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Seroprevalence of transfusion transmissible infections by ELISA in donors testing negative with rapid ICT in Asokoro District Hospital, Abuja, Nigeria

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Abstract:

Background: Blood transfusion saves lives, but it is associated with many complications which include transfusion transmissible infections (TTIs). The major objectives of this study were to determine; the prevalence of TTIs in the blood donated at Asokoro District Hospital, Abuja, Nigeria from 01 January to 31 December 2019; investigate the accuracy of rapid immunochromatographic (ICT) tests for preliminary TTIs screening of blood donors' samples and confirmed by ELISA test; and establish baseline data that will provide the impetus for improvement in equipment, infrastructure, and health system management, with the ultimate goal of ensuring safer blood transfusion practice.

Methodology: This was a retrospective cross-sectional study of records of blood donors at the Blood Bank of Asokoro District Hospital, a quasi-tertiary centre situated at Abuja, Federal Capital Territory (FCT), northcentral Nigeria, between 01 January to 31 December 2019. The socio-demographic biodata (age and gender), blood parameters (PCV and blood groups), and TTIs test results (by rapid ICT kit and ELISA) of donors were manually extracted from the record books of the hospital's blood bank. Only donor blood samples that initially tested negative for TTIs met the inclusion criteria. The data were entered into the spreadsheet of Microsoft Excel, and analysed with the Statistical Package for the Social Sciences (SPSS) for Windows version 23.0. Chi square test was used to determine association between variables and p value less than 0.05 was considered statistical significance.

Results: A total of 1400 blood samples of donors, received within the study period, met the inclusion criteria of testing negative for TTIs on rapid ICT kit test. The mean age (± SD) of the donors was 35.6±6.7 years with age range of 18-67 years. The median age was 35 years, and the predominant age group was 30-39 years, accounting for 55.4% (775/1400). Majority of the donors (97.1%, 1359/1400) were males. In terms of ABO blood group distribution, group O Rh D positive was the commonest, followed by group A Rh D positive, while the least is group AB Rh D positive. The overall prevalence of TTIs by ELISA test was 4.9% (68/1400). This prevalence was highest for HCV (1.9%, 26/1400), followed by HIV (1.2%, 17/1400), HBV (0.9%, 13/1400) and syphilis (0.9%, 12/1400).

Conclusion: The detection of TTIs by ELISA test from false negative donor samples preliminarily screened with rapid ICT kit highlighted by our study speaks to the unreliability of rapid ICT kits in screening of blood donors for TTIs. There is need therefore for health authorities in Nigeria and other LMICs to ensure widespread availability of highly sensitive blood screening methods such as ELISA to the point where it will be possible to enforce legislation against the use of the less accurate rapid ICT screening kits.

Keywords: transfusion transmissible infections; seroprevalence; ELISA; rapid ICT kit; blood donors

Séroprévalence des infections transmissibles par transfusion par ELISA chez les donneurs testés négatifs avec des TIC rapides à l'hôpital du district d'Asokoro, Abuja, Nigeria

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Résumé:

