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TRANSFUSION-TRANSMITTED INFECTIONS IN BLOOD DONORS: A ONE HEALTH PERSPECTIVE ON ABO/RH BLOOD GROUPS AND AGE

Original Research

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Acknowledgement: The authors gratefully acknowledge the support of Rehber Thalassemia Center and Railway General Hospital, Rawalpindi, for providing access to donor data.

Conflict of Interest: None

Grant Support & Financial Support: None

ABSTRACT

Background: Beyond their established importance in transfusion medicine, ABO and Rh blood group antigens have been implicated in susceptibility to various infectious and non-infectious diseases. Transfusion-transmitted infections (TTIs) such as hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), syphilis, and malaria remain critical public health concerns, particularly in low-resource settings. Understanding the association of these infections with blood group phenotypes contributes to enhancing blood safety under the One Health approach and supports Sustainable Development Goals (SDGs) 3 and 17.

Objective: This study aimed to determine the prevalence of TTIs among blood donors and assess their association with ABO and Rh blood groups, as well as with donor age categories.

Methods: A retrospective cross-sectional analytical study was conducted at the Rehber Thalassemia Center and Railway General Hospital, Rawalpindi, analyzing data from 46,453 blood donors between January 2020 and December 2024. Donor eligibility was based on standard screening criteria, and 5 mL of blood was collected from each participant. TTIs were screened using an automatic chemiluminescent microparticle immunoassay (CMIA), and malaria was screened using enzyme-linked immunosorbent assay (ELISA). ABO and Rh typing were performed using slide agglutination. Statistical analysis was conducted using the Chi-square test, with p-values <0.05 considered significant.

Results: TTIs were detected in 1,437 donors (3.09%), with HCV being the most prevalent (1.20%), followed by HBV (1.08%), syphilis (0.61%), HIV (0.20%), and malaria (0.002%). Blood group B was most common (33.7%), followed by O (31.6%), A (24.6%), and AB (10.1%), while 91% of donors were Rh positive. No significant association was found between ABO blood groups and TTI markers (HCV $p=0.60$; HBV $p=0.27$; HIV $p=0.17$; syphilis $p=0.08$), whereas Rh factor showed a significant association with all TTIs ($p<0.00001$). The highest TTI prevalence was observed in donors aged 26–35 years (n=717, 50.0%) with a statistically significant association ($p<0.05$).

Conclusion: While ABO blood groups showed no significant association with TTI prevalence, a notable relationship was identified with Rh factor. These findings underscore the importance of enhancing donor screening strategies, especially for high-risk age groups, to improve transfusion safety.

Keywords: ABO blood-group system, blood donors, blood safety, chemiluminescence immunoassay, Rh-Hr blood-group system, seroprevalence, transfusion-transmitted infections.

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INTRODUCTION

The red blood cell (RBC) membrane is composed of a range of antigens that include both polysaccharide and protein components, among which the ABO blood group system holds prominent clinical relevance. These antigens are not confined to erythrocytes alone but are also expressed on other cell types such as epithelial, vascular endothelial, and sensory neuronal cells, as well as on tissues like breast ductal epithelium and malignant cells (1). The ABO and Rh blood group antigens are well-established determinants in transfusion medicine, primarily due to their role in compatibility and immunological reactions. However, their significance extends beyond transfusion, having been associated with susceptibility to both infectious and non-infectious diseases (2). Emerging evidence has highlighted correlations between specific blood groups and the risk of developing various conditions, including breast and gastric cancers, diabetes mellitus, duodenal ulcers, *Helicobacter pylori* infection, and malaria (3,4). Furthermore, the ABO and Rh antigens have been implicated in the susceptibility to transfusion-transmitted infections (TTIs) such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), malaria, and syphilis (5,6). This link has garnered increased attention in the context of public health, particularly in developing countries where the burden of TTIs continues to rise, threatening to compromise the quality of life and increase mortality and morbidity rates (7).

