

**Seroprevalence of Zika Virus among Voluntary Blood Donors**Vijaya T.<sup>1</sup>, Ruth Jenila A.<sup>2</sup>, Deepak J.<sup>3</sup><sup>1</sup>Assistant Professor, Department of Transfusion Medicine, Govt. Villupuram Medical College Hospital, Villupuram, Tamil Nadu, India<sup>2</sup>Assistant Professor, Department of Transfusion Medicine, Govt. Stanley Medical College Hospital, Chennai, Tamil Nadu, India<sup>3</sup>Assistant Professor, Department of Transfusion Medicine, Govt. Vellore Medical College Hospital, Vellore, Tamil Nadu, India

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Conflict of interest: Nil

**Abstract****Background:** Zika is one of the emerging vector borne viral infections worldwide and it has the potential to be transmitted by transfusion of blood and blood products.**Aim:** To find the seroprevalence of Zika virus among voluntary blood donors. Also to evaluate the possibility of cross-reaction, coinfection subsequent or secondary infection with Dengue.**Materials and Methods:** 297 blood samples from voluntary blood donors were collected in one year period from July 2019 to June 2020 and were subjected to anti-Zika IgG serological tests using My Biosource ZIKV ELISA kits. All those samples were subjected to detect anti-DENV IgG serological test using In Bios dengue ELISA kits.**Results:** Among 297 VBD, 24 donors (8%) were positive for anti- ZIKV IgG. 269 samples found to be seropositive for anti-DENV IgG. 23 samples which were found to be reactive with both anti- ZIKV IgG and anti-DENV IgG.**Conclusion:** The seroprevalence of ZIKV among Voluntary blood donors in our study was around 8%. Since ZIKV infection potentially causes complications like congenital Zika syndrome and Guillain Barre Syndrome, it is worthwhile to screen blood units for ZIKV at least for high risk patients.**Keywords:** Blood donors, Zika virus, Transfusion Transmission, Immunoglobulin G, Screening, ELISA test.**DOI:** 10.25258/ijcpr.18.3.254

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**Introduction**

Blood transfusion serves as a critical, life-saving therapy in modern medicine, yet the safety of the blood supply is constantly challenged by emerging pathogens and shifting epidemiological patterns. To mitigate the risk of transfusion-transmitted infections (TTIs), the World Health Organization (WHO) and India's national blood policy emphasize reliance on voluntary, unpaid donors. [1] While mandatory screening in India currently targets Hepatitis B, Hepatitis C, HIV, Syphilis, and Malaria, [2] the risk of transmission persists due to asymptomatic carriers and donations made during the "window period" of an infection.

Zika virus (ZIKV) has recently emerged as a significant threat to blood safety worldwide. Because a high proportion of infected individuals remain asymptomatic, they may unknowingly donate blood, potentially transmitting the virus to recipients. [3]

This is a grave public health concern given the association of ZIKV with severe complications, including Guillain-Barré syndrome (GBS) and congenital microcephaly.[4] This cross sectional study was conducted to observe and analyze the seroprevalence of Zika virus among voluntary blood donors by detection of Zika IgG antibodies which may be of help to decide whether screening for Zika virus would eliminate transmission of infection to high risk groups.

Such information may be of great value to health planners and policy makers.

**Aim & Objectives:**

The aim of the study is to find the Seroprevalence of Zika virus among the voluntary blood donors.

1. To estimate the Seroprevalence of Zika virus among the voluntary blood donors.

2. To detect anti- ZIKV IgG by ELISA.
3. To evaluate the possibility of cross-reaction, subsequent or secondary infection (Zika with previous Dengue) or co-infection, all samples were subjected to detect anti-DENV IgG by ELISA.

### Materials and Methods

This Cross-sectional study was conducted over one year period from July 2019 to June 2020 in the Department of Transfusion Medicine, The Tamil Nadu Dr. M.G.R. Medical University, Guindy, and Chennai. A total of 297 voluntary blood donors were selected as per DGHS Guidelines. The donors were classified as higher, middle and lower socioeconomic status based on Modified Kuppusamy classification. [5] Five ml of blood was collected directly from voluntary blood donors in a sterile plain test tube and allowed to clot, serum was separated and stored at -20o C for ELISA.[6] The samples that were frozen earlier were thawed according to manufacturer's instruction and used. Sera were tested for anti-Zika IgG by the enzyme-linked immunosorbent assay (ELISA). Since there are no FDA (Food and Drug Administration) licensed blood donor screening ELISA IgG kits available, commercial, research-use-only My BioSource ZIKV Detect IgG Capture ELISA Kits were used in our study. All those samples were subjected to detect anti- Dengue IgG by ELISA using commercial Diagnostic InBios DENV Detect IgG. All steps were done according to the manufacturer's instructions. Data analysis was done using SPSS software.