Contexte: La transfusion sanguine sauve des vies, mais elle est associée à de nombreuses complications, notamment les infections transmissibles par transfusion (ITT). Les principaux objectifs de cette étude étaient de déterminer; la prévalence des ITT dans le sang donné à l'hôpital du district d'Asokoro, Abuja, Nigeria du 1er janvier au 31 décembre 2019; enquêter sur l'exactitude des tests immunochromatographiques rapides (ICT) pour le dépistage préliminaire des ITT des échantillons de donneurs de sang et confirmés par un test ELISA; et d'établir des données de référence qui donneront l'élan nécessaire à l'amélioration de l'équipement, de l'infrastructure et de la gestion du système de santé, dans le but ultime d'assurer une pratique plus sûre de la transfusion sanguine.
Méthodologie: Il s'agissait d'une étude transversale rétrospective des dossiers des donneurs de sang à la banque de sang de l'hôpital du district d'Asokoro, un centre quasi-tertiaire situé à Abuja, Territoire de la Capitale Fédérale (FCT), centre-nord du Nigeria, entre le 1er janvier et le 31 décembre 2019. Les biodonnées socio-démographiques (âge et sexe), les paramètres sanguins (PCV et groupes sanguins) et les résultats des tests ITT (par kit ICT rapide et ELISA) des donneurs ont été extraits manuellement des registres de la banque de sang de l'hôpital. Seuls les échantillons de sang de donneurs initialement testés négatifs pour les ITT répondaient aux critères d'inclusion. Les données ont été saisies dans le tableur de Microsoft Excel et analysées avec le progiciel statistique pour les sciences sociales (SPSS) pour Windows version 23.0. Le test du chi carré a été utilisé pour déterminer l'association entre les variables et une valeur de p inférieure à 0,05 a été considérée comme une signification statistique.
Résultats: Un total de 1400 échantillons de sang de donneurs, reçus au cours de la période d'étude, répondaient aux critères d'inclusion de test négatif pour les ITT sur le kit de test rapide ICT. L'âge moyen (± ET) des donneurs était de 35,6±6,7 ans avec une tranche d'âge de 18 à 67 ans. L'âge médian était de 35 ans et la tranche d'âge prédominante était de 30 à 39 ans, représentant 55,4% (775/1400). La majorité des donneurs (97,1%, 1359/1400) étaient des hommes. En termes de distribution des groupes sanguins ABO, le groupe O Rh D positif était le plus courant, suivi du groupe A Rh D positif, tandis que le groupe AB Rh D positif était le moins important. La prévalence globale des ITT par test ELISA était de 4,9% (68/1400). Cette prévalence était la plus élevée pour le VHC (1,9%, 26/1400), suivi du VIH (1,2%, 17/1400), du VHB (0,9%, 13/1400) et de la syphilis (0,9 %, 12/1400).
Conclusion: La détection des ITT par test ELISA à partir d'échantillons de donneurs faussement négatifs préalablement sélectionnés avec un kit ICT rapide mis en évidence par notre étude témoigne du manque de fiabilité des kits ICT rapides dans le dépistage des ITT chez les donneurs de sang. Il est donc nécessaire que les autorités sanitaires du Nigéria et d'autres PFR-PRI garantissent la disponibilité généralisée de méthodes de dépistage sanguin très sensibles telles que l'ELISA au point qu'il sera possible d'appliquer la législation contre l'utilisation des kits de dépistage rapide des TIC moins précis.

Mots clés: infections transmissibles par transfusion; séroprévalence; ELISA; kit TIC rapide; donneurs de sang

Introduction:

Blood transfusion is a very important aspect of management of patients with various medical and surgical conditions. Although blood transfusion saves lives, it is also associated with many complications which include transfusion transmissible infections (TTIs). Unsafe blood transfusion increases the risk of TTIs (1). Blood safety remains a big issue in transfusion medicine. The World Health Organisation (WHO)

recommends that all blood donors should be screened for TTIs principally human immuno-deficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis caused by *Treponema pallidum* (1).

TTIs constitute an unacceptably high public health burden. HBV and HCV caused 1.1 million deaths globally in 2019, while HIV was estimated to cause 680,000 deaths globally in 2020, with an estimated 7.1 million new cases (2). In sub-Saharan Africa, access to safe and

adequate blood transfusion services has remained a challenge (3). The National Blood Service Commission (NBSC) is the Nigerian government institution charged with the responsibility of providing safe blood and blood products, and regulating blood transfusion services nationwide. According to NBSC data, 90% of the total blood donation in Nigeria is sourced from commercial donors (4). Commercialised blood donation raises serious ethical and safety concerns, and recipients of blood from commercial donors run the risk of contracting TTIs such as HIV, HBV, HBC and syphilis (1).

The threats posed by TTIs are high, and so accurate diagnosis of infectious agents in donor blood is a major strategy for safe blood. In developing countries like Nigeria, the rapid diagnostic immuno-chromatographic techniques (rapid kits), which are easier to use and inexpensive, are widely used for donor blood screening rather than the Enzyme-Linked Immunosorbent Assay (ELISA) testing. ELISA testing is costly and requires higher technology and trained personnel, but they are more accurate for the diagnosis of TTIs (5).

