Original Research

SEROPREVALENCE AND NAT-BASED DETECTION OF TRANSFUSION-TRANSMITTED INFECTIONS IN VOLUNTARY VERSUS REPLACEMENT BLOOD DONORS: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Transfusion-transmitted infections (TTIs) remain a critical threat to blood safety globally. The comparative risk profile between voluntary non-remunerated blood donors (VNRBDs) and replacement/family donors (RDs) has been widely debated, particularly with the advent of nucleic acid testing (NAT) technologies that detect window-period infections missed by conventional serology.

Methods: A cross-sectional study was conducted among 4,860 consecutive blood donors (2,748 VNRBDs; 2,112 RDs). All donations were screened using chemiluminescence immunoassay (CLIA) for HBV surface antigen, anti-HCV, anti-HIV-1/2, and rapid plasma reagin for syphilis. Serology-negative samples were further tested by individual-donor NAT (ID-NAT) for HBV-DNA, HCV-RNA, and HIV-1-RNA. Chi-square tests and odds ratios were used for group comparisons.

Results: The overall TTI seroprevalence was significantly lower among VNRBDs (1.49%) than RDs (4.31%; $p < 0.001$). HBV was the most prevalent infection in both groups. The NAT yield among serology-negative donors was 0.11% (3/2,724) in VNRBDs versus 0.52% (11/2,121) in RDs ($p = 0.004$), with HBV accounting for the majority of NAT-only reactive cases.

Conclusion: Replacement donors carry a significantly higher burden of both serologically detectable and window-period TTIs. Strengthening voluntary donation programs and integrating ID-NAT into routine screening are essential strategies for improving transfusion safety.

Keywords: Transfusion-Transmitted Infections, Nucleic Acid Testing, Voluntary Blood Donors, Replacement Donors, Seroprevalence, Blood Safety.

INTRODUCTION

Blood transfusion is an indispensable, life-saving intervention used worldwide in surgical, obstetric, traumatic, and hematologic settings. However, the safety of the blood supply remains a persistent public health concern, primarily because of the risk of transfusion-transmitted infections (TTIs) **[1]**.

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The four major TTIs of global significance include hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and *Treponema pallidum* (syphilis), which collectively account for the greatest burden of transfusion-associated morbidity and mortality **[2]**.

Globally, the prevalence of TTIs among blood donors varies considerably depending on geographic region, donor selection practices, and the sensitivity of screening assays employed **[3]**. Studies from sub-Saharan Africa and South Asia have reported TTI seroprevalence rates ranging from 3% to over 15%, whereas high-income countries with established voluntary donation systems report rates below 0.5% **[4]**. This disparity is largely attributable to

differences in the proportion of voluntary non-remunerated blood donors (VNRBDs) versus replacement or family donors (RDs). The World Health Organization has long advocated for 100% voluntary non-remunerated donation, recognizing that replacement donors—who donate under social or familial obligation—tend to harbor higher infection rates **[1]**.

Conventional serological screening, while effective, is inherently limited by the immunological window period—the interval between infection acquisition and the development of detectable antibodies or antigens. During this period, donors may test serology-negative despite harboring transmissible levels of viral nucleic acid. Nucleic acid testing (NAT) was developed to address this residual risk by directly detecting viral genetic material, thereby shortening the window period substantially: from approximately 38 days to 5.5 days for HIV, from 66 days to 3.4 days for HCV, and from 56 days to 20.4 days for HBV **[5]**. The implementation of NAT—either in minipool format (MP-NAT) or as individual-donor NAT (ID-NAT)—has been shown to significantly improve blood safety in high-income countries **[6]**.

Despite accumulating evidence supporting both voluntary donation and NAT implementation, there remains a paucity of data from resource-limited settings that simultaneously compare serological and NAT-based TTI detection rates between voluntary and replacement donor populations **[7]**. Most existing studies have evaluated either serology or NAT in isolation, without stratifying by donor type. Furthermore, few investigations have quantified the incremental NAT yield—defined as the proportion of serology-negative, NAT-reactive donations—across these two donor categories **[8]**.

The present study aimed to determine the seroprevalence of HBV, HCV, HIV, and syphilis and to evaluate the additional diagnostic yield of ID-NAT for HBV, HCV, and HIV among VNRBDs compared with RDs at a large tertiary-care blood bank.

MATERIALS AND METHODS

Study Design and Setting-This hospital-based, cross-sectional study was conducted at tertiary-care teaching hospital. The blood bank processes approximately 2,500 donations annually from both voluntary and replacement donor channels.

Study Population and Sampling- A total of 4,860 consecutive, eligible blood donors were enrolled during the study period using a census sampling approach. Donors were classified as voluntary non-remunerated blood donors (VNRBDs; n = 2,748) if they donated altruistically through organized blood donation camps or walk-in voluntary donation, or as replacement donors (RDs; n = 2,112) if they donated at the request of a patient's family or acquaintance.