**Ethical Issues:** Human ethical clearance was obtained from the Institutional Ethical Committee of The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

### Result

This cross-sectional study showed the age distribution among the blood donors 47.5 % in 18-20 years, 41.4 % in 21-30 years, 8.4 % in 31-40 years, 2.0 % in 41-50 years, 0.7 % in >50 years. (Table:1) Demographic analysis showed out of 297 donors, 252 (84.8%) were males and 45 (15.2%) were females. Most of our donors belong to middle socioeconomic status (72%) followed by low (19.2%) and high (8.8%). Blood group distributions among the blood donors were 23.2 % of 'A' group, 33.7 % of 'B' group, 37.4 % of 'O' group, 5.7 % of 'AB' group. Rh distributions among donors were 95.3 % of Rh D positive group, 4.7% of Rh-negative group. (Table:2) Around 8 % of the donors gave history of having dengue in the past, remaining 92% denied of having dengue in the past. (Table:3) ZIKV IgG antibody screening by ELISA showed that 24 were positive and 273 were negative, giving an overall ZIKV prevalence rate of 8%. DENV IgG antibody screening by ELISA showed that 269 were positive and 28 were negative, giving an overall DENV prevalence rate of 90.6%. 269 DENV IgG positive includes 23 anti-ZIKV IgG seropositive samples. One of the samples was reactive exclusively for anti-ZIKV IgG. Of the 269 DENV IgG positive samples, 246 were exclusively reactive for DENV, while 23 showed co-reactivity with ZIKV.

**Table 1: Demographic characteristics of blood donors and their association with ZIKV Igg seropositivity**

Donor demographic details		IgG seropositive (total donors) P value	
Age (Years)	18-20	12(141)	P>0.05
	21-30	8(123)	
	31-40	4(25)	
	41-50	0(6)	
	>50	0(2)	
Gender	Male	21(252)	P>0.05
	Female	3(45)	
Socioeconomic status	High	3(26)	P>0.05
	Medium	18(214)	
	Low	3(57)	

**Table 2: Donor blood groups and their association with ZIKV IgG seropositivity**

Blood Group	A	9(69)	P>0.05
	B	6(100)	
	O	8(111)	
	AB	1(17)	
Rh Type	Positive	24(283)	P>0.05
	Negative	0(14)	

**Table 3: Dengue history and their association with ZIKV IgG seropositivity**

Dengue History	ZIKV IgG seropositive donors	Percentage %
Present	2(24)	8.3%
Absent	22(24)	91.7%

## Discussion

The emerging and reemerging infectious agents remain to be a constant threat to the safety of the blood supply. Emerging blood borne pathogens are mostly asymptomatic in healthy donors and are detected only when transfusion recipients show some clinical morbidity or mortality. Thus, any new TTI is more significant.

The present study was undertaken to define the seroprevalence of Zika infection among voluntary blood donor population. Our blood transfusion centre has 100% voluntary blood donation, hence the present study comprised only of voluntary blood donors. Voluntary blood donors with asymptomatic infection of emerging pathogens like ZIKV may contribute to the risk of Transfusion Transmitted Zika infection.

In our study, 24 out of 297 (8%) voluntary blood donors were positive for IgG anti-ZIKV antibody, which suggests the past exposure to Zika virus infection. In a similar study done by I-Ching Sam et al [7] among 1085 blood donors in Kuala Lumpur reported 7.6 % to be positive for anti-ZIKV IgG. Bouba Gake et al [8] reported 5% anti-ZIKA IgG seropositivity among blood donors in Cameroon.

In the present study, 90.6% (269 out of 297) of samples showed positivity for anti-DENV IgG and it shows clearly that our study area is known endemic for Dengue. This is somewhat closer to the study done by Oruganti et al reported 89.5% to be positive for dengue IgG. [9]

Out of 24 positive anti-ZIKA IgG positive samples, 23(96%) showed anti-DENV IgG seropositivity. This is similar to the study done by A study done by Yu-Wen Chien et al [10] among 212 participants in Southern Taiwan reported 4.2% (9/212) to be positive for anti-ZIKV IgG antibody. Eight (89%) out of 9 samples positive for anti-ZIKV IgG were also positive for anti-DENV IgG.