Studies conducted in various African regions further underscore the severity of this issue. For instance, in Malawi, the prevalence of HBV, HCV, syphilis, and HIV among donors was found to be 3.6%, 1%, 2.6%, and 1.9%, respectively (8). Even higher rates have been reported in Angola, with HBV at 8.4%, syphilis at 4.4%, HCV at 3%, and HIV at 2.1% (9). In Eritrea, syphilis led with a prevalence of 7%, followed by HBV at 5%, HIV at 0.8%, and HCV at 0.7% (10). Similarly, in Ethiopia, HBV, HCV, HIV, and syphilis were recorded at 3.9%, 0.6%, 0.5%, and 1.2% respectively in 2017 (11). These findings are alarming and align with the World Health Organization's (WHO) recommendation to rigorously screen all blood products for TTIs such as HBV, HCV, and HIV to ensure safe transfusion practices (12). Transmission of TTIs predominantly occurs through blood transfusions, unsterile needle usage, needle-stick injuries, and unprotected sexual contact (13). While these are well-established pathways, several investigations have also explored how ABO and Rh blood group antigens may influence an individual's susceptibility to such infections, likely due to the role of specific carbohydrate structures in pathogen recognition and cellular adhesion (14,15). Some antigens might act as protective barriers by preventing microbial adherence, while others could facilitate infection.

There have been conflicting findings on the association between blood groups and TTIs. A study identified blood group A-negative individuals as more susceptible to HIV and HBV, whereas B-negative individuals showed a higher risk for syphilis (16). Another study suggested that blood group B donors exhibited the highest seroreactivity to TTIs, followed by groups A, O, and AB, although these differences lacked statistical significance (17,18). Conversely, a review concluded that individuals with blood group B might have a lower risk of HBV infection (13). In Pakistan, the situation is equally concerning. National surveillance data indicated that the overall prevalence of TTIs among blood donors increased from 3.72% in 2019 to 4.61% in 2021, with HCV being the most common, followed by HBV, syphilis, and HIV (19–21). Given the disparities and limited regional data, especially from underrepresented areas, there is a pressing need to explore potential associations between ABO and Rh blood groups with TTIs in the local donor population. This study was thus undertaken to examine the relationship between transfusion-transmissible infections and different blood groups and age categories among blood donors in Pakistan. The objective was to contribute to the growing body of evidence in transfusion medicine and inform targeted screening practices and preventive strategies in resource-limited settings.

METHODS

A retrospective cross-sectional analytical study was conducted over a five-year period from January 2020 to December 2024 at the Rehber Thalassemia Center and Blood Bank, Railway General Hospital, Rawalpindi. A total of 46,453 blood donors were included in the study following a standard pre-donation screening protocol. Donors were selected based on predefined inclusion criteria: age between 18 and 60 years, body weight greater than 50 kg, and hemoglobin level above 12.5 g/dL. All individuals underwent a thorough medical history review and physical examination to assess fitness for blood donation. Only voluntary donors were recruited, ensuring that the data reflected a low-risk population profile. For laboratory analysis, 5 mL of venous blood was collected from each donor. The samples

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were tested for transfusion-transmissible infection (TTI) markers, including hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), syphilis, and malaria, in addition to determining ABO and Rh blood group status. Serum samples were separated by centrifugation and subjected to immunological screening using chemiluminescent microparticle immunoassay (CMIA) techniques. The CMIA kits used were manufactured by Roche Diagnostics (Basel, Switzerland), targeting HBsAg, anti-HCV, HIV Ag/Ab, and Treponema pallidum antibodies (anti-Tp). Screening for malarial parasites was conducted using an enzyme-linked immunosorbent assay (ELISA) method provided by Bio-Rad Laboratories (California, USA). Reactive and non-reactive results were interpreted by comparing the chemiluminescent signal to a predefined cutoff value (S/Co ratio) according to the manufacturer's instructions (14).

ABO and Rh blood grouping were performed using both forward and reverse typing methods via the slide agglutination technique. Monoclonal antisera (anti-A, anti-B, and anti-D) were utilized along with a 5% red cell suspension to ensure accurate blood group identification. The conventional classification system was applied, and all initial results were subsequently confirmed to maintain consistency and reliability (22). Ethical approval for the study was obtained from the Ethical Committee of Riphah International University, Rawalpindi, the study adhered to ethical standards for human research. All participants provided informed consent prior to sample collection, in accordance with institutional and international guidelines.

