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Transfusion Transmissible Viral Infections Risk at Two Teaching Hospitals Blood Banks in Nigeria

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Abstract **Background/ Objectives:** Transfusion Transmissible viral infections such as HIV, HBV and HCV are infectious agents that can pose a threat to blood safety. The aim of this study is to determine the prevalence and coinfection of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) among blood donors tested negative to these viruses by rapid test devices. **Materials and Methods:** Two hundred and twelve (212) healthy blood donors (108 and 104 from UATH and NAUTH respectively) certified fit for blood donation were recruited for this study. One hundred and nine eighty (198) samples that were seronegative for HBsAg, HIV, HCV and Syphilis by rapid test were examined for the presence of HBsAg, Anti-HCV and HIV (Ab + Ag) using 4TH generation ELISA. Qualitative conventional PCR was used to confirm ELISA positive sera for HBV DNA, HIV RNA and HCV RNA. **Results:** Out of 198 blood donor samples negative for HBV, HCV and HIV by rapid test, 13.7%, 13.7% and 8.1% were positive by ELISA for HBV, HIV and HCV respectively. Twelve samples (6.1%) have coinfection of TTIs. One (1) sample out of the 12 samples is co-infected with these three viral (HBV, HCV and HIV) agents. Of the 28 samples positive for HBV by ELISA, 20 (71.4%) were positive for HBV DNA, 8 (36.4%) of 22 positive samples by HIV ELISA were positive for HIV RNA and 4 (28.6%) of 14 samples were positive for HCV RNA (p=0.0001). **Conclusion and Recommendations:** There is a high prevalence of transfusion transmissible viral infections among blood donors at our study population. This study therefore recommends blood donors in these Hospitals in particular and Nigeria in general be screened for HIV, HCV and HBsAg using ELISA 4th generation test kits and Nucleic Acid Testing (NAT).

Keywords: *blood donors, screening, transfusion transmissible viral infections*

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1. Introduction

Transfusion transmissible infections can be simply defined as infections acquired through transfusion of blood and blood products from donors to recipients [1]. Screening for transfusion-transmissible infections (TTIs) to exclude blood donations at risk of transmitting infection from donors to recipients is a critical part of the process of ensuring that transfusion is as safe as possible. Effective screening for evidence of the presence of the most common and dangerous TTIs can reduce the risk of transmission to very low levels [2]. Blood transfusion services should therefore establish efficient systems to ensure that all donated blood is correctly screened for specific TTIs and that only non-reactive blood and blood components are released for clinical use. Screening of donated blood for TTIs represents one element of strategies for blood safety and availability. The first line of defense in providing a safe blood supply and minimizing the risk of transfusion-transmitted infection is to collect blood from well-selected, voluntary non-remunerated blood donors from low-risk populations, particularly those who donate regularly. The prevalence of TTIs in voluntary non-remunerated blood donors is generally much lower than among family/replacement and paid donors [3,4].

Screening for transfusion transmissible infections (TTIs) is an established procedure in Blood Banks globally before accepting an individual to donate blood for clinical use. Most important of these TTIs screened for include hepatitis viruses (including Hepatitis B and C), HIV, and syphilis [1,5]. Hepatitis B virus (HBV) infection screening is particularly important because it is a major public health problem affecting over 350 million people worldwide [6]. Studies have shown that the carrier rate of hepatitis B virus (HBsAg marker) in blood donors in Nigeria appears to be between 5% and 17% depending on the geographical location [7,8]. The prevalence of HBV infection in Nigeria was estimated to be 2.4-18.4% of the population [9,10].

The risk of transfusion-transmitted HBV infection has been reduced by screening all blood donations for HBV surface antigen (HBsAg) since 1970 as most blood banks in resource limited economies screen for hepatitis B virus infection mainly by screening for the hepatitis B surface antigen (HBsAg) and bleed donors based on its negativity [11].

Transfusion Transmitted Infections (TTIs) are a major problem associated with blood transfusion. An unsafe blood transfusion is very costly both from human and economic point of view. Morbidity and mortality resulting from the transfusion of infected blood have far reaching consequences, not only for the recipients themselves but also for their families, their communities and the wider society [12]. The economic cost of the failure to control transfusion transmissible viral infections includes increase requirement for medical care, higher level of dependency, loss of productive labor force and placing heavy burden on already overstretched health and social services on national Economy [13].