In our study, 23 (96%) anti-DENV IgG seropositivity among 24 anti- ZIKV IgG positive samples could suggest the possibility of cross-reactivity or Zika with previous dengue infection (ZIKVwpDENV) or co-infection.

These data are consistent with the literature on the possible cross- reactivity [11] as the envelope (E) proteins elicits the major antibody response after flaviviral infection. ZIKVs and DENVs are closely related, with the amino acids in their envelope (E) proteins sharing a homology of 51–54%. [12] Serological tests for flaviviruses have previously focused on the E protein, using recombinant E protein, inactivated virions, or virus- like particles. [11] The greatest limiting factor in the IgG E

protein-based ELISAs is the cross- reactivity of antibodies between different flaviviruses.

Among 269 DENV IgG positive donors only 23 were found to be positive for anti-Zika IgG and the remaining 253 were negative for anti-Zika IgG. So, the cross reactivity cannot be concluded. Hence, 23 samples which were found to be reactive with both ZIKV and DENV to be considered as co- infection or secondary/ subsequent infection (eg: ZIKVwpDENV), rather than cross-reaction. Exact conclusion of Zika virus existence could not be derived with these analysed data without confirmatory tests.

Co-infections by DENV and ZIKV and by DENV and CHIKV have been previously reported in a study in Colombia. [13]

Diagnostic testing of secondary flavivirus infections is further complicated by the rapid reappearance of cross-reactive antibodies. In addition, anamnestic responses resulting from previous flavivirus infection boost high avidity antibody titres that makes the serology even more complicated. So most sensitive assays are needed to distinguish the recent Zika infection from ZIKV infection with previous flavivirus infections like dengue in patients. [14]

Only one donor among 297 donors showed positivity exclusively for anti-ZIKV IgG. Furthermore, anti- DENV IgG or anti- ZIKV IgG reactivity should not be employed solely without confirmatory testing because of high rate of false positivity and false negativity in serological platform. So, the serological results are presumptive not confirmatory.

According to criteria used by AABB, Zika virus should be classified as a high-risk agent that threatens the safety of blood recipients. 15 Blood donations that positive for Zika virus is quarantined and removed from blood supply.

India is considered as the vulnerable country to Zika with 67,422 travelers arriving per year across the globe. Approaches like implementation of stringent donor eligibility criteria is needed. At present in India according to NBTC guidelines, Zika infected donor is deferred for 4 months following recovery and also following travel to Zika virus outbreak zone.

Implementation of an effective pathogen reduction technology may offer a better level of safety. Since it would be expensive to perform pathogen inactivation of all blood products for this emerging infection, high risk group approach (pregnant women and foetuses) may be made practically available.

**Table 4: Comparison of ZIKV seroprevalence findings with other studies**

Study	Place	IgG seropositivity	DENV seropositivity for ZIKV IgG Positive samples	IgM seropositivity	RT-PCR
Present study	Chennai, India (n=297) (Blood donors)	8% (24/297)	96% (23/24)	-	-
Bouba Gake et al [8]	Cameroon (n=1084) (Blood donors)	5% (54/1084)	-	-	-
Yu-Wen Chien et al [10]	Southern Taiwan (n =212) (General population)	4.2% (9/212)	89% (8/9)	0% (0/212)	-
I-Ching Sam et al. [7]	Kuala Lumpur (n= 1085) (Blood donors & Inpatients)	7.6% (82/1085)	90.2% (74/82) (PRNT)	-	-

However, to prevent transfusion transmissible Zika virus infection it is prudent to elicit proper history, other signs and symptoms suggestive of Zika and to perform confirmatory tests by RT-PCR and anti-Zika IgM to screen out asymptomatic donors.

### Conclusion

The seroprevalence of ZIKV among Voluntary blood donors in our study was around 8%. Since ZIKV infection potentially causes complications like congenital Zika syndrome and Guillain Barre syndrome, it is worthwhile to screen blood units for ZIKV at least for high-risk patients like pregnant women and fetuses. However, to prevent transfusion transmissible Zika virus infection it is prudent to elicit proper donor history, other signs and symptoms suggestive of Zika. The 8% seroprevalence, coupled with the limitations of serological testing, supports the need for confirmatory testing (RT-PCR/IgM) to screen out asymptomatic/presymptomatic donors for high-risk recipients, despite the current unavailability of approved screening kits. To arrive at a definitive conclusion, it is imperative to do a study on large number of voluntary blood donors with preferably approved test kits.

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