RESULTS

Out of 46,453 blood donors included in the study, the overwhelming majority were male (99%), while only 0.56% were female. The largest proportion of donors (43.9%) were within the age group of 26–35 years, followed by 39.6% in the ≤25 age group, 11.4% aged 36–45 years, 3.9% between 46–60 years, and 1.2% aged over 60 years. The mean age was 25.8 years, with minimum and maximum ages recorded as 18 and 70 years, respectively. The most represented occupational group was students, comprising 52.4% of the donor population. In terms of ABO blood group distribution, blood group B was most common, accounting for 33.7% of donors, followed by group O (31.6%), group A (24.6%), and group AB (10.1%). Regarding Rh factor, 91% of donors were Rh positive, while 9% were Rh negative. The overall prevalence of transfusion-transmissible infections (TTIs) among the donor population was 3.09% (n=1,437). Among these, hepatitis C virus (HCV) was the most prevalent TTI, affecting 1.20% (n=553) of donors. This was followed by hepatitis B virus (HBV) at 1.08% (n=504), syphilis at 0.61% (n=283), HIV at 0.20% (n=96), and malaria at 0.002% (n=1). The distribution of TTIs across ABO blood groups revealed the highest number of HCV cases in blood group B (34.9%), followed by O (32.9%), A (23.3%), and AB (8.9%). HBV was most prevalent in group O (33.4%), followed by B (29.6%), A (26%), and AB (11.1%). For HIV, blood group A had the highest proportion (34.4%), followed by B (29.2%), O (27.1%), and AB (9.4%). Blood group B also showed the highest prevalence of syphilis (40.3%), with decreasing proportions in groups O (26.1%), A (24.4%), and AB (9.2%).

In relation to Rh typing, Rh-negative donors had a higher positivity rate for all TTIs: 2.96% for HBV, 4.12% for HCV, 1.41% for HIV, and 1.69% for syphilis, compared to significantly lower positivity among Rh-positive donors. Statistical analysis showed significant associations between Rh blood group and all TTIs ($p < 0.00001$ for HBV, HCV, HIV, syphilis, and malaria). However, the association between ABO groups and TTIs was not statistically significant, though variations were observed ($p = 0.273$ for HBV, $p = 0.60$ for HCV, $p = 0.17$ for HIV, and $p = 0.08$ for syphilis). The prevalence of TTIs across age groups showed significant association ($p < 0.05$). Donors aged 26–35 years accounted for the highest proportion of TTI cases (50%, n=717), followed by those under 25 years (29.7%, n=427), 36–45 years (11.4%, n=164), 46–60 years (6.6%, n=95), and over 60 years (2.3%, n=34). Notably, HCV was more prevalent in donors younger than 25 years (1.02%), while HBV (1.27%), syphilis (0.87%), and HIV (0.12%) were most common in the 26–35 year age group.

Table 1: Characteristics of Blood Donor by different Age range, years of donation, Sex, ABO, and Rh Groups (n=46,453)

Variables	Age Range 18–25 years n=18,396 (39.6%)	26–35 years n=20,391 (43.9%)	36–45 years n=5,296 (11.4%)	46–60 years n=1,812 (3.9%)	≥60 years n=558 (1.2%)	Total
Years of donation						
2020	4,094	4,938	1,531	486	144	11,193
2021	3,846	3,920	1,209	461	124	9,560

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Variables	**Age Range**					
	18–25 years n=18,396 (39.6%)	**26–35 years n=20,391 (43.9%)**	**36–45 years n=5,296 (11.4%)**	**46–60 years n=1,812 (3.9%)**	**≥60 years n=558 (1.2%)**	**Total**
2022	1,791	1,920	408	153	074	4,346
2023	3,964	4,167	703	411	114	9,359
2024	4,701	5,446	1,445	301	102	11,995
Total	18,396	20,391	5,296	1,812	558	46,453
Sex						
Male	15,014	20,997	8,383	1,756	43	46,193 (99.44)
Female	102	93	65	0	0	260 (0.56)
Total	15,116 (32.5%)	21,090 (45.4%)	8,448 (18%)	1756 (3.8%)	43 (0.09%)	46,453
Rh	ABO					Total
	A	AB	B	O		
Negative	981 (2.1%)	467 (1.0%)	1,297 (2.8%)	1,429 (3.1%)		4,174 (9.0%)
Positive	10,438 (22.5%)	4,255 (9.1%)	14,339 (30.9%)	13,247 (28.5%)		42,279 (91%)
Total	11,419 (24.6%)	4,722 (10.1%)	15,636 (33.7%)	14,676 (31.6%)		46,453 (100.0%)

Table 2 Distribution of Participants Based on Their Occupations

Occupation	**Frequency**	**Percentage %**
Student	24,342	52.4
Farmer	2,788	6
Government employees	10,308	22.2
Doctors	6,968	15
Others	2,047	4.4
Total	46, 453	100

Table 3 Association between ABO and Rh blood group and TTI marker presence (n=46,453).

