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Prevalence of Hepatitis C Virus Infection Among Voluntary Blood Donors in Sikkim: Comparison of Third-Generation ELISA and RT-PCR

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ABSTRACT

Background: Blood transfusion carries a potential risk of transmitting transfusion-transmissible infections, including hepatitis C virus (HCV). Although serological screening has substantially reduced this risk, infections during the diagnostic window period may be missed.
Aim: To determine the prevalence of HCV infection among voluntary blood donors in Sikkim and to compare the effectiveness of third-generation enzyme-linked immunosorbent assay (ELISA) for HCV screening.
Methods: This cross-sectional study enrolled 302 voluntary blood donors using systematic random sampling. The sample size was calculated assuming an HCV prevalence of 0.27%, with 95% confidence level and 5% absolute precision. Serum samples were screened for anti-HCV antibodies using third-generation ELISA, followed by real-time PCR capable of detecting HCV genotypes 1–6. Prevalence was calculated with 95% confidence intervals (CI). Data were analyzed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA).
Results: The overall anti-HCV seroprevalence was 2.0% (6/302; 95%CI: 0.4%–3.6%). Male donors constituted 82.8% of the study population. Due to the small number of seroreactivity cases, subgroup comparisons were exploratory and not powered for inferential statistical testing.
Conclusion: The prevalence of active HCV infection among voluntary blood donors in Sikkim is relatively high compared to the East Himalayan Region. Third-generation ELISA appears adequate for routine donor screening in this low-prevalence setting, while molecular testing may be reserved for selected high-risk scenarios.

Keywords: Blood donors; ELISA; Hepatitis C virus; RT-PCR; Sikkim; Transfusion-transmissible infections.

INTRODUCTION

Blood transfusion is an essential component of modern medical care but continues to pose a risk of transmitting transfusion-transmissible infections (TTIs) despite significant improvements in donor selection and screening strategies. Among TTIs, hepatitis C virus (HCV) is of particular importance due to its efficient blood-borne transmission and its potential to cause chronic hepatitis, cirrhosis, and hepatocellular carcinoma [1,2].

Hepatitis C remains a major global public health problem, with many infected individuals remaining asymptomatic for prolonged periods [1]. The identification of HCV as the causative agent of non-A, non-B hepatitis led to major advances in blood safety worldwide [3]. Routine screening of blood donors using serological assays has substantially reduced transfusion-associated HCV transmission. Third-generation ELISA is widely used due to its high sensitivity and specificity and is recommended for blood donor screening in India by the National AIDS Control Organization (NACO) [4]. However, antibody-based assays may fail to detect infection during the early window period when viral RNA is present in the absence of detectable antibodies [5].

Molecular techniques such as RT-PCR directly detect HCV RNA and further shorten the diagnostic window period. Nucleic acid testing enhances transfusion safety by identifying HCV infection in seronegative donors [6]. Despite these advantages, routine molecular screening is not universally practiced in resource-limited settings, including many regions of India (4). The prevalence of HCV infection among blood donors in India ranges from 0.2% to 2%, with marked regional differences [7-9]. Studies from Northeast India have reported variable seroprevalence among blood donors [10-13]. Data from Sikkim remain limited [14,15].

The present study aimed to assess the prevalence of HCV infection among voluntary blood donors in Sikkim and to compare the diagnostic yield of third-generation ELISA and RT-PCR in this low-prevalence setting.

MATERIALS AND METHODS

Study design and setting

This cross-sectional study was conducted in the Departments of Pathology and Microbiology at Sikkim Manipal Institute of Medical Sciences after obtaining approval from the Institutional Ethics Committee.

Study population

Voluntary blood donors fulfilling eligibility criteria as per NACO guidelines [4] and providing written informed consent were included.

Sampling technique and sample size

Sample Size Calculation

The sample size was estimated using the single-proportion formula:
n=Z2×p×(1−p)/d2n = Z^2 \times p \times (1-p) / d^2n=Z2×p×(1−p)/d2
Where:

- Z = 1.96 (for 95% confidence level)
- p = expected prevalence (0.27%)
- d = allowable error (5%)

The minimum required sample size was calculated, and 302 donors were ultimately enrolled to enhance precision and compensate for potential exclusions.