The use of unscreened or inadequately screened blood for transfusion keeps the patient at risk of acquiring many transfusion transmitted infections (TTIs) like Hepatitis viruses (HBV, HCV), Human Immuno-deficiency Viruses (HIV), syphilis, malaria, to mention but a few [14]. Most blood banks in Nigeria screen blood donor using rapid test device and only the antibodies to infectious agents is screened. Antigen and viral DNA/RNA are not tested. The blood donors who are apparently healthy and HBsAg, HIV, HCV and Syphilis negative by rapid test kit devices may be infected with Hepatitis B virus and other transfusion transmitted infections such as HIV and HCV. Hence the need to use ELISA/ molecular diagnostics tool, a more sensitive and specific approach cannot be overemphasized. It is against this background that we embarked on this study to investigate the prevalence of HBV, HCV and HIV among blood donors certified fit for donation in these Hospitals using ELISA and PCR. This will enable us make evidence-based recommendations for effective screening of blood donors to prevent transmission of TTIs from infected blood donors to recipients during blood transfusion thereby ensuring blood safety in transfusion service in Nigeria.

2. Materials and Methods

Study sites: The study sites comprise the blood banks of University of Abuja Teaching Hospital (UATH) and Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi from Northern and Southern Nigeria respectively.

Subjects: These include 212 (108 from UATH and 104 from NAUTH) apparently healthy blood donors who had been screened and found eligible by the respective blood banks for donation were recruited over a period of five months, from June 2016 to October, 2016. Subjects who had hepatitis B vaccination in the previous one month were excluded. Relevant sociodemographic information was obtained from blood donors using structured questionnaire.

Ethical Considerations: Ethical approval was obtained from the ethics committee of both hospitals and informed consent was obtained from the subjects.

Study Design: This is a descriptive, cross-sectional, laboratory based study conducted at Nnamdi Azikiwe University Teaching Hospital, Nnewi and University of Abuja Teaching Hospital, Gwagwalada Abuja, Nigeria.

Specimen: Five milliliters of venous blood samples were taken from each blood donor into a clean dry tube. Blood samples were allowed to stand at room temperature for clotting and retraction. Thereafter, the samples were centrifuged to give a clear serum.

2.1. Serological Analysis

The samples were screened for HBV, HCV and HIV using Fortress Diagnostics 4th generation ELISA kit and read with Stat Fax 2100 Plate Reader.

2.2. DNA/RNA Extraction from Plasma Samples

DNA/RNA extraction was performed according to Kwok and Higushi, [15] using Zymo extraction kit (Zymo Rnesearch Corporation) and marketed by Ingaba Biotech Ltd Pretoria, South Africa. DNA was extracted from HBsAg negative/positive samples and then PCR was used for detection of HBV-DNA. The extraction was done according to the guidelines of the manufacturer. RNA was extracted from HIV and HCV negative/positive samples and then PCR was used for detection of HCV RNA and HIV RNA.

2.3. Detection of HBV DNA by Conventional PCR

PCR amplifications were carried out in 20µl reaction volumes with the following PCR components: Master Mix 10ul, forward primer 0.4ul, Reverse primer 0.4ul, DNA Template 2ul and 7.2 ul of water making it up to 20ul on an Applied Biosystems GeneAmp PCR System 9700 Thermal cycler. Primer pairs were designed from the highly conserved overlapping regions of the *S* and *P* regions of the HBV genome. A conventional PCR was performed: Forward primer sequence: TCA CCA TAT TCT TGG GAA CAA GA and Reverse Primer sequence: CGA ACC ACT GAA CAA ATG GC. PCR conditions include the following: Thermal cycling parameters were: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 30sec at 95°C denaturation, 30 seconds at 53°C annealing temperature, 30 seconds at 72°C extension, followed by a final extension of 2 minutes at 72°C. Thermal cycling parameters remained the same as in the all PCR round done. A positive control (HBV plasmid DNA) and a negative control of the master mix were integrated to each run to validate the PCR products that produce a 340bp fragment. The detection limit of the HBV DNA by conventional PCR is approximately 2.5 copies per reaction (between30-40copies/mL)

2.4. Detection of PCR Product

For analyses of the PCR amplification, 10ul of the amplified samples was electrophoresed on a 2% agarose gel made in Tris acetated EDTA buffer (pH=8.0-8.5) and visualized by UV illumination after ethidium bromide (10 g/ml) staining. Positive and negative controls were also treated as samples.