ABO	**HBV**		**HCV**		**HIV**		**Syphilis**		**Malaria**	
	Negative (n, %)	**Positive (n, %)**	**Negative (n, %)**	**Positive (n, %)**	**Negative (n, %)**	**Positive (n, %)**	**Negative (n, %)**	**Positive (n, %)**	**Negative (n, %)**	**Positive (n, %)**
A	11,289 (98.9%)	131 (1.1%)	11,290 (98.9%)	129 (1.1%)	11,386 (99.7%)	33 (0.3%)	11,350 (99.4%)	69 (0.6%)	11419 (100%)	0%
AB	4666 (98.8%)	56 (1.2%)	4673 (99.0%)	49 (1.0%)	4713 (99.8%)	9 (0.2%)	4696 (99.5%)	26 (0.5%)	4722 (100%)	0%
B	15,487 (99.05%)	149 (0.95%)	15443 (98.8%)	193 (1.25%)	15608 (99.8%)	28 (0.2%)	15522 (99.3%)	114 (0.7%)	15636 (100%)	0%
O	14,508 (98.9%)	168 (1.1%)	14494 (98.8%)	182 (1.26%)	14,650 (99.8%)	26 (0.2%)	14602 (99.5%)	74 (0.5%)	14675 (99.93%)	1 (0.007%)
Total	45,950	504 (1.08%)	45,900 (98.8%)	553 (1.20%)	46,357 (99.8%)	96 (0.20%)	46,170 (99.39%)	283 (0.61%)	46,452	1 (0.002%)

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ABO	HBV		HCV		HIV		Syphilis		Malaria	
	Negative (n, %)	Positive (n, %)	Negative (n, %)	Positive (n, %)	Negative (n, %)	Positive (n, %)	Negative (n, %)	Positive (n, %)	Negative (n, %)	Positive (n, %)
	X2 = 3.88, p = 0.273		X2 =1.85, p= 0 .60		X2 =5.00, p=0.17		X2 = 6.65, p=0.08			
Rh										
Positive	44,865 (98.96)	471 (1.04)	44,785 (98.8)	505 (1.12)	45,245 (99.82)	80 (0.18)	45,064 (99.42)	264 (0.58)	44,367	1 (0.002)
Negative	1,085 (97.04)	33 (2.96)	1,115 (95.81)	48 (4.12)	1,11 (98.58)	16 (1.41)	1106 (98.31)	19 (1.69)	2085	0
Total	45,950 (98.92%)	504 (1.08%)	45,900 (98.80%)	553 (1.20%)	46,357 (99.80%)	96 (0.20%)	46,170 (99.39%)	283 (0.61%)	46,452	1 (0.002%)
	X2 =37.5, p= < .00001		X2 = 87.74, p= .00001		X2 =83.4, p= < .00001		X2 =22.3, p= < .00001		X2 =37.5, p= < .00001	

Table 4: Association between different age groups with TTIs prevalence (n=46,453).

Age Group	HBV Negative	HBV Positive (%)	HCV Negative	HCV Positive (%)	HIV Negative	HIV Positive (%)	Syphilis Negative	Syphilis Positive (%)	Malaria Negative	Malaria Positive	Total Donors	TTI Positive Total
<25 years (39.6 %)	18,232	164 (0.89%)	18,208	188 (1.02%)	18,365	31 (0.16%)	18,352	44 (0.23%)	18,396	0	18,396	427
26–35 years (43.9 %)	20,132	259 (1.27%)	20,138	253 (1.24%)	20,366	25 (0.12%)	20,212	179 (0.87%)	20,390	1	20,391	717
36–45 years (11.4 %)	5,254	42 (0.79%)	5,240	56 (1.05%)	5,274	22 (0.41%)	5,252	44 (0.83%)	5,296	0	5,296	164
46–60 years (3.9%)	1,779	33 (1.82%)	1,773	39 (2.15%)	1,803	9 (0.49%)	1,798	14 (0.77%)	1,812	0	1,812	95
≥60 years (1.2%)	552	6 (1.07%)	541	17 (3.04%)	549	9 (1.61%)	556	2 (0.35%)	558	0	558	34
Total	45,949	504 (1.08%)	45,900	553 (1.20%)	46,357	96 (0.20%)	46,170	283 (0.61%)	46,452	1	46,453	1,437 (3.09%)

