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## **Research Paper**

# Seroprevalence and Trend of Transfusion Transmissible Infections (TTIS) Among Blood Donors At A Teaching Hospital In Abuja, Nigeria: A Five Years' Review.

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#### ABSTRACT.

Questionable quality of blood and blood components for clinical use pose great risks to patients who need transfusion. There is limited information on the seroprevalence of transfusion transmissible infections among blood donors in the Federal Capital Territory. This retrospective descriptive study determined the seroprevalence and trend of human immunodeficiency virus, hepatitis B and C viruses, and syphilis infection serological markers among blood donors at the Blood Bank unit of the University of Abuja Teaching Hospital, Gwagwalada, Federal Capital Territory, Nigeria. This baseline data provides one of the essential information required for monitoring and evaluation of the safety of blood and blood components for clinical use. Materials and Methods: Rapid test strips were used to screen all prospective blood donors for HIV 1/2 antibodies, HBsAg, HCV antibodies, and Syphilis respectively. Data abstracted from blood donor register at the UATH blood bank included HIV 1/2 antibodies, HBsAg, anti-HCV antibodies, and Syphilis screening tests results; age; sex; and blood donation type from  $1^{st}$ , January,  $2014 - 31^{st}$ , December, 2018. Data analysed with Statistical Package for Social Science (SPSS) vs 26 and Microsoft Excel 16. Chi-square test was used for comparison and p value< 0.05, 95% CI wasconsidered statistically significant. Results: A total of 26,712 blood donors were screened for TTIs, 25,139 (94.11%) of them were males and 1,573 (5.89%) females, with blood donors' mean age of 32.3 years (range 16 - 65 years). Majority 25,534 (95.59%) were family replacement blood donors (FREPBDs) and 1,178 (4.41%) voluntary non-remunerated blood donors (VNRBDs). The overall seroprevalence rates of TTIs and TTI coinfections among blood donors in this study was 11.88% and 1.02% respectively, statistically significant declining trend was noted in TTIs seroprevalence rates from 14.94% to 10.03% (p < 0.05%, 95% Cl) in 2014to 2018 respectively. Conclusion: The study revealed a high seroprevalence of TTIs and TTI coinfections, which is consistent with reported findings among high risks blood donor groups like FREBDs (95.80% in our study) and paid donors (p < 0.05, 95% Cl) and the declining trend in TTIs seroprevalence rates during the study period.

**KEYWORDS:** Transfusion transmissible infections (HIV, HBV, HCV and Syphilis), trend, blood donation, FREPBDs, VNRBDs and TTIs coinfection.

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#### I. INTRODUCTION:

Transfusion transmissible infections (TTIs) such as human immunodeficiency virus (HIV 1&2), hepatitis B and C viruses (HBV and HCV), and treponema pallidum constitute a major challenge for transfusion safety especially in Sub-Saharan-Africa where these diseases are endemic <sup>1,2,3,4</sup>. Questionable quality of blood and blood components for clinical use pose great risks to transfused recipients <sup>5,6</sup>. Blood components therapies have been reported to be the route of infection in 5% - 10% of all people living with Human immunodeficiency virus (PLWHIV) and post-transfusion hepatitis in 12.5% of patient <sup>7,8,9</sup>, <sup>10,11</sup>, <sup>12</sup>.

Blood is a vital healthcare resource used for many clinical interventions, and has no alternative for patients in real need of blood transfusion, thus, timely and affordable access to screened blood and blood components for clinical use is essential for effective and efficient healthcare service delivery globally<sup>6,10,13</sup>. Nigeria being a member of the 46 World Health Organization African Region (WHO-AR) countries, adopted