In as much as rapid tests are being used to detect TTIs in many blood banks in Nigeria to overcome poor funding and equipment constraints, they raise major concerns on their accuracy to diagnose TTIs in the blood donors. Therefore, this retrospective study was done to determine the prevalence of TTIs in the blood donated at Asokoro District Hospital, Abuja, Nigeria from 01 January to 31 December 2019; investigate the accuracy of rapid kit tests for four TTIs namely HIV, HCV, HBV and syphilis in blood donors' samples subjected to confirmatory testing using ELISA technique; and establish baseline data that will hopefully provide impetus for improvements in health system management, equipment and infrastructure that will ultimately ensure safer blood transfusion practice

Materials and method:

Study location:

The study was conducted at Asokoro District Hospital Blood Banking Service (ADHBBS). Asokoro District Hospital is a quasi-tertiary centre situated in Abuja, Federal Capital Territory (FCT), Nigeria. It serves as a referral centre for cases from primary and secondary healthcare facilities in the FCT, and other neighbouring states such as Kogi, Nasarawa and Niger States. The ADHBBS provides TTI-tested blood and blood products. All prospective donors are screened for TTIs with rapid kits and only those who test negative to all four TTIs

are allowed to donate. The donor samples are then further tested by ELISA and only donor blood whose samples are confirmed negative for all four TTIs are retained in the blood bank for use. All the procedures conducted by the ADHBBS are in strict compliance with the guidelines in the Nigerian National Blood Policy issued by the National Blood Service Commission (6).

Study design and population:

This is a retrospective cross-sectional study of records of blood donors at ADHBBS from 01 January to 31 December 2019. Donors are predominantly voluntary non-remunerated and family replacement donors. The socio-demographic biodata (age and gender), blood parameters (PCV and blood groups), and TTIs test results (rapid kit and ELISA) of the donors were manually extracted from the record books of the ADHBBS. Only donor blood samples that initially tested negative for these TTIs (HIV, HCV, HBV and syphilis) met the inclusion criteria. Cases with missing or incomplete biodata and test results were excluded.

Ethical consideration:

Ethical clearance was obtained from the Medical Ethics Committee of Asokoro District Hospital with approval number; FCTA/HHSS/HMB/ADH/114/22.

Screening methods:

Rapid tests for TTIs were conducted using lateral flow immuno-chromatographic (ICT) assay for qualitative detection of antibodies. The following rapid kits were used during the study period; Abbot Determine HIV-1/2, Atlas Medicals Rapid Test Strips (IgG and IgM) for *Treponema pallidum* (VDRL), HCV and HBsAg. ELISA tests were carried out using fourth generation kit (ADALTIS/EIAgen) for *in-vitro* diagnostic screening in human serum and plasma, to detect antibodies to HBsAg, HCV, syphilis, HIV-1, HIV-2 and HIV-1 p24 antigen. Diagnostic sensitivity and specificity rates for these tests hover between ≥ 99.7% and 100%. Detection limit for p24-antigen was 1 IU/ml, based on the WHO 1st International Reference code 90/636 (7). The manufacturers' instructions, as stated in the inserts of all the test kits, were strictly followed in the performance of these tests.

Data analysis:

The data retrieved from the blood bank record book were entered using the spreadsheet of Microsoft Office 365 version of Excel (Microsoft Corporation, Redmond, Washington, USA), and exported to the Statistical Package

for the Social Sciences (SPSS) for Windows, version 23.0 (SPSS Inc. Chicago, Illinois, USA) for analysis. Chi square test was used for determining association between variables and *p* value less than 0.05 was considered statistical significance.

Results:

A total of 1400 blood samples of donors, received within the study period met the inclusion criteria of testing negative for TTIs on rapid kit testing. The mean age (± SD) of the participants was 35.6 ± 6.7 years with a range of 18-67 years while the median age was 35 years. The preponderance of blood donors was in the 30-39 years age group, accounting for 55.4% (775/1400) while the least (0.4%, 6/1400) was in the <20 years age group. Majority of the donors, accounting for 97.1% (1359/1400), were males (Table 1).

With respect to ABO blood group distribution of donors, group O Rhesus D positive, accounting for 61.1% (855/1400), was the commonest, followed by group A Rhesus D positive (17.9%, 250/1400), while AB Rh D positive (0.1%, 1/1400) was the least common (Table 2). The prevalence of HIV among donors using ELISA was 1.2% (17/1400) while that of HBV, HCV and syphilis were 0.9% (13/1400), 1.9% (26/1400), 0.9% (12/1400) respectively (Fig 1, Table 3).