Chi-Square Test Results:

- HBV: $\chi^2 = 26.29$, p = 0.000028
- HCV: $\chi^2 = 36.27$, p = 0.00001
- HIV: $\chi^2 = 80.36$, p = 0.00001
- Syphilis: $\chi^2 = 71.57$, p = 0.00001

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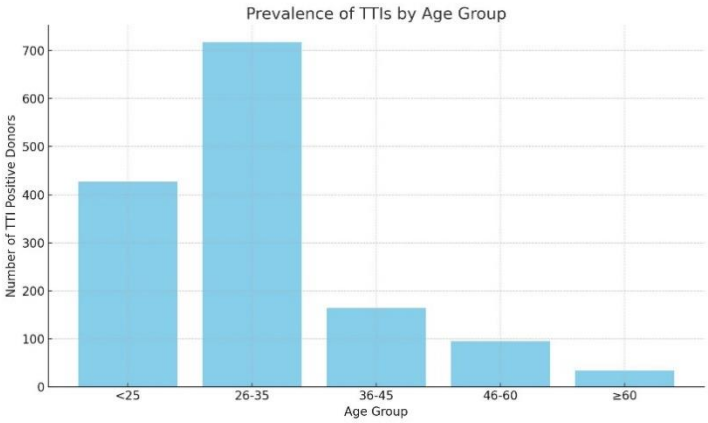


Figure 1 Prevalence of TTIs by Age Group

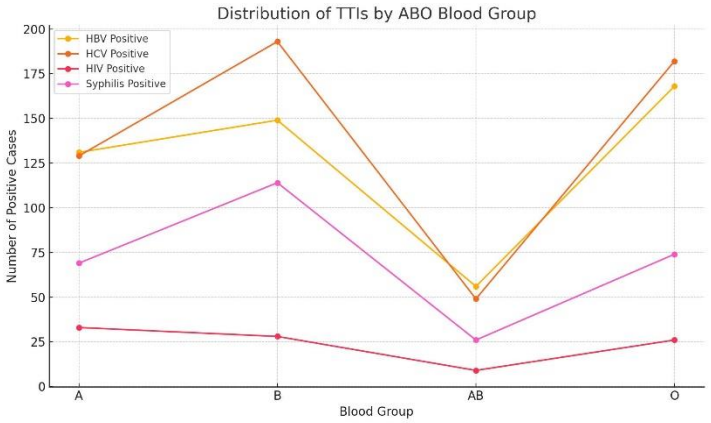


Figure 2 Distribution of TTIs by ABO Blood Group

DISCUSSION

The transfusion of blood and its components remains a vital, life-saving medical procedure globally; however, the transmission of blood-borne infections continues to pose a substantial threat, particularly in low- and middle-income countries where the prevalence of transfusion-transmissible infections (TTIs) remains significantly high (13). In these regions, despite advances in screening protocols, achieving a zero-risk transfusion environment remains a challenge. The estimated risk of TTI transmission through a single unit of blood is approximately 1%, a figure that gains significance given the severe and sometimes incurable nature of infections like hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, syphilis, and malaria (10,14). Education and awareness efforts aimed at mitigating high-risk behaviors, especially among vulnerable donor groups, play a pivotal role in addressing this issue (15). In Pakistan, blood safety remains a pressing public health concern due to the persistent incidence of TTIs coupled with logistical and infrastructural limitations in ensuring voluntary, low-risk donor pools (16,17). Although many studies have explored associations between ABO and Rh blood groups with infectious diseases, regional data examining these relationships remain limited, particularly within blood donor populations. The present study aimed to bridge this gap by investigating the association between ABO/Rh blood groups and the prevalence of TTIs in a large donor population.

