

Original Research Article

Seroprevalence of Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Syphilis among Voluntary Blood Donors in Rural Southwestern Uganda: A Retrospective Study

ABSTRACT

Despite the improvement with blood screening, transfusion transmissible infectious (TTIs) agents such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis are still among some of the challenges in the blood safety for the recipient in Uganda. The aim of this study was to determine the prevalence of the four TTIs among the voluntary blood donors in southwestern Uganda. A retrospective study was conducted using one year data (January 2014 to December 2014) from a regional blood bank in southwestern Uganda. Routine screening by the blood bank included anti HIV, hepatitis B surface antigen, anti-hepatitis C using the Abbott ARCHITECT i2000 SR analyzer. Both Rapid Plasma Reagin (RPR) test and Treponema Pallidum Haemagglutination (TPHA) tests were used for detection of syphilitic infection. 5.67% of blood donors were positive for any of the screening tests and 0.34% had multiple infections. The overall seroprevalence was 1.03%, 1.87%, 2.22%, and 0.54% for HIV, HBV, HCV and syphilis respectively. The most common dual combinations were HBV-HCV 51.8%, HIV-HCV 22.7% and HIV-HBV 10.0%. Triple infection with HIV-HBV-HCV was 3.7% and HIV-HBV-Syphilis was 1.3%. There was no quadruple infection detected in this study. There was a statistically significant increase of HIV seropositivity among the age group of donors above 47 years ($p=0.001$). A substantial prevalence of TTIs was found among the blood donors in southwestern Uganda.

Key words: HIV, HBV, HCV, syphilis, Prevalence, TTIs, Uganda

Introduction

Blood transfusion practices worldwide emphasize on safety and protection of human life¹. One of the biggest challenges to blood safety particularly in sub-Saharan Africa is accessing safe and adequate quantity of blood and blood product¹. Transmission of infectious diseases through donated blood is of paramount concern in order to provide safe blood for transfusion which forms an integral part of medical and surgical treatment. Blood transfusion carries the risks of transfusion induced transmissible infections (TTIs) including HIV, hepatitis, syphilis, malaria and less frequently toxoplasmosis, brucellosis and some other viral infections like Epstein Barr virus, cytomegalovirus and herpes³. Transfusion associated infections; mainly hepatitis B virus (HBV), hepatitis C virus (HCV), Human Immunodeficiency Virus (HIV) and syphilis among donors are of public health concern but in particular the three viruses namely HIV, HBV and HCV are of great concern because of their prolonged viraemia and carrier state.

In Uganda, the Blood Transfusion Services (UBTS) which was established in 1989, is a semi-autonomous centrally coordinated organization in the ministry of health, sufficiently decentralized to render services to all regions in the country. UBTS is mandated to provide sufficient and efficacious blood and blood components through voluntary non-remunerated blood donation for appropriate use in health care services in Uganda. UBTS has developed an improved blood programme that has significantly contributed to the prevention and reduction of transmission of HIV/AIDS, hepatitis B, hepatitis C and syphilis in Uganda⁴. Despite the screening of blood and blood products, blood transfusion still accounts for 5 to 10% of HIV infections in sub-Saharan Africa and 12.5% of patients who receive blood transfusions are at risk of post transfusion hepatitis².

Hepatitis B is a DNA virus, enveloped which belongs to the family hepadnaviridae and can infect the liver of hominoidae, including humans and can cause acute and chronic disease with high tendency to progress to cirrhosis and hepatocellular carcinoma. An estimated 240 million people are chronically infected with hepatitis B (defined as hepatitis B surface antigen positive for at least 6 months). More than 780,000 people die every year due to complications of hepatitis B including cirrhosis and hepatocellular carcinoma⁵. Hepatitis B prevalence is highest in sub-Saharan Africa and East Asia, where between 5-10% of adult people are chronically infected⁶.

Hepatitis C virus (HCV) is a small (55-65nm in size), enveloped positive sense single stranded RNA virus of the family Flaviviridae. Hepatitis C virus is one of the causes of viral hepatitis (cirrhosis) and some cancers such as hepatocellular carcinoma (HCC) and lymphomas in humans⁷. The hepatitis C virus like hepatitis B virus is a blood borne virus and the most common modes of infection are through unsafe injection practices; inadequate sterilization of medical equipment; and transfusion of unscreened blood and blood products⁸. Hepatitis C virus causes both acute and chronic infection. About 15-45% of infected persons spontaneously clear the virus within 6 months of infection without any treatment⁸. Hepatitis C virus is found worldwide and about 130-150 million people globally have chronic hepatitis C infection and significant number of those who are chronically infected will develop liver cirrhosis or hepatocellular carcinoma. Approximately 500,000 people die each year from hepatitis C related complications⁸.