Laboratory testing

Five milliliters of venous blood were collected aseptically. Serum was separated by centrifugation at 3000 rpm for 5 minutes. All samples were screened for anti-HCV antibodies using a third-generation ELISA kit. Subsequently, all samples were tested for HCV RNA by real-time PCR using a commercially available assay capable of detecting HCV genotypes 1–6, according to the manufacturer's instructions [5].

Follow-up of reactive donors

Sero-reactive donors were recalled and referred for further clinical evaluation and management.

Statistical analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA).

- Categorical variables were expressed as frequencies and percentages.
- Prevalence estimates were calculated with 95% confidence intervals using the Wilson method.
- Due to the limited number of seroreactivity cases (n = 6), inferential statistical comparisons between demographic groups were not performed to avoid type II error and unstable estimates.

RESULTS

The anti-HCV seroprevalence was 2.0% (6/302; 95%CI: 0.4%–3.6%). The prevalence of active viremia detected by RT-PCR was 0% (95%CI: 0%–1.2%).

Among ELISA-reactive donors, 33.3% (2/6) reported a prior history of treated HCV infection. No statistically meaningful association between demographic variables and seroreactivity could be established due to the limited number of positive cases. The detailed information is tabulated in Table 1.

Characteristic	Frequency (n)	Percentage (%)
Gender		
Male	250	82.8
Female	52	17.2
Age group (years)		
18–25	72	23.8
26–30	90	29.8
31–35	58	19.2
36–40	46	15.2
> 40	36	11.9
HCV screening		
ELISA reactive	6	2.0
RT-PCR positive	0	0
Previously treated HCV	2	0.7
Newly detected HCV	4	1.3

Table 1 Demographic characteristics and HCV screening results of voluntary blood donors (n = 302)

DISCUSSION

The observed anti-HCV seroprevalence of 2.0% falls within the upper range reported in Indian blood donor populations (0.2%–2%) [7-9]. However, the absence of detectable HCV RNA suggests that active viremic infection in this cohort is likely below 1.2% (upper bound of the 95% CI), supporting a low residual transfusion risk in this setting.

Studies from Northeast India have reported anti-HCV seroprevalence ranging from 0.4% to 1.8%, reflecting regional variability [10-13]. Previous studies from Sikkim have similarly documented low prevalence of transfusion-transmissible infections among blood donors [14,15]. The predominance of male donors and the higher proportion in the 26–30-year age group align with trends observed in Indian donor populations [8,9].

None of the ELISA-reactive samples was positive by RT-PCR. Persistence of anti-HCV antibodies despite the absence of detectable viremia in treated or spontaneously resolved infections is well documented [5]. The remaining RNA-negative seroreactive cases may represent resolved infections or false-positive ELISA results. From a transfusion safety perspective, the absence of detectable HCV RNA indicates that none of the seropositive donors was actively infectious, suggesting a negligible risk of transfusion-transmitted HCV in this cohort. This highlights the efficiency of current donor screening protocols.

The absence of RNA-positive, antibody-negative donors indicates that no additional yield from universal molecular screening was demonstrated in this cohort. While nucleic acid testing reduces the diagnostic window period, its incremental benefit appears minimal in low-prevalence populations, consistent with prior transfusion safety modelling studies [6,16,17].

Given infrastructure and cost considerations, selective implementation of molecular screening based on epidemiological risk assessment may be a more cost-effective strategy in similar low-prevalence settings.

LIMITATIONS:

- The relatively small sample size limits the precision of prevalence estimates.
- The low number of seroreactive cases restricts inferential statistical analysis and risk factor modelling.
- A single-centre design may affect external validity.
- The study was not powered to detect rare window-period infections.

CONCLUSION

The upper bound of the 95% confidence interval for active HCV infection suggests an extremely low residual transfusion risk in this population. Third-generation ELISA is sufficient for routine donor screening in this low-prevalence setting, while RT-PCR may be reserved for selected high-risk or high-prevalence scenarios. Future multicenter studies incorporating cost-effectiveness analyses may better define the role of universal nucleic acid testing in low-prevalence regions. These findings emphasize the importance of confirmatory molecular testing in blood donor screening to avoid overestimation of infection burden.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING STATEMENT

No external funding was received for this study.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was reviewed and approved by the Institutional Ethics Committee of Sikkim Manipal Institute of Medical Sciences (Approval No.: SMIMS/IEC/2023-105).

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