the WHO resolutions on blood safety, and the recommended strategies for blood safety for developing countries. Thus, it is mandatory to screen all donor blood components for HIV, HBV, HCV and Syphilis infection serological markers (using Enzyme Linked Immunosorbent assay/EIA and Venereal Disease Research Laboratory test (VDRL) with supplementary and/or confirmatory tests for reactive donor blood (WHO guidelines for TTIs screening algorithm)<sup>14</sup>, <sup>15</sup>, <sup>16</sup>. However, the National Blood Transfusion Policy which is imperative for full implementation of the National Blood Transfusion Service (NBTS) established since 2006 "to coordinate blood transfusion services on a countrywide bases within the National Health Plan"is yet to be enacted <sup>17</sup>. Therefore, blood banking and blood transfusion services are still fragmented, uncoordinated and unregulated in both public and private healthcare facilities. Studies have shown that 75% - 95% of blood donation is nurtured and premised on the culture of emergency blood collection from high risk blood donor groups (familyreplacement and paid blood donors)<sup>18</sup>, <sup>19</sup>, <sup>20</sup>, <sup>21</sup>, <sup>22</sup>.

Studies from various parts of Nigeria have reported wide seroprevalence range of HBV, HCV, HIV and Syphilis infection markers among prospective blood donors ranging from 0.9% - 22.7% <sup>23</sup>, <sup>24</sup>, <sup>25</sup>, <sup>26</sup>. There is limited information on the seroprevalence rate of transfusion transmissible infections (TTIs) among blood donors in the Federal Capital Territory (FCT). Thus, the objective of this retrospective descriptive study was to determine the seroprevalence and trend of HIV, HBV, HCV and syphilis infection serological markers among blood donors at the Blood Bank unit of the Department of Haematology and Blood Transfusion, University of Abuja Teaching Hospital (UATH), Gwagwalada, Abuja, Federal Capital Territory (FCT), Nigeria., during the study period 1<sup>st</sup>, January, 2014 to 31<sup>st</sup>, December, 2018. And generate the baseline data on TTIs among blood donors in the FCT that is vital for monitoring and evaluation of blood safety and availability.

#### II. MATERIALS AND METHODS:

In this retrospective descriptive study, data was abstracted from blood donor register at the blood bank unit, UATH, Gwagwalada between 1<sup>st</sup>, January, 2014 and 31<sup>st</sup>, December, 2018. UATH is 500 bedded Tertiary Hospital located in Gwagwalada City, Head Quarters of Gwagwalada Area Council, Federal Capital Territory, North Central Nigeria. The hospital serves as a referral hospital to patients from the FCT and other states in Nigeria.

Predonation screening of all blood donors were performed with rapid diagnostic test (RDT) strips: Determine (Alere Medical Co. LTD) for HIV 1/2 antibodies, OfaStep (Polymed Therapeutics, Inc. Houston, TX, USA) for HBsAg and Anti-HCV, and Aria (CTK Biotech Inc. Poway. CA, USA) for Syphilis. All tests were performed according to manufactures' instructions. Data abstracted from the blood donor register at the UATH blood bank included the screening tests results for HIV 1/2 antibodies, HBsAg, anti-HCV antibodies, and Syphilis antibodies and the demographic characteristics: age, sex and type of blood donation for the period of the study. Microsoft Excel 2016 and Statistical Package for Social Science (SPSS), version 26.0, were used for data analysis. Chi-square test was used for comparison of frequency values, a P-value of <0.05 and 95% confidence interval (CI) considered statistically significant. The ethical approval for conducting the study was obtained from the University of Abuja Teaching Hospital Health Research Ethics Committee.

## III. RESULTS:

A total of 26,712 prospective blood donors were screened for HIV, HBV, HCV and Syphilis infection markers during the five years' period, 94.11% (n = 25,139) of them were males and 5.89% (n = 1,573) females with a male: female ratio of 16:1. Family replacement donors (FREPDs) accounted for 95.59% (n = 25,534) of them, while 1,178 (4.41%) were voluntary non-remunerated blood donors (VNRBDs). The mean age of the blood donors was 32.3 years (range 16-65 years), 94.85% (n = 25,337) were between the ages of 16years – 45years, while 5.15% (n =1,375) were in ages46-65 years, Table 1 showed the demographic characteristics blood donors.