Given the regional variations of transfusion transmissible infections, screening for TTIs such as human immunodeficiency virus, hepatitis B virus, hepatitis C virus and syphilis among blood donors is essential for blood transfusion safety and in extension for protecting human life ⁸.

The knowledge of the prevalence of TTIs among the blood donors in southwestern Uganda will harness the understanding of the frequency of these highly infectious transfusion transmissible infections in the region, which in turn will foster the design and the implementation of the Uganda Blood Transfusion Safety Strategies.

Methods

Design and sample population

A retrospective study was conducted using one year data (January 2014 to December 2014) from a regional blood bank in rural southwestern Uganda. Records of 25,504 voluntary blood donors previously recruited from the nine districts were reviewed. The donors were recruited from the nine districts in southwest Uganda namely Bushenyi, Ibanda, Isingiro, Kabale, Kiruhura, Mbarara, Ntungamo, Rukungiri and Shema, that have a total population of 3,334,260⁹. Prior to donating blood the donors were first assessed for physical and health wellbeing. The first step in screening for potential donors is taking past medical history of the client. Individuals are required to give answers to a panel of questions on previous illness and medical conditions. Past history of blood transfusion and questions targeted to ascertain risky sexual behavior and practice are also part of the questionnaire. The physical assessment criteria required that the donors were: body weight >45 kg; hemoglobin levels, male 13.5–17.0 g/dl and female 12.5–16 g/dl, a blood pressure of up to 160/90 mmHg and healthy subjects of age 17 to 65 year were accepted. Only donors who satisfied these criteria were recruited. The medical and sociodemographic histories of the donors were recorded in the logbook and venous blood was collected in blood banking bags

following standard operating procedures. The donated blood samples were then screened for the TTIs using the serological methods described for each test below. Blood samples that were found to be infected with any of the TTIs were discarded using standard procedure. The aim of this study was to determine the seroprevalence of HIV, HBV, HCV and Syphilis among the voluntary blood donors in southwestern Uganda.

Comment [san1]: Why were the TTIs not screened for before collecting the donor's blood into the blood bag? Unnecessary waste of the donors' blood could have been avoided.

Serological analysis

Blood samples were routinely screened by blood transfusion staff in the blood bank for detection of hepatitis B virus surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV), HIV antibody (anti-HIV) and antibodies to *Treponema pallidum*.

Laboratory diagnosis for syphilis

Both Rapid Plasma Reagin (RPR) test and Treponema Pallidum Haemagglutination (TPHA) tests are used for the detection of syphilis infection. Serum from all donors was tested for the presence of treponemal antibodies using rapid plasma reagin (RPR) test following the manufacturer's instructions (RPR, Wampole Laboratories, Princeton, N.J., USA). Antibodies to *Treponema pallidum* were confirmed with Treponema pallidum haemagglutination test (TPHA, Lorne Laboratories, UK). A result was considered positive if both the first and the second tests were positive and vice versa.

Laboratory tests for hepatitis B surface antigen (HBsAg) and hepatitis C antibodies (anti-HCV)

Detection of HBsAg using Architect HBsAg qualitative assay

The Architect HBsAg Qualitative II assay using Architect i2000 system (Abbott Laboratories, Diagnostic Division, Abbott Park, IL), is a one- step immunoassay for the qualitative detection of HBsAg in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA)

technology, with flexible assay protocols, referred to as chemiflex. The assay is done on the fully automated Architect instrument as per the manufacturer's protocol. In the Architect HBsAg Qualitative II assay, sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium –labelled conjugate are combined to create a reaction mixture. HBsAg present in the sample binds to anti-HBs coated microparticle and to the anti-HBs acridinium –labelled conjugate. After washing, ancillary wash buffer is added to the reaction mixture. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the optical system. The presence or absence of HBsAg in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the sample is considered reactive for HBsAg. All positive sera for HBsAg were retested by an ELISA system using hepatitis B surface antigen (Murex Biotech Ltd, Dartford UK), following manufacturer's instructions.

Detection of anti-HCV using Architect Anti-HCV assay.