2.5. Detection of HCV RNA by RT-PCR

Three different regions of HCV RNA genome namely 5′ UTR, core and NS5B were PCR amplified with genome

specific primers by nested RT-PCR method. The primer sequences for Forward primer: ACTGTCTTCACGCAGA AAGCGTCTAGCCAT and Reverse Primer: CGAGA CCTCCCGG GGCACTCGCAAGCACCC

Conventional nested RT-PCR for the detection of HCV RNA was performed with normal ABI *Taq* polymerase. For rapid amplification of HCV RNA, RT-PCR was carried out in a total volume of 20ul with the following PCR components: Master mix (1X) 10ul, Forward Primer (0.2uM) of 0.16ul volume, Reverse Primer (0.2Um) of 0.16ul, Template 1ul and water 8.68ul making the final volume to 20ul. The RT-PCR conditions were initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds. Annealing was done at 50°C for 30 seconds, extension at 72°C for 30 seconds and the final extension was carried out at 72°C for 2 minutes in an ABI 9700 thermal cycler. The PCR products were electrophoresed in 2% agarose gel stained with ethidium bromide and observed under gel documentation system (Bio-Rad, USA), for HCV 5′ UTR, core and NS5B, bands were obtained at 256 bp, 405 bp and 389 bp respectively.

2.6. Amplification of HIV V3 Region (Nested PCR)

Conventional nested RT-PCR for the detection of HIV RNA was performed with normal ABI *Taq* polymerase. The primers for the primary or 1st round or nest are: Forward primer: GGCATCAAACAGCTCCAGGCAAG and Reverse Primer: AGCAAAGCCCTTTCTAAGCCCTGTCT. For rapid amplification of HIV RNA, 1st round one-step RT-PCR was carried out in a total volume of 20 µl containing PCR components: Master Mix (1X) of 10ul volume, Forward Primer (0.2uM) of 0.16ul, Reverse Primer (02.uM) of 0.16ul, Template 1ul and H_2O 8.68ul Final volume to 20ul. The PCR Conditions: Initial denaturation 95°C for 5 minutes, Denaturation 95°C for 30 seconds, Annealing 55°C for 30 seconds, Extension 72°C for 30 seconds, Final Extension 72°C 2minutes. The total number of cycles is 25 cycles. This was followed by the 2nd round nested PCR with this primers sequence: Forward primer: TCCTGGCTGTGGAAAGATACCTA and Reverse Primer: GTCCCCTCGGGGCTGGGAGG in a 20 µl total reaction volume containing the following PCR components: Master Mix (1X) of 10ul, Forward Primer (0.2uM) of 0.16ul volume, reverse Primer (0.2uM) of 0.16ul, Template 0.5ul volume, H2O 9.18ul making the final volume to 20ul. The PCR conditions are as follow: initial denaturation 95°C for 5 minutes, Denaturation 95°C for 30 seconds, Annealing 58°C for 30 seconds, Extension 72°C for 30 seconds, Final Extension 72°C at 2minutes. The total number of cycles is 35 cycles. The PCR products were electrophoresed in 2% agarose gel stained with ethidium bromide and observed under gel documentation system (Bio-Rad, USA), for HIV 5′ UTR, core and NS5B, bands were obtained at 256 bp, 405 bp and 389 bp respectively.

2.7. Statistical Analysis

Data obtained from this study were entered on Microsoft excel, validated on SPSS version 20 for windows and analyzed on Epi Info version 7 for windows. Also Graph pad prism software version 7.0 was used for some data analysis. Descriptive statistics (frequency, means, and standard deviation) was used to estimate participants' socio-demographic characteristics at 95% confidence interval. Inferential data analysis such as Chi-Square was used to establish the association/relationship between two variables. Statistical significance was determined at a probability level of $p< 0.05$.

3. Results

Table 1. HBsAg Positivity by ELISA Technique among Blood Donors Tested Negative by Rapid Test Device and its Relationship with HBV DNA Assay

Study Sites	ELISA No. of Samples Tested	No. (%) Positive	PCR No. of Samples Tested	No. (%) Positive	Chi-Square (p-value)
UATH Abuja	100	5 (5%)	5	0 (0%)	*12.95 (0.0003)*
NAUTH Nnewi	102	23 (22.5%)	23	20 (87%)	*0.70 (0.4)*
TOTAL	202	28 (13.7%)	28	20 (71.4%)	

Statistically Significant (alpha< 0.05).