Table 1: Socio-demographic characteristics of blood donors in Asokoro District Hospital, Abuja, Nigeria

Variables	Gender		Total (%)
	Male (%)	Female (%)	
Age range (years)	18-67	21-43	18-67
Mean age ± SD (years)	35.7±6.6	29.6±5.2	35.6±6.7
Median age (years)	35	29	35
95% CI	35.4-36.1	27.9-31.3	35.2-35.9
Age group (years)			
<20	6 (0.4)	0	6 (0.4)
20-29	219 (15.6)	23 (1.6)	242 (17.3)
30-39	758 (54.1)	17 (1.2)	775 (55.4)
40-49	339 (24.2)	1 (0.1)	340 (24.3)
≥50	37 (2.6)	0	37 (2.6)
Total	**1359 (97.1)**	**41 (2.9)**	**1400 (100.0)**

Table 2: ABO Blood group distribution of donors

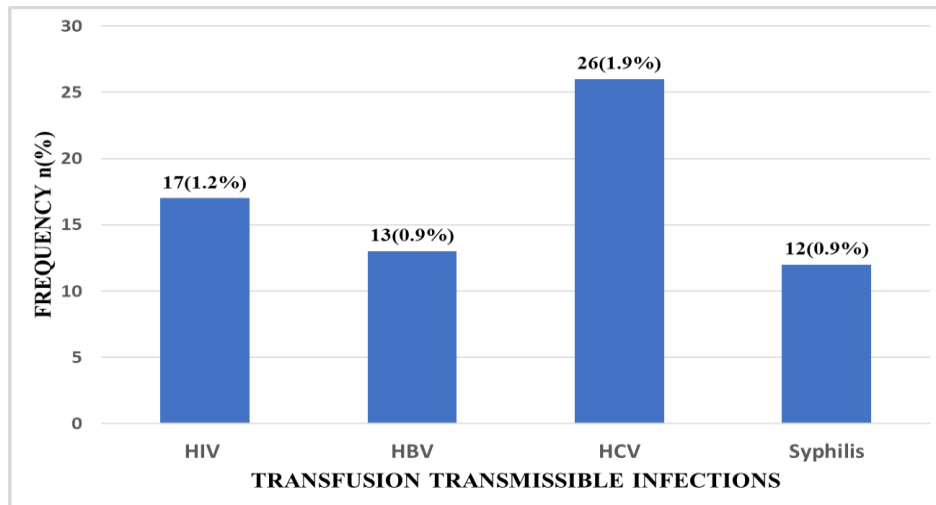| Blood group	Frequency	Percent
AB+	1	0.1
A-	10	0.7
A+	250	17.9
B-	14	1.0
B+	219	15.6
O+	855	61.1
O-	51	3.6
Total	**1400**	**100.0**

Fig 1: Prevalence of TTIs with ELISA in donors who tested negative with rapid ICT kits

Table 3: Prevalence of transfusion transmissible infections by ELISA test with respect to age group of donors with false negative rapid ICT kits

Age group (years)	HIV (%)		HBV (%)		HCV (%)		Syphilis (%)	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
<20	0	6 (100.0)	0	6 (100.0)	0	6 (100.0)	0	6 (100.0)
20-29	2 (0.8)	240 (99.2)	4 (1.3)	238 (98.3)	5 (2.1)	237 (97.9)	4 (1.7)	238 (98.3)
30-39	10 (1.3)	765 (98.7)	4 (0.5)	771 (99.5)	17 (2.2)	758 (97.8)	6 (0.8)	769 (99.2)
40-49	5 (1.5)	335 (98.5)	5 (1.5)	335 (98.5)	2 (0.6)	338 (99.4)	2 (0.6)	338 (99.4)
≥50	0	37 (100.0)	0	37 (100.0)	2 (5.4)	35 (94.6)	0	37 (100.0)
Total	**17 (1.2)**	**1383 (98.7)**	**13 (0.9)**	**1387 (99.1)**	**26 (1.9)**	**1374 (98.1)**	**12 (0.9)**	**1388 (99.1)**
Chi square	1.056		4.302		6.212		2.527	
p value	0.901		0.376		0.184		0.640	

HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus

The prevalence of TTIs by ELISA test was not significantly associated with age group of the donors (*p*>0.05) for all TTIs, although age groups 20-29, 30-39, and 40-49-years were most frequently affected, except for HCV in which age group >50 years had the highest prevalence of 5.4% (2/37) while the prevalence in age group <20 years was 0% for all the TTIs (Tables 3).