The overall seroprevalence rate of TTIs serological markers among blood donors in this study was 11.88% (n = 3174) and 1.02% (n = 273) tested positive for two or more TTIs markers. Year wise, there was declining trend in the cumulative seroprevalence rates from 14.94% (n = 487) in 2014to 13.66% (560), 11.7% (n = 772), 11.87% (n = 731) and 10.03% (624), in 2015, 2016, 2017 and 2018 respectively. However, the trend for coinfection increased from 0.13% (36) in 2014 to 0.23% (n = 61) and 0.33 (n =89) in 2015 – 2016, and declined significantly to 0.22% (n =58) and 0.11% (n = 29) in 2017 –2918 respectively. The cumulative seroprevalence rates for the specific major TTIs: HIV; HBsAg; Anti-HCV and Syphilis, were 1.45% (n = 388); 4.40% (1176); 3.52% (n = 939); and 2.51% (n = 671) respectively, (table 2).

Evaluation of TTIs seroprevalence rates by prospective blood donor gender showed no significant difference in the overall seroprevalence rates of TTIs between males 11.94% (n = 3002) and females 10.93% (n = 172). TTIs serological markers was more prevalent among FREPBDs 12.25% (n = 3129) than VNRBDs 3.82% (n = 45). Age group 26-35 years constituted the predominant blood donor population 43.79% (n = 11698), they also had the highest TTIs seroprevalence rate of 13.83% (n= 1618) among the age groups. Age

groups 16 - 25 years, 36 - 45 years and 46 - 55 years had 10.61% (n= 706), 10.51% (n = 734) and 8.92% (n = 116) respectively, while all blood donors in age group 56 - 65 years tested negative for the major TTIs serological markers, (table 3).

#### IV. DISCUSSION:

Studies have shown that Nigeria is hyperendemic for HBV infection with a wide prevalence rates range of 1.2% – 26%; while HCV, HIV and syphilis are endemic with prevalence rates of 0.5% - 12.3%; 0.3% - 5.6% and 0.1% - 4% respectively, in adult population<sup>27, 28, 29</sup>. Our study revealed high seroprevalence rates of TTIs among blood donors at the University of Abuja Teaching Hospital, with overall seroprevalence rates of 11.88% and 1.02% for TTIs and TTIs coinfectionsrespectively (Table 2). This finding is consistent with the reported high seroprevalence rates of 12.3 and 12.9% from similar studies conducted by Fasola et al (South Western Nigeria)<sup>19</sup> and Keleta YT et al (Eritrea)<sup>30</sup>. It is however less than the seroprevalence rates reported from other studied in Nigeria, 22.7% and 17.7% (Daramola et al Ekiti and Olusola et al Abeokuta, South Western Nigeria)<sup>25</sup>, <sup>26</sup>; 15.5% (Onoja AM et al - Jos, North-Central Nigeria)<sup>31</sup>; 15% (Okoroiwu HU - Calabar, South-South Nigeria)<sup>22</sup>. Other African countries have also reported higher seroprevalence rates than ours, 29.5% (Bisetenge FS et al - Ethiopia)<sup>32</sup>, 18.04% (Fouelifack Ymele F et al - Cameroon)<sup>33</sup> 15.9% (Matee MIN et al -Dar Es Salaam, Tanzania)<sup>34</sup>, 15% (William Ampofo – Ghana)<sup>35</sup> and 14.76% (Arzouma Paul et al – Burkina Faso)<sup>36</sup>. The difference may be attributed to the screening methods in these facilities and/or higher prevalence of these infections in their general population. Our finding is however, in contrast with the reported low seroprevalence rates of TTIs among blood donors of 0.9% (Ijeoma Nwokeukwu et al - Enugu, South-East, Nigeria)<sup>23</sup>, 3,1% (Mohamed Osman Abdelaziz - North state, Sudan)<sup>37</sup> and 0.43% (Irm Yasmeen et al – India)<sup>38</sup>. Statistically significant (p < 0.05) declining trend in the seroprevalence rates of TTIs was noted from 2016 -2018, with a surge in TTIs between 2016 and 2017 that was not statistically significant (table 2).