Table 2. HIV Positivity by ELISA Technique among Blood Donors Tested Negative by Rapid Test Device and its Relationship with HIV RNA Assay

Study Sites	ELISA No of Samples Tested	No. (%) Positive	PCR No. of Samples Tested	No. (%) Positive	Chi-Square (p-value)
UATH Abuja	99	10 (10.1%)	10	4 (40%)	*2.1 (0.14)*
NAUTH Nnewi	98	17 (17.3%	12	4 (33.3%)	*0.0487*
TOTAL	197	27 (13.7%)	22	8 (36.4%)	*(0.9759)*

Statistically Significant (alpha< 0.05).

Table 3. HCV Positivity by ELISA Technique among Blood Donors Tested Negative by Rapid Test Device and its Relationship with HCV RNA Assay

Study Sites	ELISA No. of Samples Tested	No. (%) Positive	PCR No. of Samples Tested	No. (%) Positive	Chi-Square (p-value)
UATH Abuja	93	2 (2.2%)	2	1 (50%)	*8.8 (0.003)*
NAUTH Nnewi	92	13 (14.1%)	12	3 (25%)	*0.2571*
TOTAL	185	15 (8.1%)	14	4 (28.6%)	*(0.8794)*

Statistically Significant (alpha< 0.05).

Table 4. Transfusion Transmissible Viral Infections Risk Assessment at Blood Banks in the Study Population

Viral Agents	Blood units screened and Certified safe for Transfusion	Positive Samples (PCR)	% Frequency	Chi-Square (p-value)
HBV	198	20	10.1%	*53.92 (<0.0001)*
HCV	198	4	2.0%	
HIV	198	8	4.0%	

Statistically significant ($p < 0.05$).

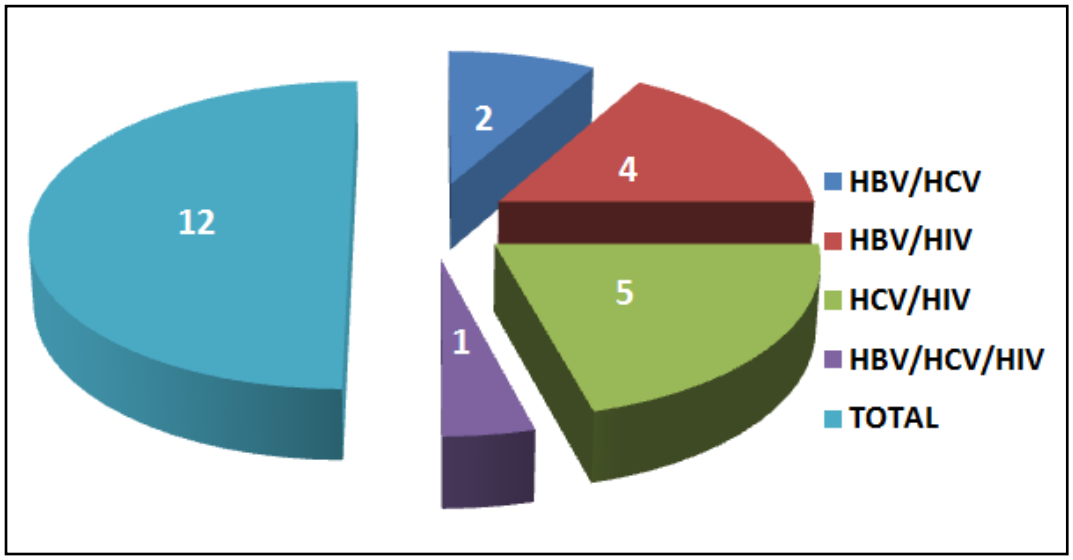

Figure 1. Coinfection of Transfusion Transmissible Viral Infections among Blood Donors

