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HEPATITIS B, HEPATITIS C, AND SYPHILIS PREVALENCE AMONG BLOOD DONORS IN RAWALPINDI CITY

BY

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Abstract

Background:
The spread of infectious diseases through blood and blood products is a global health issue, especially in developing nations. Hepatitis B, hepatitis C, HIV, and syphilis are some of the most common infections that can be passed on through blood transfusions. The only way to decrease the transfusion-transmissible infections is the safe blood transfusion, which means blood should be properly screened before transfusion. The current study sought to ascertain the prevalence of HBS, HCV, and Syphilis among blood donors in Rawalpindi City, Pakistan.

Methodology:
A total of 385 voluntary blood donors were screened over a six-month period at Alkhidmat Raazi Hospital, Rawalpindi, Pakistan. The obtained samples were analyzed serologically for hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV) antibodies, and anti-Treponema pallidum (the causative agent of syphilis) antibodies using the Chemiluminescent Microparticle Immunoassay (CMIA) technique.

Results:
Our research studies revealed that the prevalence of HCV, HBV, and syphilis among blood donor volunteers was 4.1%, 4.1%, and 4.41%, respectively. Our research studies show that the seropositivity of HBV and HCV was higher in males, while the seropositivity of syphilis was higher in females. HBsAg rate was slightly higher in unmarried donors, but HCV and syphilis seropositivity rates were higher in married donors. The seropositivity of HBV and syphilis was slightly higher in donors whose age was below 35 years, and the seropositivity of HCV was higher in those donors whose age was greater than 35 years.

Conclusion:
We concluded that the prevalence of hepatitis C virus (HCV), hepatitis B virus (HBV), and syphilis is significantly higher in blood donors. Additionally, our analysis indicates those low socioeconomic conditions, a lack of health education significantly contribute to the increased risk of transfusion-transmissible infections (TTIs). To control the spread of these infections and ensure the safety of recipients, it is essential to enhance the procedures and practices related to blood donor selection. Furthermore, blood donors must undergo comprehensive screening for TTIs using accepted techniques.

Keywords: *Hepatitis B virus (HBV), Hepatitis C virus (HCV), chemiluminescent immunoassay (CMIA), Transfusion transmitted infections (TTIs).*

Introduction

Blood transfusion is one of the most essential therapeutic interventions in modern medicine, playing a crucial role in saving lives during trauma, surgical procedures, hematological disorders, and other medical conditions. Blood transfusion is clinically significant but poses the risk of transmitting transfusion-transmissible infections (TTIs), potentially leading to severe adverse outcomes for recipients. Contaminated blood can carry a wide range of viruses, bacteria, and parasites. Hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and Treponema pallidum (Causative agent of syphilis) are considered the most significant due to their global health impact. These infections remain major causes of morbidity and mortality worldwide, particularly in developing countries where healthcare systems face challenges in ensuring safe transfusion practices. In response, the World Health

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Organization (WHO) strongly recommends that all donated blood be mandatorily screened for TTIs, including HBV, HCV, HIV, and syphilis, before transfusion (1).

Hepatitis, an inflammation of the liver, is most usually caused by viral infections. Among these, HBV and HCV pose the greatest concern to transfusion safety. HBV is a double-stranded DNA virus from the *Hepadnaviridae* family that remains a major cause of chronic liver disease. Globally, it is estimated that 3.5% of the population is chronically infected with HBV, and HBV-related consequences, notably hepatocellular carcinoma and liver cirrhosis, account for almost 800,000 deaths each year (2). In Pakistan, the prevalence of HBV is worrying. According to recent forecasts for 2023, over 9 million people, or 3.87% of the population, are infected with HBV (3).

HCV, a single-stranded RNA virus of the *Flaviviridae* family, is another major global health concern. According to WHO, around 50 million people were living with chronic HCV infection worldwide in 2022 (4). Similar to HBV, HCV infection poses a significant risk in transfusion medicine, particularly in regions where routine screening is inconsistently implemented.