Table 1: Demographic Characteristics of Blood Donors at UATH, Gwagwalada: 2014 -2018.							
<b>Demographic Characteristics</b>	No.	%					
Gender							
Males	25139	94.11%					
Females	1573	5.89%					
Total	26712	100.00%					
Type of Blood Donation							
VNRBD	1178	4.41%					
FREPBD	25534	95.59%					
Total	26712	100.00%					
Blood Donor Age Group							
16 - 25	6654	24.91%					
26 - 35	11698	43.79%					
36 - 45	6985	26.15%					
46 - 55	1301	4.87%					
56 - 65	74	0.28%					
Total	26712	100%					

	2: Year wis						: 2014 -2				0		
	Blood Donors	HIV		HBsAg		Anti-HCV		Syphilis		All TTIs		Coinfections	
YEAR	No.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
2014	3260	64	1.96%	195	5.98%	138	4.23%	90	2.76%	487	14.94%	36	0.13%
2015	4099	76	1.85%	214	5.22%	171	4.17%	99	2.42%	560	13.66%	61	0.23%
2016	6076	87	1.25%	293	4.20%	202	2.90%	190	2.72%	772	11.07%	89	0.33%
2017	6157	97	1.58%	259	4.21%	200	3.25%	175	2.84%	731	11.87%	58	0.22%

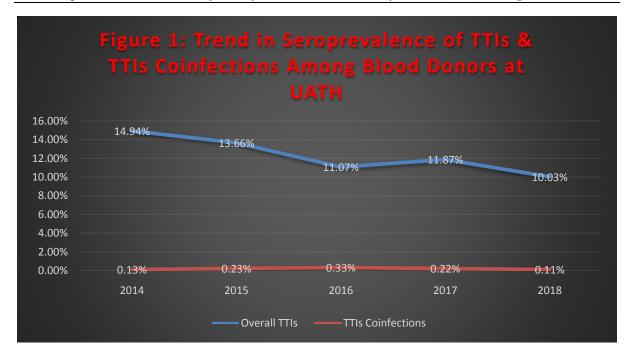
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2018	6220	64	1.03%	215	3.46%	228	3.67%	117	1.88%	624	10.03%	29	0.11%
P-value			< 0.05		< 0.05		< 0.05		< 0.05		< 0.05		< 0.05
2014- 2018	26712	388	1.45%	1176	4.40%	939	3.52%	671	2.51%	3174	11.88%	273	1.02%