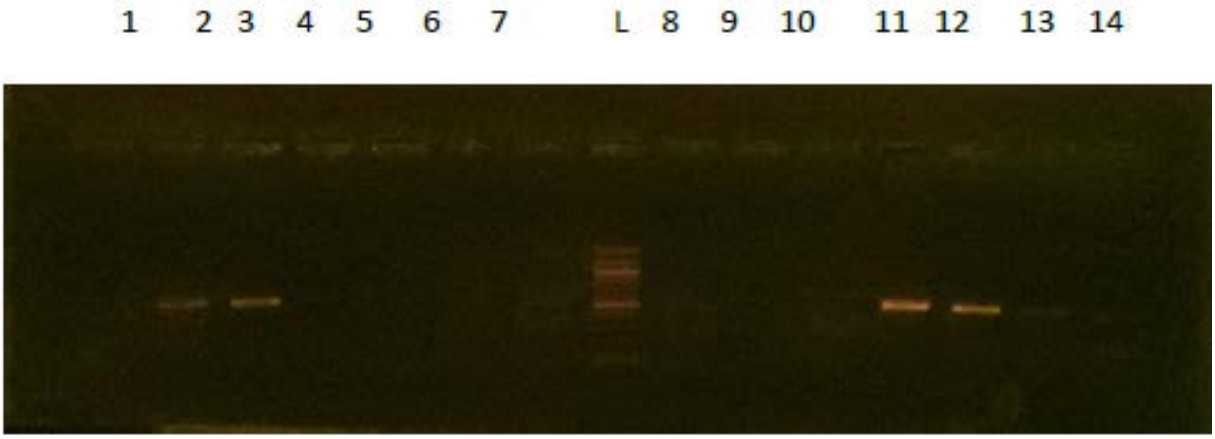

Figure 2. Agarose Gel Electrophoresis Showing the Amplified HCV Bands (L represents a 100bp ladder, lanes 2,3, 11, and 12 represents)

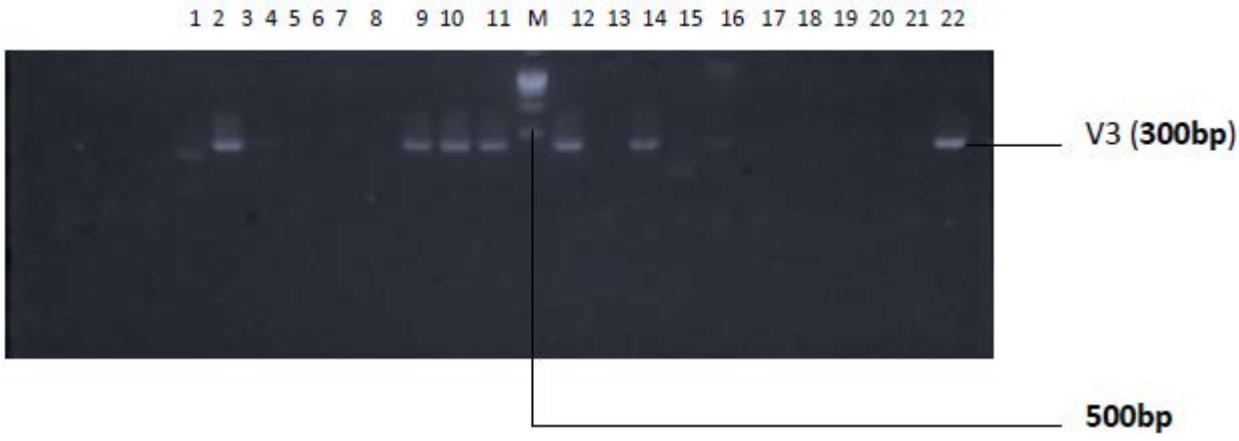

Figure 3. Agarose Gel Electrophoresis Showing the V3 Bands of the HIV Virus from Blood Donors (M represents the 1kb molecular ladder, while lanes 2,9,10,11,12,14 and 22 represent the amplified V3 region)

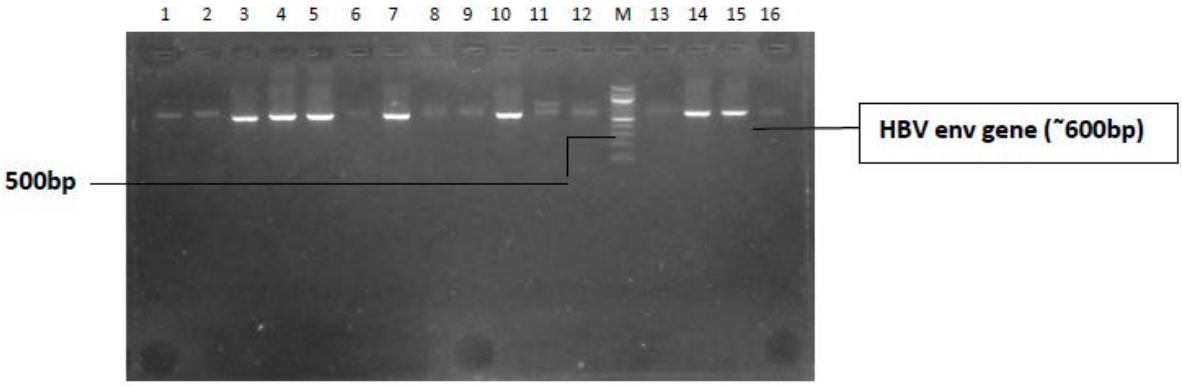

Figure 4. Agarose Gel Electrophoresis Showing Amplified HBV Gene of Blood Donors with HBV Infection (Lanes 1,2,3,4,5,6,7, 10, 11 13 14, 15 showing successful amplification, Lane M represents a 100bp ladder.)