Syphilis, caused by the motile Gram-negative spirochete *Treponema pallidum*, is a chronic, systemic sexually transmitted infection that can also be transmitted through infected blood donations. *T. pallidum* exclusively infects humans, and studies suggest that 16% to 30% of individuals exposed to a syphilis-infected partner acquire the disease within 30 days (5). The pathogen typically enters through breaches in the skin or mucous membranes of the genital, rectal, or oral regions. Syphilis frequently starts without noticeable symptoms, yet it continues to pose a significant public health concern in many countries, including Pakistan, where it is considered one of the most prevalent sexually transmitted infections. An estimated 29.5% of the Pakistani population—approximately 70 million individuals—are affected by syphilis. Moreover, it has been reported that among the approximately 3.5 million annual blood donations in Pakistan, about 1% of donors carry the risk of TTIs, including syphilis (6).

To address these issues, the WHO has emphasized a comprehensive approach to safe blood transfusion protocols. This method entails recruiting frequent, volunteer, non-remunerated blood donors and implementing rigorous screening processes for all main TTIs under strict quality control systems. Despite these recommendations, screening coverage and adherence vary, particularly in low- and middle-income countries such as Pakistan (7).

Given the high prevalence of HBV, HCV, and syphilis in the general population, as well as Pakistan's heavy reliance on blood transfusions, ongoing monitoring of TTI prevalence among blood donors is critical for improving transfusion safety and public health policies. As a result, the purpose of this study is to evaluate the prevalence of HBV, HCV, and syphilis among blood donors, as well as to compare their distribution among gender categories.

Methods:

A total of 385 blood donors were screened over a 6-month period at Alkhidmat Razi Diagnostic Center, Rawalpindi, after the approval of the institute's ethical committee. Blood donors who donate blood voluntarily were included, while all other blood donors were excluded. Individuals with suspected HBV, HCV, and Syphilis cases and with a previous history of HBV, HCV, and Syphilis cases were also excluded.

Venous blood samples were collected from 385 blood donors at Alkhidmat Raazi Diagnostics and Blood Bank in Rawalpindi. A blood sample of 5 ml was collected by Needle Size 21 G * 1 ½ inch") from an antecubital vein at room temperature and shifted to a BD Vacutainer tube size 13 * 100 mm). Collected samples were being stored at 2-8 °C in a refrigerator until further processing of CMIA. After the collection, the blood samples were run on CMIA.

The collected samples were analyzed using CMIA for the qualitative detection of HBsAg and anti-HCV antibodies. During the CMIA procedure, the analyzer probe aspirated a specific volume of the specimen and passed it through a reaction column, where the target antigens bound to their corresponding antibodies. The resulting antigen–antibody complexes were detected by the analyzer, and the outcomes were displayed on the screen. Samples with a signal-to-cutoff (S/CO) ratio greater than or equal to 1.00 were considered reactive (positive), whereas those with a ratio less than 1.00 were classified as non-reactive (negative).

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25, and the findings were summarized in tabular form.

Results:

HBS Ag among different age groups:

Participants were categorized based on age. Among the 279 donors aged 18–35 years, 266 tested negatives for HBsAg, while 13 tested positive. In the 35–55 years age group (n = 106), 103 donors were negative for HBsAg, and 3 donors were positive (see Table 1.1).

HBS Ag Gender wise distribution:

Of the 385 donors, 377 were male and only 8 were female. Among the 377 male donors, 16 (4.2%) tested positive for HBsAg, while 361 (95.8%) tested negative. All eight female donors (100%) tested negative for HBsAg, as indicated in Table 1.2.

HBsAg Positivity by Marital Status:

Among the 385 donors, 255 were married and 130 were unmarried. Eight (3.1%) of the 255 married donors tested positive for HBsAg, while 247 (96.9%) tested negative. Table 1.3 shows that 8 (6.1%) of the 130 unmarried donors tested positive for HBsAg, while 122 (92.8%) tested negative.