The overall TTI prevalence in this study was 3.09%, with HCV emerging as the most prevalent infection (1.20%), followed by HBV (1.08%), syphilis (0.61%), HIV (0.20%), and malaria (0.002%). These findings are in alignment with previous regional reports from Islamabad and northern Pakistan, where TTI prevalence ranged between 3.3% and 3.7% (18,19). Compared to broader national and international studies, the prevalence observed here was lower than in studies conducted in Faisalabad (6.55%), Lahore (4.61%), Karachi (5.8%), and northern India (5.59%), but higher than the prevalence reported in Delhi (0.238%). Differences in donor demographics, awareness levels, socio-economic conditions, and the rigor of screening protocols may contribute to this variation (20). A striking demographic feature of the donor population was the overwhelming predominance of male donors (99.44%), a trend consistent with similar studies across developing regions (15). This gender imbalance is often attributed to physiological factors such as menstruation, pregnancy, and lactation, which limit female eligibility for blood donation. Additionally, anemia and low hemoglobin levels frequently disqualify female donors (12).

Analysis of blood groups revealed that group B was most prevalent, followed by O, A, and AB. While statistical associations between ABO blood groups and TTIs were not significant, a relatively higher prevalence of HCV was observed in blood group O. Although this aligns with findings from some studies that reported no definitive link between ABO blood groups and infection susceptibility (17,18), others have demonstrated an elevated risk of HCV among group O individuals (16). Similarly, blood groups B and AB exhibited higher rates of syphilis and HIV, though without statistical significance. HBV showed greater prevalence in blood groups A, O, and AB, which is consistent with earlier findings from local studies indicating a similar distribution (20). One of the notable findings was the significant association between Rh factor and TTI prevalence. Rh-negative donors had a comparatively higher burden of all four major infections analyzed. This was particularly prominent in HCV cases, echoing findings from earlier studies suggesting a possible biological or

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immunological basis for increased susceptibility in Rh-negative individuals (16). In contrast, other studies failed to establish such an association (11), indicating the need for further investigation to elucidate the mechanisms involved.

Malaria had the lowest prevalence among all TTIs, consistent with national trends and previous literature. This could be attributed to the urban origin of the majority of study participants, where malaria incidence tends to be lower compared to rural and under-resourced areas (6,21). The age distribution analysis revealed that the 26–35-year age group exhibited the highest prevalence of TTIs (50%), followed by the ≤25-year group. This trend is comparable to findings from earlier research, indicating that individuals in their most socially and occupationally active years are more exposed to risk factors associated with TTIs. HBV, HCV, and syphilis were particularly prominent in this age group. This pattern contrasts with other studies that observed higher prevalence in older age brackets (22), suggesting regional variation and potential differences in transmission dynamics and donor recruitment strategies.

The findings of this study support the hypothesis that blood group and age are potential factors associated with TTI prevalence, although not all associations reached statistical significance. The study's strengths include a large sample size, a well-defined donor population, and standardized testing protocols. However, several limitations warrant acknowledgment. The cross-sectional and retrospective design limits causal inference, and potential confounders such as socio-economic status, prior medical history, or behavioral risk factors were not assessed. Furthermore, while associations were explored, no molecular or immunogenetic investigations were conducted to elucidate mechanisms underlying these patterns. Future research should focus on multicenter studies with broader geographic representation and incorporate molecular diagnostics to explore host–pathogen interactions related to blood group antigens. In addition, targeted education and donor screening strategies may benefit from insights into demographic trends identified in this and similar studies. Overall, this research contributes valuable data to transfusion medicine and epidemiology in Pakistan by highlighting demographic and serological trends among blood donors and underscoring the need for further in-depth investigations into TTI transmission dynamics.

CONCLUSION

This study concluded that transfusion-transmitted infections remain a critical concern among blood donors, with hepatitis C emerging as the most prevalent. While no significant association was found between ABO blood groups and TTIs, a notable link was observed with the Rh factor. Additionally, the highest infection rates occurred in younger age groups, underscoring the need for focused health education and early intervention. These findings highlight the importance of robust donor screening protocols to ensure safer blood transfusion practices. Strengthening such preventive measures not only enhances patient safety but also contributes to broader public health goals, aligning with global health and sustainability initiatives.

AUTHOR CONTRIBUTION

Author	Contribution
Muhammad Adeel*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Nimra Ismaeel	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Mehrish Waheed	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Arsalan khan	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Zahra Khadim Hussain	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Mehak Irfan	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Tariq Shahzad	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Obaid Ullah	Writing - Review & Editing, Assistance with Data Curation

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