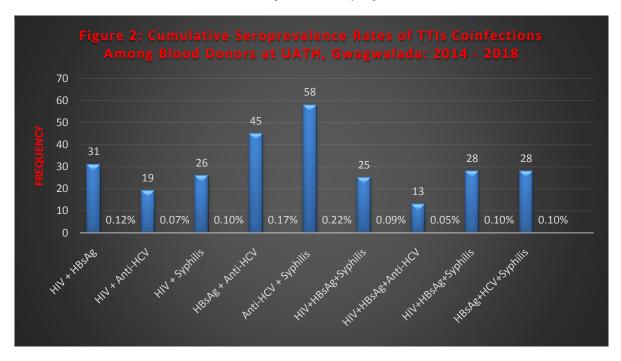
		-	2014 - 2018	1	1	1	
<b>Blood Donors</b>		HIV	HBsAg	Anti-HCV	Syphilis	ALL TTIs	
Blood Donor Gender	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Male	25139 (94.11%)	363 (1.44%)	1120 (4.46%)	889 (3.54%)	630 (2.51%)	3002 (11.94%)	
Female	1573 (5.89%	25 (1.59%)	56 (3.56%)	50 (3.18%)	41 (2.61%)	172 (10.93%)	
P-value	p = 0.000	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05	
Total	26712 (100.00%)	388 (1.45%)	1176 (4.40%)	939 (3.52%)	671 (2.51%)	3174 (11.88%)	
Type of Blood De	onation	1	1	I.	I		
VNRBD	VNRBD 1178 (4.41%)		25 (2.22%)	20 (1.70%)	0 (0.00%)	45 (3.82%)	
FREPBD	25534 (95.59%)	388 (1.52%)	1151 (4.51%)	919 (3.60%)	671 (2.67%)	3129 (12.25%)	
P-value	p = 0.000	p = 0.000	p < 0.002	p < 0.002	p = 0.000	p < 0.001	
Total	26712 (100.00)	388(1.45%)	1176 (4.40%)	939 (3.52%)	671 (2.51%)	3174 (11.88%)	
Age groups	1	1	1	· L	· L	1	
16 - 25	6654 (24.91%)	98 (1.47%)	259 (3.89%)	196 (2.95%)	153 (2.30%)	706 (10.61%)	
26 - 35	11698 (43.79%)	184 (1.57%)	615 (5.26%)	485 (4.15%)	334 (2.86%)	1618 (13.83%)	
36 - 45	6985 (26.15%)	89 (1.27%)	259 (3.71%)	225 (3.22%)	161 (2.31%)	734 (10.51%)	
45 -55	1301 (4.87%)	17 (1.31%)	43 (3.31%)	33 (2.54%)	23 (1.78%)	116 (8.92%)	
56 -65	74 (0.28%	0 (0.00%)	0 (0.00%)	0.00 (0%)	0 (0.00%)	0 (0.00%)	
Total	26712 (100.00%)	388 (1.45)	1176 (4.40%)	939 (3.52%)	671 (2.51%)	3174 (11.88%)	
	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.05	

The overall seroprevalence rate of TTIs coinfections in our study was comparable with the reported 2.2% by Onoja et al (Nigeria)<sup>31</sup>, 1.8% Matee MIN et al (Tanzania)<sup>34</sup> and 1.1% Fouelifack Ymele F et al (Cameroon)<sup>33</sup>. The high seroprevalence rates of coinfections could be attributed to the fact that the risk factors for infection with these major TTIs are similar, thus, infection with one infectious agent may facilitate infection with the other TTIs. Despite the significant decline in TTIs seroprevalence rates, statistically significant increased trend was noted in TTIs coinfections seroprevalence rates in 2015 and 2016 which dropped significantly in 2017 and 2018 (As shown in table 2 and figure 1). Dual infections with HCV and syphilis, was the most common coinfection observed in this study (figure 2).

HBsAg was the most prevalent serological marker of TTIs observed in this study, followed by HCV, Syphilis and lastly HIV with 4.40%, 3.52%, 2.51% and 1.02% respectively (as shown in table 2 and figure 2). This is in agreement with reports from some parts of Nigeria by Fasola et al 19; Olusola BA et al 25; Daramola et al 26; Onoja AM et al 31,0kon K et al 39; AO Ugwu et al 40 and Dike Kingsley Ugochukwu et al 41. This finding is also in agreement with reports from some African countries by Belay Tessema et al 10, F. S. Bisetegen et al 32. Contrary to our finding, syphilis was the predominant major TTI found in a study from Eritrea 33. There was year wise decline in the seroprevalence rates of HIV from 2015, however, statistically significant declinewas noted only in 2016 and 2018. Seroprevalence rates of HBV also declined slightly in 2015, then significantly from 2016 to 2018. Although there was decline in seroprevalence of HCV from 2015, marked decline (p < 0.05) was seen only in 2016 and 2017. The highest seroprevalence rate for syphilis was observed in 2017, thedecline in seroprevalence of syphilis in 2015 and 2017 were not statistically significant, however, in 2018, a significant decline in seroprevalence rate was noted. This irregular and inconsistent pattern of TTIs seroprevalence could be attributed to poor blood donor selection process (as often encountered with FREPBDs and paid blood donors).