4. Discussion

The ELISA results of blood donors tested for HBsAg, HIV and anti-HCV in this study population showed that out of 204 samples tested for HBsAg, 28 samples representing 13.7% were positive, HIV ELISA result gave 27 (13.7%) positive out of 197 samples tested. Anti-HCV ELISA result shows that out of 185 samples tested, 15 samples representing 8.1% were positive. These values were quite high compared with 1% positivity for HBsAg and 0% positivity for HCV reported by Olotu *et al.*, [16] after retesting with ELISA format. Oluyinka *et al.*, [17] also reported that all samples tested negative by rapid test device were negative by ELISA technique. The reason for this difference could be we used 4th generation ELISA test kits which is more sensitive than the 3rd generation kit they used. The ELISA HIV 4th generation kit also contains antibody and antigen (p24) making it more sensitive in detection of small concentration of HIV in the blood specimen [18]. The results obtained from ELISA testing for HBV, HIV and HCV show that ELISA format is more sensitive than rapid test kit but PCR is also highly sensitive and specific in detection DNA/RNA of infectious agents. This also signifies that the rapid test kits being used for screening for these viral agents in the blood banks where the subjects were recruited are not adequate. This is because they could allow transfusion of HBsAg positive blood for example in at least 10 out of every 100 blood donations. The inadequacy in sensitivity and variation in performance between different locations of some rapid test kits used in resource poor settings for HBsAg screening has been shown by Bjoerkvoll *et al.*, [19]. In their cross-sectional epidemiological study they compared the accuracy of rapid test immunochromatographic kits in the detection of HBsAg, anti-HBc and anti-HCV against ELISA, in two populations of 1200 potential blood donors in rural Cambodia and Vietnam. For HBsAg specifically, they found the rapid test kits to be high in specificity (99.8–99.9 %) but lower in sensitivity (86.5 %). They also found a difference in its sensitivity between both countries. In Cambodia the sensitivity was 93.5 % and in Vietnam 81.8 %. This collaborates with this study that showed that ELISA is more sensitive than rapid test in detection of Transfusion Transmissible viral infections such as HIV, HBV and HCV.

In addition, results from this study also showed evidence of coinfection of transfusion transmissible viral infectious agents (HBV, HCV and HIV) among blood donors. Of the 198 blood donors screened with ELISA 4th generation kits (HBsAg, Anti-HCV and HIV with Ag+Ab), 12 blood donors have coinfection of transfusion transmissible viral infections representing 6.1% prevalence of multiple infections. One (1) out of the 12 has coinfection of these three viruses (HIV, HBV & HCV) representing 8.3% prevalence. This finding disagrees with the result of Akpulu *et al.*, [20] that recorded 26 (1.4%) out of 1875 blood donors with co-infection of transfusion transmissible infectious agents in Zaira, Nigeria. The reason for this difference could be attributed to high sample size they used compared to ours. However, our result correlates with their finding in relation to HIV/HCV coinfection having the highest prevalence. The reason for this multiple infection could be attributed to the fact that these viral infections have common routes of transmission such as sexual contacts, vertical transmission from mother to child and occupational hazard/ accidents such as needle prick [21].

The Risk and Implications of transfusion of infected blood units to recipients cannot be overemphasized. Accurate estimates of risk of TTIs are essential for monitoring the safety of blood supply and evaluating the efficacy of currently employed screening procedures [22]. The risk assessment of transfusion of transmissible viral infections such as HBV, HCV and HIV from this study is high. Of the 212 valid blood donors that gave consent, 8 were confirmed positive for HBV and 6 were confirmed positive for HCV by rapid test kits and were excluded from the study. The remaining 198 blood units have been confirmed suitable for transfusion and possibly their blood units have been transfused to their recipients at these two Teaching Hospitals under study. The findings from this study have shown that 20 blood donor samples that were ELISA positive for HBsAg were positive for HBV DNA, 8 HIV ELISA positive samples were positive for HIV RNA and 4 HCV ELISA positive samples were positive for HCV RNA. In summary, of the 198 blood units that have been screened, certified safe and possibly transfused to their patients in these two Teaching Hospitals in Nigeria, 32 blood units representing 16.2% are infected with transfusion transmissible viral infections. What this means is 16 out 100 blood units transfused in our study population have risk of transmission of infectious viral agent such as HBV, HCV and HIV. Of these 32 infected blood units, HBV infected blood units is 20 representing 62.5% of infected blood units being transfused to patients followed by HIV which have 8 blood units with HIV