Discussion:

Transfusion transmissible infections poses a major challenge to safe blood transfusion services especially in sub-Saharan Africa. Most laboratories and blood bank service providers in developing countries still rely on the use of rapid kits for pre-donation screening of blood donors due to the unavailability of ELISA testing facilities in most of these blood banks (8). The mean age of the participants of 35.6±6.7 years in our study is similar to the findings of Buseri et al., (8) in southwest Nigeria, while the age range of 18-67 years is similar to the reports of studies done by Buseri et al., (8), Khan et al., (9) in northwest Frontier Province, India, and Mukhtar et al., (10) in northwest Nigeria, who reported age range of 18-64 years, 18-61 years and 18-60 years respectively. Majority (55.4%, 775/1,400) of the donors in our study were in the 30-39 years age group, findings similar to the studies of Buseri et al., (8) and Mukhtar et al., (9). The reasons for this are unclear but could be related to the hospital policy that encourages blood donation from spouses of women who attend

the antenatal clinic. Majority of the blood donors were males (97.1%, 1,359/1,400) and this is similar to reports of studies done by Mukhtar et al., (10), Egah et al., (11) and Nwo-kediuko et al., (12) with 98%, 95% and 91.8% being males respectively.

After ELISA testing, the prevalence of HIV infection in donor samples in this study was 1.2% (17/1400), while the prevalence of HBV, HCV and syphilis were 0.9% (13/1400), 1.9% (26/1400), and 0.9% (12/1400) respectively. These findings highlight the reduced accuracy of rapid kit screening. This discordance between the rapid kit test and ELISA was also reported in studies done by Al-Matary et al., (13) in Yemen, Agrawal et al., (14) in India and Erhabor et al., (15) in northwest Nigeria. Of the four TTIs, the prevalence of detecting false negative ICT test was highest for HCV (1.9%, 26/1400), which may indicate that accuracy of rapid HCV ICT kits is particularly low, but could also be due to the fact that HCV has not received adequate attention in Nigeria as awareness, screening, and vaccination programs, which are in place for both HIV and HBV infections, are largely absent for HCV infection. Although the high prevalence of HCV infection was accounted for by age group >50 years (5.4%, 2/37), there was no statistically significant association between age group of donors and prevalence of all the TTIs ($p > 0.05$). The use of rapid kit tests for HBV and the other TTIs is clearly associated with high false negatives and a probable risk of increase TTIs in transfused blood to recipients. This is not surprising as HBV markers in surface antigen negative blood donors were found to contain the viral core antigen in the study done by Salawu et al., (16).

The ABO blood group distribution of donors showed a preponderance of O Rhesus D positive blood group with 61.1% (855/1400), a finding which agrees with several studies in Nigeria (17,18,19), confirming that O Rhesus D positive blood group is the commonest blood group in Nigerians. The major limitation of our study is that rapid kits from only a few manufacturers were tested against the fourth generation ELISA and as such, our findings only speak to the accuracy of the rapid kits made by these specific manufacturers and may not necessarily reflect on the accuracy of rapid kits by other manufacturers. Another limitation is the retrospective nature of our study design which may be associated with incomplete or inaccurate data. In addition, there was no 'gold standard' test (e. g. PCR) to truly compare the diagnostic performance (sensitivity, specificity, predictive values, likelihood ratio etc) of the two screening methods (rapid ICT kit and ELISA) for the four TTIs.

Conclusion:

The significant detection of false negative donor samples with ELISA highlighted by our study speaks to the unreliability of rapid kit screening in the prevention of TTIs. There is need therefore for health authorities in Nigeria and elsewhere to accelerate the widespread availability of highly accurate blood screening methods like ELISA to the point where it will be possible to enforce the legislation against the use of the less accurate rapid kit screening.

Future studies on diagnostic accuracy of these tests should be conducted using a 'gold standard' test and the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool as guide. Local research and development efforts should also be funded to enable affordable highly accurate alternatives to ELISA. Furthermore, the high prevalence of HCV infection deduced from this study should be combated by aggressive awareness and screening campaigns

Contributions of authors:

CGN contributed to study concept, design, definition of intellectual content, literature search, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review; YDO contributed to data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review; EOS contributed to the study concept, design, definition of intellectual content, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review; KNE made substantial contributions to the study concept, design, definition of intellectual content, literature search, manuscript preparation, manuscript editing and manuscript review; BA contributed to the study concept, design, definition of intellectual content, manuscript editing and manuscript review; and AFA made substantial contributions to concept, design, manuscript editing and manuscript review.

Conflict of interest:

Authors declare no conflict of interest

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