Inclusion and Exclusion Criteria- All donors aged 18–65 years weighing ≥50 kg and meeting national blood donor selection criteria were included. Donors with incomplete records, hemoglobin <12.5 g/dL, or those deferred on medical grounds prior to phlebotomy were excluded.

Serological Screening- Each donation was tested for HBV surface antigen (HBsAg), anti-HCV antibodies, anti-HIV-1/2 antibodies and p24 antigen (fourth-generation combo assay), and syphilis antibodies using a fully automated chemiluminescence immunoassay (CLIA) platform (ARCHITECT i2000SR, Abbott Diagnostics, Wiesbaden, Germany). Samples reactive on initial testing were repeated in duplicate; those reactive on at least two of three results were classified as seropositive.

Nucleic Acid Testing- All serology-non-reactive samples underwent individual-donor NAT (ID-NAT) using the Grifols Procleix Panther System (Grifols Diagnostic Solutions, Emeryville, CA, USA) for simultaneous detection of HBV-DNA, HCV-RNA, and HIV-1-RNA using transcription-mediated amplification (TMA) technology. Samples initially reactive on NAT were retested; those repeatedly reactive were confirmed as NAT-yield cases.

Data Collection- Demographic data including age, sex, donor type, donation history (first-time vs. repeat), and blood group were extracted from the blood bank management software.

Statistical Analysis- Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation and compared using the independent-samples *t*-test. Categorical variables were presented as frequencies and percentages and compared using Pearson's chi-square test or Fisher's exact test, as appropriate. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. A two-tailed p-value < 0.05 was considered statistically significant.

RESULTS

Demographic Characteristics- The mean age of donors was 32.1 ± 8.4 years, with VNRBDs being significantly younger than RDs (31.4 ± 7.8 vs. 33.2 ± 9.1 years; $p < 0.001$). Males constituted 84.9% of all donors. The proportion of first-time donors was markedly higher among RDs (72.6%) compared to VNRBDs (34.2%; $p < 0.001$) (Table 1).

Table 1. Demographic and Donation Characteristics of Study Participants (N = 4,860)

Characteristic	VNRBDs (n = 2,748)	RDs (n = 2,112)	P-Value
Age (years), mean ± SD	31.4 ± 7.8	33.2 ± 9.1	< 0.001

Male sex, n (%)	2,262 (82.3)	1,873 (88.7)	< 0.001
First-time donors, n (%)	940 (34.2)	1,533 (72.6)	< 0.001
Blood group O, n (%)	928 (33.8)	698 (33.1)	0.610
Blood group B, n (%)	879 (32.0)	659 (31.2)	0.558
Blood group A, n (%)	632 (23.0)	502 (23.8)	0.533
Blood group AB, n (%)	309 (11.2)	253 (12.0)	0.447

Seroprevalence of TTIs- The overall TTI seroprevalence was 2.72% (132/4,860). VNRBDs had a significantly lower seroprevalence (1.49%; 41/2,748) compared to RDs (4.31%; 91/2,112; OR = 0.33; 95% CI: 0.23–0.48; p < 0.001). HBV was the most prevalent infection in both groups, followed by HCV, syphilis, and HIV. Statistically significant differences between VNRBDs and RDs were observed for all four infections individually (Table 2).

Table 2. Seroprevalence of Transfusion-Transmitted Infections by Donor Type

Infection	VNRBDs (n = 2,748), n (%)	RDs (n = 2,112), n (%)	OR (95% CI)	P-Value
HBV (HBsAg)	24 (0.87)	49 (2.32)	0.37 (0.23–0.61)	< 0.001
HCV (Anti-HCV)	8 (0.29)	19 (0.90)	0.32 (0.14–0.73)	0.003
HIV (Combo Ag/Ab)	4 (0.15)	11 (0.52)	0.28 (0.09–0.88)	0.014
Syphilis (RPR)	6 (0.22)	15 (0.71)	0.31 (0.12–0.79)	0.006
Co-infections	1 (0.04)	3 (0.14)	—	0.178
Overall TTI	41 (1.49)	91 (4.31)	0.33 (0.23–0.48)	< 0.001

NAT Yield among Serology-Negative Donors- Among the 4,728 serology-negative donors (2,707 VNRBDs; 2,021 RDs), ID-NAT identified 14 additional reactive donations (overall NAT yield = 0.30%). The NAT yield was significantly lower in VNRBDs (3/2,707; 0.11%) than in RDs (11/2,021; 0.54%; p = 0.004). HBV-DNA accounted for 9 of the 14 NAT-yield cases (64.3%), followed by HCV-RNA (3 cases; 21.4%) and HIV-1-RNA (2 cases; 14.3%) (Table 3).