Large population of high-risk blood donors increase the prevalence of TTIs in donated blood. It was estimated that replacement blood donation will contribute 75% - 80% of all the blood donated for clinical use in the WHO African region countries<sup>42</sup>. Studies have also shown that 83.3%, 16.4% and 0.3% of whole blood were donated by VNRNBDs, FREPBDs and Paid donors respectively, globally. While more than 90% donated blood is by VNRBDs in developed countries, only 17 countries in the WHO Africa region reported collection of > 90% whole blood from VNRBDs, 22 countries are considered dependent on FREPBDs and paid blood donors. Dependence on FREPBDs may affect timely supply of safe and quality blood and blood components for clinical use, which sometimes may be fatal<sup>5</sup>. FREPBDs accounted for 95.59% of blood donor population in this study while 4.41% were VNRBDs (table 1). Our finding was relatively higher than the WHO estimates for the African

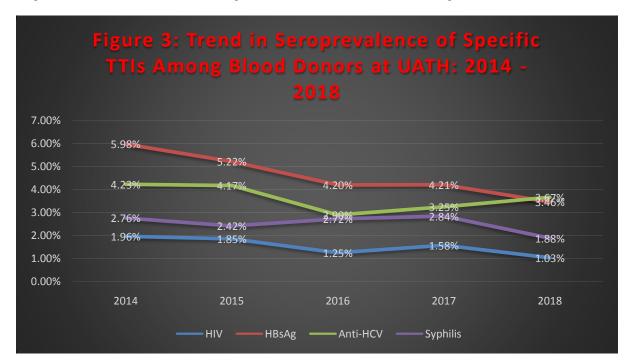


regions. Our finding was however, consistent with reports from various regions of Nigeria 19, 20, 21, 23, 24, 25, 26, 27, 28 and some African countries such as Ethiopia, Ghana and Sudan that showed FREPBDs constituted 88.0%, 96.3%, and 100% of their blood donor population respectively 32, 35, 43. There were no paid donors among the blood donors in our study and most similar studies from other regions of Nigeria. This finding could be due to the fact that paid blood donors often pose as relatives or friends of patients/recipients. Nonetheless, A O Ugwu

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et al (South Eastern Nigeria) reported 59.2%, 22.1% and 18.7% of blood donors were FREPBDs, Paid blood donors and VNRBDs respectively in their study<sup>40</sup>. Studies have shown that FREPBDs are twice as likely to test positive for TTIs as VNRBDs<sup>44</sup>. We found statistically significant higher seroprevalence rates of TTIs among FREPBDs (12.25%) compared with VNRBDs (3.82%) (as shown in table 3). The observed high seroprevalence of TTIs in this study is also comparable with reports from studies both within Nigeria<sup>22, 23, 31, 39</sup> and other African countries<sup>30, 34, 43, 45</sup>. We however observed that, though the TTIs were more prevalent among FREPBDs, the risk of transmitting HBV and HCV is substantial among VNRBDs. This finding could be attributed to the higher burden of the two major TTIs in Nigeria relative to HIV and Syphilis<sup>27, 28, 29</sup>. In contrast to our observation, similar study from India found no significant difference in the prevalence of TTIs between VNRBDs and FREPBDs<sup>46</sup>.

The data in this study revealed that males were the major blood donor population, they constituted 94.11% while females accounted for 5.89%, with male female ratio 16:1 (table 1). This finding was concordant with the reported median blood donations from female blood donors in Africa of 25% (range: 2 – 73%)<sup>5</sup>. Similar studies conducted in Nigeria and other countries were also agreeable with our observation <sup>19, 20, 21, 23, 24, 30, 32, 35</sup>. Despite the disparity in the gender distribution, there was no statistically significant difference in the gender specific seroprevalence rates of TTIs between males and females ( shown in table 3). It was also reported that persons aged less than 24 years accounted for 42% of blood donors in Africa, and globally, 40% of blood donations were by donors aged 25 – 44years<sup>5</sup>. The predominant blood donor age group in this study was 26 – 35 years, which accounted for 43.97% of the blood donors, followed by 36-45 years, 16 – 25 year, 46 – 55 years and 56 – 65 years age groups, these constituted 26.15%, 24.91%, 4.87% and 0,28% of the blood donors reportively, this finding concorded with above reports<sup>5</sup>. It was noted that 94.85% of blood donors were within 16 – 45 age range, studies from within and outside Nigeria also showed similar blood donor age bracket <sup>19, 20, 21, 23, 24, 32, 35, 43</sup>.