All sera were analyzed using the commercially available anti-HCV automated chemiluminescent microparticle immunoassay (CMIA) systems for the detection of immunoglobulin G (IgG) antibodies to the hepatitis C virus. The Architect anti-HCV assay using Architect i2000 system (Abbott Laboratories, Diagnostic Division, Abbott Park, IL) uses automated chemiluminescent detection technology (Chemiflex), and the reactive component contain recombinant antigens representing the core non-structural 3 (NS3) and non-structural 4 (NS4) proteins HCr43 and C100-2. The ARCHITECT Anti-HCV assay is a two-step

immunoassay, using chemiluminescentmicroparticle immunoassay, using qualitative detection of anti-HCV in human serum and plasma. In the first step, sample, recombinant HCV antigens coated paramagnetic microparticles and assay diluent are combined. Anti-HCV present in the sample binds the HCV coated microparticles. After washing, antihuman acridinium labelled conjugate is added in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti HCV in the samples and the RLUs detected by the ARCHITECT immunoassay optics. The presence or absence of anti-HCV in the specimen is determined by the ARCHITECT machine by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from the ARCHITECT Anti-HCV calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the specimen is considered reactive for anti-HCV. The IgG antibodies to HCV were retested using an ELISA technique (Murex anti HCV version 4.0) according to manufacturer's instructions.

Laboratory diagnosis for HIV1 and HIV2 antibodies

The Architect HIV Ag/Ab Combo assay is a two-step immunoassay to determine the presence of HIV p24 antigen and antibodies to HIV-1 (Group M and Group O) and HIV -2 in human serum and plasma using Architect i2000 system (Abbott Laboratories, Diagnostic Division, Abbott Park, IL) using chemiluminescentmicroparticle immunoassay (CMIA) technology with flexible protocols, referred to as Chemiflex. In the first step, sample, Architect I Wash Buffer, assay diluent, and paramagnetic microparticles are combined. HIV p24 antigen and HIV-1/HIV-2 antibodies present in the sample bind to the HIV-1/HIV-2 antigen and HIV p24 monoclonal (mouse) antibody coated microparticles. After washing, the HIV p24 antigen and

HIV-1/HIV2 antibodies bind to the acridinium labelled conjugates (HIV-1/HIV-2antigens [recombinant], synthetic peptides, and HIV p24 antibody [mouse, monoclonal]). Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as a relative light units (RLUs). A direct relationship exists between the amount of HIV antigen and antibodies in sample and the RLUs detected by the Architect I system optics. Results and interpretations of test performance. The presence or absence of HIV p24 antigen or HIV-1/HIV-2antiboies in the specimen is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an Architect HIV Ag/Ab Combo calibration. Specimen with signal cutoff (S/CO) values greater than or equal to 1.00 are considered reactive for HIV p24 antigens and HIV-1/HIV-2 antibodies. Specimens with cutoff values less than 1.00 considered non-reactive for HIV p24 antigens or HIV-1/HIV-2 antibodies. Specimens that are initially reactive in the Architect HIV Ag/Ab Combo assay should be retested in duplicate. Repeat reactivity is highly predictive of the presence of HIV p24 antigen and HIV-1/HIV-2 antibodies. Like with all other immunoassays, the Architect HIV Ag/Ab Combo assay may yield nonspecific reactions due to other causes, particularly when testing in low prevalence population. A repeatedly reactive specimen should be investigated further with more sensitive tests such as recombinant immunoblots, antigen tests, and HIV nucleic acid tests which by themselves are difficult to perform and having high percentage of indeterminate results and high cost, which make them deterrent to use in developing countries and as a routine diagnostic procedures. All reactive samples to HIV-1 and HIV-2 were retested using an ELISA technique Vironostika HIV Uni-Form II Ag/Ab (Biomerieux, Boxtel, The Netherland) following the manufacturer's instructions.

Analysis

Analysis of the data was performed using STATA version 13¹⁰ software. Two-sided chi-square tests for association were computed to detect differences in categorical variables (age groups and gender) with probability values (p-values) calculated at < 0.05 level of significance.

Ethics

The research proposal was reviewed and approved by the Research and Ethics Committee (REC) of Mbarara University of Science and Technology. Permission to use the blood bank data was granted by the Director Uganda Blood Transfusion Services (UBTS). However, because of the retrospective nature of the study, a waiver of informed consent was obtained from REC. In order to maintain anonymity, codes but not names of the voluntary non remunerated blood donors were used during entire work of the study.

