best explained by that cohort simply being the largest, rather than by a genuinely elevated per-person risk [29]. This pattern is epidemiologically important: it suggests that transmission in this population is not confined to an older, more heavily exposed subgroup but is instead broadly distributed across young adult men, consistent with community-level exposure through unsafe injections, cosmetic and dental procedures, and shared instruments [30]. The absence of a significant HBV–HCV association, together with the observation of only two co-infected donors, indicates that the two infections are being acquired largely independently in this population, rather than clustering in a shared high-risk group such as injecting drug users, who would be expected to show marked co-infection [31]. This again points toward diffuse iatrogenic and community transmission as the dominant route. From a blood-safety perspective, a

combined HBV/HCV reactive rate approaching one in ten donors screened represents a considerable residual risk, particularly because ICT screening, while practical and inexpensive, is less sensitive than enzyme immunoassay or nucleic-acid amplification testing and can miss early-window-period and low-titer infections. The true prevalence, and the true residual transfusion risk, may therefore be higher than the ICT-based estimates reported here [32, 33].

Strengths and limitations: The principal strengths of this study are its reasonable sample size and balanced sampling across governmental and charitable blood banks, which improves the representativeness of Lahore's donor picture. The authors reported statistical estimates with confidence intervals and formally tested them, rather than relying on raw percentages alone. Several limitations must, however, be acknowledged. First, screening relied solely on ICT rapid kits without confirmatory EIA or PCR testing, so both false positives and, more importantly, window-period false negatives are possible [33]. Second, the study enrolled only male donors, so the findings cannot be generalized to female donors. Third, the study did not capture key behavioral and exposure variables, injection history, surgical and dental history, tattooing, and vaccination status, limiting the ability to identify modifiable risk factors. Fourth, the donor-level age distribution used for the box-plot and non-parametric analyses was reconstructed from the published age-stratified counts; while the marginal totals are exact, individual-level associations should be interpreted with appropriate caution.

Conclusion

Among 600 male blood donors in Lahore, HBsAg seroprevalence was 4.8%, and anti-HCV was 4.5%, with HBV marginally more common. Neither infection was significantly associated with ABO/Rh blood group or with donor age, and the two viruses co-occurred no more often than expected by chance. The broadly even distribution of infection across blood groups and young-adult age bands points to diffuse community and iatrogenic transmission rather than a concentrated high-risk subpopulation. These results reaffirm that HBV and HCV remain a substantial threat to blood safety in Lahore and support adopting more sensitive confirmatory screening, robust voluntary-donor programs, vaccination, and sustained public-health education.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the subject(s) to publish this paper.

Data Availability Statement: Additional data will be made available upon reasonable request to the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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