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Table. 1.1. HBS Ag among different age groups

Age Groups	Hepatitis B Surface Antigen		Total
	Negative	Positive	
18-35 years	266	13	279
35-55 years	103	03	106
Total	369	16	385

Table. 1.2. HBS Ag Gender wise distribution

Gender	Hepatitis B Surface Antigen		Total
	Negative	Positive	
Female	08	0	08
Male	361	16	377
Total	369	16	385

Table. 1.3. HBsAg Positivity by Marital Status

Marital Status of the Participants	Hepatitis B Surface Antigen		Total
	Negative	Positive	
Married	247	8	255
Unmarried	122	8	130
Total	369	16	385

Hepatitis C among different age groups:

Participants were classified according to their age. 279 donors ranged in age from 18 to 35, with 8 (2.9%) testing positive for HCV and 271 (97.1%) testing negative. As shown in Table 2.1, 106 people between the ages of 35 and 55, of which 8 (7.5%) tested positive for HCV, while 98 (92.5%) tested negative.

Hepatitis C gender wise distribution:

Of the 385 donors, 377 were male and eight were female. Among the 377 male donors, 16 (4.2%) tested positive for HCV, while 361 (95.8%) tested negative. All eight female donors (100%) tested negative for HCV, as indicated in Table 2.2.

HCV Positivity by Marital Status:

Out of 385 donors, 255 were married and 130 were unmarried. Among the married donors, 13 (5%) tested positive for HCV, while 242 (95%) tested negative. As shown in Table 2.3, 3 of the 130 unmarried donors (2.3%) were HCV-positive, whereas 127 (97.7%) were HCV-negative.

Table 2.1. HCV among different age groups

Age Groups	Hepatitis C antibodies		Total
	Negative	Positive	
18-35 years	271	08	279
35-55 years	98	08	106
Total	369	16	385

Table 2.2. HCV gender wise distribution

Gender	Hepatitis C Virus Antibodies		Total
	Negative	Positive	
Female	08	00	08
Male	361	16	377
Total	369	16	385

Table 2.3. HCV Positivity by Marital Status

Marital Status of the Participants	Hepatitis C Virus Antibodies		Total
	Negative	Positive	
Married	242	13	255
Unmarried	127	03	130
Total	369	16	385

Syphilis among different age groups in blood donors:

Out of 385, 279 participants falls in the age group between 18-35 years, and 106 participants in the age 35-55 years. Out of 279 donors aged 18 to 35, 14 (5%) tested positive for syphilis, while 265 (95%) tested negative. Table 3.1 shows that out of 106 donors between the ages of 35 and 55, 5 (4.7%) tested positive for syphilis, whereas 101 (95.3%) tested negative for syphilis.

Syphilis, gender wise distribution:

Among the 385 blood donors assessed, the majority were male (n=377), whereas only eight were female. Of the male donors, 17 (4.5%) were seropositive for syphilis, while 360 (95.5%) tested negative. In contrast, one out of eight female donors (12.5%) was positive for syphilis, and the remaining seven (87.5%) were non-reactive, as presented in Table 3.2.

Syphilis Positivity by Marital Status:

The study involved a total of 385 volunteer blood donors. Of these, 255 individuals were married, while 130 were unmarried. Of these 15 tested positive for syphilis, whereas 240 tested negatives. Conversely, among the unmarried donors, 4 individuals were found to be positive for syphilis, with 126 testing negatives. These findings, summarized in Table 3.3.

Table 3.1. Syphilis among different age groups

Age Groups	*Treponema pallidum* (Syphilis)		Total
	Negative	Positive	

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18-35 years 35-55 years	265 101	14 05	279 106
Total	366	19	385

Table 3.2. Syphilis gender wise distribution

Gender Of the Participants	*Treponema pallidum* (Syphilis)		Total
	Negative	Positive	
Female Male	07 359	01 18	08 377
Total	366	19	385

Table 3.3. Syphilis Positivity by Marital Status

Marital Status of the Participants	*Treponema pallidum* (Syphilis)		Total
	Negative	Positive	
Married Unmarried	240 126	15 04	255 130
Total	366	19	385