Age group 26-35 years had the highest age group specific TTIsseroprevalence rates(All TTIs -13.83%; HIV – 1.57%; HBsAg – 5.26%; Anti-HCV – 4.15% and Syphilis – 2.86%(table 3 showed the age group distribution of TTIs among blood donors). Statistically significant difference was found in the seroprevalence rates of TTIs between age group 26-35 compared withage groups 46-55 years (8.92%) and 56-65 years (0.00%). All blood donors in age group 55-65 yearstested negative for serologic markers of TTIs. This observation could be attributed to the low-risk life styles of these persons, despite the high prevalence of the TTIs in the general population  $^{27}$ ,  $^{28}$ ,  $^{29}$ .

The prevalence of TTIs in donated blood is directly dependent on the prevalence of the TTIs in the general population, the type of blood donation, the effectiveness of blood donor recruitment and selection process. There is high burden of TTIs in Sub-Saharan Africa, the WHO Global status report on blood safety and availability 2016 showed that low-income countries have the highest prevalence of the four major TTIs (HIV, HBV, HCV and syphilis) in blood donated for clinical use<sup>5</sup>. The high overall seroprevalence rate of TTIs found

in this study (11.88%) could be attributed to the high burden of these major TTIs among adults in Nigeria<sup>27</sup>, <sup>28</sup>, <sup>29</sup> as well as the large proportion of relatively high-risk blood donor population(table 1).

#### V. CONCLUSION:

This study revealed high overall seroprevalence rate (11.88%) of serologic markers of TTIs among blood donors at the University of Abuja Teaching Hospital, Gwagwalada. The data alsoshowed that blood and blood components for clinical use were donated mainly by family replacement blood donors and males, both accounted for 95.59% and 94.11% ofblood donors at UATH respectively. The study also generated for the first time, the requisite baseline data for monitoring and evaluation of blood and components afety, and policy formulation to improve the current blood safety status. The study has thus, highlighted the lack of compliance and the ability to comply with the current NBTS recommended mandatory TTIs screening algorithm for testing donor blood in Nigeria due to resource constrains. The dire need for enactment the National Blood Transfusion Policy by the legislature cannot be over emphasized. We advocate for adequate funding of the NBTS to ensure sustainable and affordable blood transfusion program which can provide timely adequate, safe and quality blood and blood components for clinical use. This is imperative for the achievement of the sustainable development goal 3.

#### LIMITATIONS:

The NBTS recommended screening algorithm for all donor blood is not operational in this center due to lack the requisite facilities occasioned by limited resources in our setting. Studies have shown that further screening with automated ELISA tests and confirmatory tests techniques after the initial screening with rapid diagnostic test (RDTs) kits, captured missed reactive as well as false positive donated blood. Fasola et al found additional 3.9% seroprevalence among blood donors during the post-donation screening with automated ELISA after the pre-donation RDTs screening<sup>19</sup>. Thus, the seroprevalence rates of TTIs found in our study might not be the true reflection of seroprevalence rates of TTIs among blood donors at UATH.

#### **AUTHORS' CONTRIBUTIONS:**

All the authors participated in the research. T. I. OTU conceptualized and designed the study, involved in data abstraction and analysis, and wrote the manuscript, U. G. EJIKEME contributed in data abstraction and manuscript writing and P. Onyeka contributed in data abstraction and manuscript writing. All authors read and approved the final manuscript.

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