Results

Demographic characteristics of the study population

The sample participants was 23,504, had about 72.8% (n=17,116, CI: 72.2-73.4) Males and

27.2%(n=6,388;CI:26.6-27.8)females. Age groups were 17-25, 26 -36, 37-47 and >47, the largest age group was 17-25 years, 88.4%(n=20,766;CI:87.9-88.8) and the least number was in the age group of 47 years and above 149 (0.6%). There were eight districts from which blood was donated and these were Bushenyi, Ibanda, Isingiro, Kabale, Kiruhura, Mbarara, Ntungamo and Rukungiri. Rukungiri district had the largest number of donors, followed by Mbarara district, Kabale district and Bushenyi with 25.2% (n=5,843), 22.2% (n=5,142), 14.4% (n=3,102) and 13.2% (n=3,054) respectively.

Comment [san2]: Change this to 10-year age grouping of 17-26; 27-36; 37-46; 47&above.

Table 1: Demographic characteristics of study population

Variables (n=23,504)	n (%)	95% CI
Age :		
17 - 26	20,768 (88.4)	87.9 – 88.8
27 – 36	1,964 (8.4)	8.0 – 8.7
37 – 47	624 (2.6)	2.5 – 2.8
>47	149 (0.6)	0.5 – 0.7
Sex:		
Male	17,116 (72.8)	72.2-73.4
Female	6,388 (27.21)	26.6-27.8
Districts:		
Bushenyi	3,054 (13.2)	12.7 – 13.6
Ibanda	1,429 (6.2)	5.8 – 6.5
Isingiro	1,868 (8.1)	7.7 – 8.4
Kabale	3,102 (13.4)	13.0 – 13.8
Kiruhura	1,320 (5.6)	5.4-6.0
Mbarara	5,142 (22.2)	21.6-22.7
Ntungamo	1,420 (6.1)	5.8-6.4
Rukungiri	5,843 (25.2)	24.7-25.8

Comment [san3]: Change to 37-46

Overall Prevalence of TTIs among the blood donors in southwestern Uganda

From the total of 23,504 donors in 2014, 5.67% (n=1,332) were infected with at least one pathogen. The overall prevalence of TTIs (HIV, HBV, HCV and Syphilis) was 1.03%, 1.87%, 2.22%, 0.54% respectively as shown in table 2 below.

Table 2: Overall Prevalence of TTIs among the blood donors in southwestern Uganda

Parameter	Total sample	TTIs			
	n(%)	HIV n(%)	HBV n(%)	HCV n(%)	Syphilis n(%)
Positive	1,332 (5.67)	243 (1.03%)	440 (1.87)	522 (2.22)	127 (0.5)
Negative	22,172 (94.33)	23,261 (98.97)	23,064 (98.13)	22,982 (97.78)	23,377 (99.46)
Total	23,504 (100%)	23,504 (100%)	23,504 (100%)	23,504 (100%)	23,504 (100%)

Prevalence of TTIs by Age groups, Gender, ABO and Rhesus (D) Blood groups and Districts.

Despite the overall prevalence of TTIs being low among the donors, results in Table 3 below shows high risks for HBV, HCV, HIV and syphilis among donors aged 47 years and above with 2.7% for HIV, 2.4% for HBV and 2.0% for syphilis. The prevalence for HCV was more pronounced among the middle age group with the highest being 2.6% for the blood donors aged 27-36 years but the age group above 47 years had zero incidence. The prevalence of TTIs (HIV, HBV, HCV, and syphilis) in males was 1.1%, 2.1%, 2.6% and 0.5% respectively. Meanwhile among the female donors the prevalence of TTIs (HIV, HBV, HCV, and syphilis) was 0.9%, 1.4%, 1.3% and 0.6% respectively. Of all the TTIs infections detected among the donors, syphilis infection

recorded the least prevalence (0.54%). Among the eight districts where blood was being donated, Kiruhura district had the highest prevalence of most of the TTIs with HBV (2.9%), HCV (5.5%) and HIV (1.1%). Meanwhile the prevalence of syphilis was highest among the blood donors from Rukungiri district (1.1%).

Table 3: Prevalence of TTIs by Age groups, Gender, ABO and Rhesus (D) Blood groups and Districts.