RNA representing 25% and HCV infected blood with HCV RNA is 4 units representing 12.5% of infected blood units. The percentage risk of transfusion of viral agents, HBV, HIV and HCV in relation to the 198 blood units certified fit for transfusion in our study population is 10.1%, 4% and 2% respectively and is statistically significant. What this mean is 10, 4, and 2 out 100 blood units transfused at these two centers have transmission risk of HBV, HIV and HCV respectively. The implication of transfusion of infected blood units is the resultant increase in the prevalence of overt infection of these viral agents in the population with its attendance public health consequences. The residual risk obtained from this study correlates with the study by Candotti and Allain, [23] which reported that the estimated residual risk of HBV transfusion transmission remains significantly higher than the risk of either HIV-1 or HCV. Whether residual risk estimates translate into true rate of infection is largely unknown since estimates are generally based on the simplification that all HBV DNA-containing donations are infectious [23]. They concluded by saying that all blood units containing HBV DNA have been shown to be infectious in immunocompromised individuals, such as organ- or bone marrow-transplant recipients. HBV transmission was previously reported from OBI donors who had circulating HBV DNA at a low level [24,25]. These authors emphasized that the lack of a clear relationship between infectivity and viral load in blood components may be related to immune factors affecting the susceptibility to infection in recipients. In addition, HBV infectivity is related to the amount of plasma transfused and the viral load in the product [23].

5. Conclusion/Recommendations

The study has established that there is a high prevalence of HIV, Hepatitis B and C infections among blood donors tested negative by rapid test devices using ELISA 4th generation kit. Moreover, there are coinfections of these viral agents among blood donors in our study population. However, availability of safe blood for transfusion is a must for the recipients and community as well and can be achieved by vigorous and cautious screening of donors / or donated blood units with highly sensitive and specific laboratory tests that can detect transfusion transmissible viral infections. This study therefore recommends blood donors in these Hospitals in particular and Nigeria in general be screened for HIV, HCV and HBsAg using ELISA 4th generation test kits as rapid test device is less sensitive. Screening of blood donors by Nucleic Acid Testing (NAT) for HBV, HCV and HIV is also recommended. Implementation of these findings in blood transfusion services will minimize transfusion transmissible viral infections risk in Nigeria.

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Conflicting Interests

The authors declare that they have no conflicting interests.

References

[1] World Health Organization (2009a) Hepatitis B Fact sheet No 204. Accessed 10 June 2009. Available: http://www.who.int/csr/disease/hepatitis.

[2] Dodd RY (2007). Current risk for transfusion-transmitted infections. *Current Opinion in Hematology*. 14(6): 671-676.

[3] Panda M Kar K (2008). HIV, hepatitis B and C infection status of the blood donors in a blood bank of a tertiary health care centre of Orissa. *Indian Journal of Public Health*. 52(1): 43-44.

[4] World Health Organization (2008). Global Database on Blood Safety, 2004–2005 report. Geneva, World Health Organization, 2008.

[5] Acheson NH (2011). Fundamentals of Molecular Virology. 2nd Ed. John Wiley and Sons Inc. USA. Pages 365-375.

[6] Asim M Ali R Khan LA Husain SA Singla R Kar P. (2010). Significance of anti-HBc screening of blood donors and its association with occult hepatitis B Virus infection: Implications for blood transfusion. *Indian Journal of Medical Research.* 132: 312-317. Sept 2010.

[7] Baba MM Hassan AW Gashau W (2010). Prevalence of hepatitis B antigenaemia and Human Immunodeficiency Virus in blood donors in Maiduguri, Nigeria. *Nigerian Journal of Medicine.* 9: 10-12.

[8] Okwesili A Usman I Abubakar W Onuigue F Erhabor O Buhari H Abdulrahaman Y Isaac Z Mainasara Y Aghedo F Ikhuenbor D Mohammed H Y Dallatu K Mainasara A Gwarzo S (2014). Prevalence of Transfusion -Transmissible Hepatitis B Infection among Blood Donors in Sokoto, North Western, Nigeria. *Health Sciences Research.* 1(4): 113-118.