Discussion

Our Research shows that the burden of transfusion-transmissible infections (TTIs) was driven by HBsAg, HCV, and syphilis, each with a prevalence of 4–5%. When compared with recent Pakistani reports, our HBsAg (4.1%) and HCV (4.1%) rates are higher than several large studies that generally report ≤2% for HBV and HCV in donor populations. For example, a regional transfusion center in northern Pakistan (2017–2021) found HBsAg 0.68% and anti-HCV 1.40%, with syphilis 0.90% (13). Similarly, a multi-year analysis from Islamabad reported HBsAg 0.4–0.7%, HCV 1.1–1.9%, and syphilis 0.5–1.0% in repeat cross-sections (14). A recent analysis from Rawalpindi reported declining HBV and HCV trends among asymptomatic donors over 2015–2021 (HBV ~1%, HCV ~0.5%) (15). In contrast, a district-level study from Swabi (Khyber Pakhtunkhwa) in 2024 documented HCV 4.89% and HBV 2.13% which is closer to our HCV estimate and highlights that higher TTI prevalence may cluster outside major urban transfusion hubs (16). Our figures, therefore, likely reflect local epidemiology, donor selection practices, and differences in first-time vs repeat donors rather than assay performance alone (13-16).

The syphilis seroprevalence (4.41%) in our donors is particularly higher than most recent reports that typically find <1% among Pakistani donors. Northern Pakistan centers reported 0.8–0.9% (13,14). Research in Khyber Pakhtunkhwa has found ~0.8% positivity using treponemal testing (17), and Swabi reported 0.27% (16). This discrepancy could stem from: (i) test algorithm (e.g., use of a non-treponemal screen without confirmatory treponemal testing inflates apparent prevalence from biologic false positives), (ii) donor mix (higher proportion of first-time/replacement donors), and (iii) localized sexual health dynamics. Given the transfusion safety implications, centers witnessing syphilis ≥2–3% should review their two-step algorithm (screen + confirmatory treponemal assay) and post-test counseling pathways per good practice (13-17).

HCV was significantly higher in older donors; older Pakistan-born adults experienced peak community transmission decades earlier via healthcare-related exposures, unsterile injections, or barbering, with risk now decelerating in younger cohorts as injection safety and infection control improved (14,18). Conversely, HBsAg was modestly higher in younger donors, which is unexpected in the context of HBV infant immunization (introduced nationally in the early 2000s). Recent multi-center data show HBV generally decreasing in younger donors (15). Our findings may reflect small-area clustering, suboptimal adolescent catch-up coverage, or overrepresentation of first-time donors who lack prior deferral. This pattern warrants audit of vaccination history during donor screening and targeted health education (14,15,18).

Sex and marital status gradients should be interpreted cautiously. The donor pool was overwhelmingly male (98%), limiting inference for women; the apparently higher female syphilis proportion is numerically unstable. Pakistani studies uniformly report a male-dominant donor base (>95%), with low female participation due to sociocultural and hemoglobin/weight deferrals (13-15). Marital status in our study showed higher HCV among married donors and higher HBsAg among unmarried. Prior reports rarely find strong, consistent marital-status effects after adjusting for age and donor type; instead, first-time vs repeat status and geography are stronger predictors of positivity (13,14,18). Strengthening donor education, voluntary repeat donation, and risk-questionnaire fidelity remains key to reducing window-period donations (13–15,18).

Our prevalence underscores the importance of rigorous screening and continuous quality assurance. WHO recommends mandatory screening of all donations for HBV, HCV, HIV and syphilis using quality-assured assays, with standard operating procedures, external quality assessment, and traceability; Pakistan's documented gaps historically included variable SOP adherence and inconsistent QA, though the national landscape has been improving with provincial blood transfusion authorities and consolidated centers. Where feasible, adding NAT for HBV/HCV can reduce the residual risk in settings with ≥2–3% seroprevalence, though cost–cost-effectiveness depends on local incidence and reactivity rates (18,19).

Conclusion

We concluded that the prevalence of hepatitis C virus (HCV), hepatitis B virus (HBV), and syphilis in our study was 4.1%, 4.1%, and 4.4%, respectively. Additionally, our analysis indicates that low socioeconomic conditions, a lack of health education, and the transfusion of untested blood significantly contribute to the increased risk of transfusion-transmissible infections (TTIs). To control the spread of these infections

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and ensure the safety of recipients, it is essential to enhance the procedures and practices related to blood donor selection. Our research strongly recommends carefully choosing blood donors, emphasizing obtaining voluntary donors. Furthermore, blood donors must undergo comprehensive screening for TTIs using accepted techniques.

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