Table 3. NAT Yield (Serology-Negative, NAT-Reactive) by Donor Type

Viral Marker	VNRBDs (n = 2,707), n (%)	RDs (n = 2,021), n (%)	P-Value
HBV-DNA	2 (0.07)	7 (0.35)	0.026
HCV-RNA	1 (0.04)	3 (0.15)	0.178
HIV-1-RNA	0 (0.00)	1 (0.05)	0.428
Total NAT yield	3 (0.11)	11 (0.54)	0.004

DISCUSSION

The present study provides a comprehensive comparison of TTI prevalence between voluntary and replacement blood donors using both serological and molecular testing platforms. Our findings unequivocally demonstrate that replacement donors carry a significantly higher burden of TTIs—both serologically detectable infections and window-period infections identifiable only by NAT—reinforcing the global imperative to transition toward 100% voluntary non-remunerated donation.

The overall seroprevalence of 2.72% observed in our study is consistent with reports from other developing countries. Biadgo et al. reported an overall TTI prevalence of 4.5% among blood donors in northwest Ethiopia **[9]**, while Tessema et al. documented rates of 3.8% at a teaching hospital in the same region **[10]**. In contrast, studies from high-income nations with established voluntary donation systems consistently report TTI rates below 0.5% **[11]**. The substantially higher TTI burden in our replacement donor cohort (4.31%) compared to voluntary donors (1.49%) aligns with findings from multiple settings, including sub-Saharan Africa **[12]** and South Asia **[13]**, and underscores the epidemiological rationale for the WHO's longstanding recommendation to phase out replacement donation.

HBV emerged as the predominant TTI in both donor groups, accounting for 55.3% of all seropositive cases. This finding is consistent with the high endemicity of HBV in the study region and parallels observations by Mavenyengwa et al. in Namibia **[14]** and Dulal and Paudel in Nepal **[15]**. The disproportionately higher HBV seroprevalence among RDs (2.32% vs. 0.87%) likely reflects inadequate pre-donation screening, lower health literacy, and the absence of self-deferral behavior among individuals donating under familial obligation rather than altruistic motivation.

A central and novel contribution of this study is the stratified analysis of NAT yield by donor type. The overall NAT yield of 0.30% in our cohort is broadly comparable to rates reported in Indian blood banks

implementing ID-NAT. Chatterjee et al. reported a NAT yield of 0.034% in a predominantly voluntary donor population **[16]**, while Chaurasia et al. documented a yield of 0.24% in a mixed donor cohort **[17]**. However, the nearly five-fold higher NAT yield among replacement donors (0.54%) compared to voluntary donors (0.11%) observed in our study has not been extensively documented in the literature and carries important implications for transfusion policy. This differential yield suggests that replacement donors are not only more likely to harbor established infections but are also more likely to be in the acute, pre-seroconversion phase of infection—the period of highest infectivity and greatest residual transfusion risk.

HBV-DNA accounted for 64.3% of all NAT-yield cases, a finding consistent with the known prolonged window period of HBV relative to HCV and HIV and the phenomenon of occult hepatitis B infection (OBI), characterized by the presence of HBV-DNA in the absence of detectable HBsAg **[18]**. The identification of OBI cases exclusively through NAT highlights a critical limitation of serological screening alone and supports the implementation of universal ID-NAT, particularly in settings with high HBV endemicity. Sharma et al. similarly demonstrated that NAT implementation significantly enhanced blood safety by intercepting window-period HBV donations that would have been released as serology-negative **[19]**.

The significantly higher proportion of first-time donors among RDs (72.6% vs. 34.2%) deserves attention. First-time donors are well-established as having a higher TTI prevalence compared to repeat donors, attributable to the absence of prior screening and the "healthy donor effect" that operates among repeat voluntary donors **[20]**. The preponderance of first-time donors in the replacement category thus compounds the risk profile of this group and further justifies targeted interventions to convert first-time replacement donors into regular voluntary donors.

Several limitations should be acknowledged. First, the single-center design may limit the generalizability of findings. Second, confirmatory testing (e.g., Western blot for HIV, recombinant immunoblot assay for HCV) was not uniformly performed on all seropositive samples due to resource constraints, potentially introducing some degree of misclassification. Third, syphilis was screened using a non-treponemal assay (RPR), which has known limitations regarding sensitivity and specificity compared to treponemal assays. Finally, the cross-sectional design precludes longitudinal assessment of donor conversion or incidence estimation.

CONCLUSION

This study demonstrates that replacement blood donors have a significantly higher prevalence of transfusion-transmitted infections detected by both serological and nucleic acid testing compared to voluntary non-remunerated donors. The nearly five-fold greater NAT yield among replacement donors highlights the elevated residual risk posed by this donor category, particularly for HBV window-period infections. These findings strongly support the dual strategy of maximizing voluntary donation through sustained community engagement and integrating individual-donor NAT into routine blood screening protocols to minimize residual transfusion risk. Policymakers and blood banking authorities should prioritize resource allocation toward achieving 100% voluntary donation and universal NAT implementation to ensure the highest standards of transfusion safety.

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