[9] Olokoba AB Salawu FK Danburam A Desalu OO Olokoba LB Wahab KW Badung LH Tidi SK Midala J Aderibigbe S Abdulrahman MB Babalola OM Abdukkarim A (2009). Viral Hepatitis in Voluntary Blood Donors in Yola, Nigeria. *European Journal of Scientific Research.* 31 (3): 329-334.

[10] Ndako JA Nwankiti OO Echeonwu GON Junaid SA Anaele O Anthony TJ (2011). Studies on Prevalence and Risk Factors for Hepatitis B Surface Antigen among Secondary School Students in North-central, Nigeria. *Sierra Leone Journal of Biomedical Research.* 3 (3): 163-168.

[11] Badur S Akgun A (2001). Diagnosis of Hepatitis B Infection and monitoring of Treatment. *Journal of Clinical Virology.* 21: 229-237.

[12] World Health Organization (2007). Status of blood safety in the WHO African Region: Report of the 2004 Survey WHO Regional Office for Africa, Brazzaville. 2007; 1-25:25.

[13] World Health Oorganization (2002). Safe Blood and Blood Products. Introductory Module: Guidelines and Principles for Safe Blood Transfusion Practice. Geneva, World Health Organization, 2002.

[14] WHO (2009b). Blood Safety Indicators, 2007. Geneva, World Health Organization, 2009.

[15] Kwok S Higushi R (1989) Avoiding false positives with PCR. *Nature.* 339 (6221): 237-238.

[16] Olotu AA Oyelese AO Salawu L Audu RA Okwuraiwe AP Aboderin AO (2016). Occult Hepatitis B Virus infection in previously screened, blood donors in Ile-Ife, Nigeria: Implications for blood transfusion and stem cell transplantation. *Virology Journal.*13: 76-86.

[17] Oluyinka OO Tong HV Bui Tien S Fagbami AH Adekanle O Ojurongbe O Bock CT Kremsner PG Velavan TP (2015). Occult Hepatitis B Virus Infection in Nigerian Blood Donors and Hepatitis B Virus Transmission Risks. *PLoS ONE 10(7): e0131912.*

[18] Constantine NT Saville RD Dax EM (2005). Retroviral Testing and Quality Assurance Essential for Laboratory Diagnosis. 1st Ed. A non-profit supported through an educational grant from MedMira Laboratories. Pages 133-151.

[19] Bjoerkvoll B Viet L Ol HS Lan NT Sothy S Hoel H Gutteberg T Husebekk A Larsen S Husum H (2010). Screening test accuracy among potential blood donors of HBsAg, anti-HBc and anti-HCV to detect hepatitis B and C virus infection in rural Cambodia and Vietnam. *Southeast Asian Journal of Tropical Medicine and Public Health*. 41(5): 1127-1135.

[20] Akpulu SP Adamu M Oledinma S Yaro EG Ajayi IT (2017). Prevalence of Transfusion Transmissible infections among blood donors attending Hajiya Gambo Sawaba General Hospital, Zaria, Nigeria. *Nigerian Biomedical Science Journal*.14 (3): 24-37.

[21] Levinson, W (2014). Review of Medical Microbiology and Immunology. 13TH Ed. McGraw Lange Medical Book, USA. Pages 331- 341.

[22] Sharma DC Rai S Bharat S Iyenger S Gupta S Sao S Jain B (2014). Transfusion Transmissible Infections among Blood Donors at the Blood Bank of Medical College of Gwalior: A 5 Year Study. *International Blood Research and Reviews*. 2 (5): 235-246.

[23] Candotti D Allain JP (2009). Transfusion-transmitted hepatitis B virus infection. *Journal of Hepatology*. 51: 798-809.

[24] Manzini P Girotto M Borsotti R Giachino O Guaschino R Lanteri M Testa D Ghiazza P Vacchini M Danielle F (2007). Italian blood donors with anti-HBc and occult hepatitis B virus infection. *Haematologica*. 92: 1664-1670.

[25] Bouike Y Imoto S Mabuchi O Kokubunji A Kai S Okada M Taniguchi R Momose S Uchida S Nishio H (2011). Infectivity of HBV DNA positive donations identified in look-back studies in Hyogo-Prefecture, Japan. *Transfusion Medicine*. 21: 107-115.