Variables	HBV		HCV		HIV		SYP	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Study population (n=23,504)	440 (1.87)	1.7 –2.1	522(2.22)	2-2.4	243(1.03)	0.9-1.2	127(0.54)	0.5-0.6
Age (n=23,504)								
17 – 26	381(1.8)	1.7-2.0	456(2.2)	2.0-2.4	194(0.9)	0.82-1.1	107(0.5)	0.4-0.6
27 – 36	42(2.1)	1.6-2.9	51(2.6)	2.0-3.4	37(1.9)	1.3-2.6	13(0.7)	0.4-1.1
37-46	12(1.9)	1.1-3.4	15(2.4)	1.4-3.9	8(1.3)	0.6-2.4	4(0.6)	0.2-1.7
>47	5(2.4)	1.4-7.9	(0)	0	4(2.7)	1.0-7.0	3(2.0)	0.7-6.7
Sex (n=23,504)								
Male	351(2.1)	1.8-2.3	84(2.6)	2.3-2.8	55(1.1)	1.0-1.3	91(0.5)	0.4-0.7
Female	89(1.4)	1.1-1.7	438(1.3)	1.1-1.6	188(0.9)	0.7-1.1	36(0.6)	0.4-0.8
ABO blood groups (n=23,504)								
A	90(1.5)	1.2-1.9	122(2.1)	1.7-2.5	70(1.2)	0.9-1.5	48(0.8)	0.6-1.1
AB	16(1.6)	0.9-2.6	22(2.2)	1.4-3.3	9(0.9)	0.5-1.7	2(0.2)	0.05-0.8
B	91(1.9)	1.5-2.3	122(2.5)	2.1-3.0	39(0.8)	0.6-1.1	22(0.5)	0.3-0.7
O	243(2.1)	1.8-2.3	256(2.2)	1.9-2.4	125(1.1)	0.9-1.3	55(0.5)	0.4-0.6
Rhesus (D) blood groups (n=23,504)								
Rh(D) positive	425(1.8)	1.7-2.0	507(2.2)	2.0-2.4	239(1.0)	0.9-1.2	122(0.5)	0.4-0.6
Rh (D) negative	15(3.1)	1.9-5.2	15(3.1)	1.9-5.2	4(0.8)	0.3-2.4	5(1.0)	0.4-2.5
Districts (n=23,504)								
Bushenyi	54(1.8)	1.4-2.3	65(2.1)	1.7-2.7	33(1.1)	0.8-1.5	5(0.2)	0.01-0.4
Ibanda	12(0.8)	0.5-1.5	46(3.2)	2.4-4.3	17(1.2)	0.7-1.9	7(0.5)	0.2-1.0
Isingiro	43(2.3)	1.7-3.1	52(2.8)	2.1-3.6	13(0.7)	0.4-1.2	2(0.1)	0.03-0.4
Kabale	49(1.6)	1.2-2.1	27(0.8)	0.6-1.2	27(0.9)	0.6-1.3	12(0.4)	0.2-0.7
Kiruhura	38(2.9)	2.1-3.9	72(5.5)	4.4-6.8	15(1.1)	0.7-1.9	8(0.6)	0.1-1.2
Mbarara	106(2.1)	1.7-2.5	119(2.3)	1.9-2.8	70(1.4)	1.1-1.7	24(0.5)	0.3-0.7
Ntungamo	22(1.5)	1.0-2.3	34(2.4)	1.7-3.3	14(1.0)	0.6-1.6	3(0.2)	0.07-0.7
Rukungiri	107(1.8)	1.5-2.2	86(1.5)	1.2-1.8	50(0.9)	0.6-1.1	66(1.1)	0.9-1.4

ASSOCIATION BETWEEN SOCIODEMOGRAPHIC VARIABLES AND PREVALENCE OF TTIs AMONG THE BLOOD DONORS IN SOUTHWESTERN UGANDA

Results in table 4 below shows Males within the age group above 47 years had predominant HIV infections than other age groups ($p=0.001$). There was an association between blood group A donors and having syphilitic infection ($p=0.006$). This was a statistically significant association between male gender and having HBV and HCV infections ($p=0.001$ and $p<0.001$) respectively. There was no statistically significant association between the Rhesus (D) blood groups and the different transfusion transmissible infections among the blood donors.

Table 4: ASSOCIATION BETWEEN SOCIODEMOGRAPHIC VARIABLES AND PREVALENCE OF TTIs AMONG THE BLOOD DONORS IN SOUTHWESTERN UGANDA

Age groups	HIV (%)	p-value	HBV (%)	p-value	HCV (%)	p-value	Syphilis (%)	p-value
17-26	0.93	0.001*	1.83	0.431	2.20	0.187	0.52	0.072
27-36	1.88		2.14		2.60		0.66	
37-46	1.28		1.92		2.40		0.64	
>47 years	2.70		3.38		0.00		2.03	
SEX								
Female	0.86	0.109	1.39	0.001*	1.31	0.001*	0.56	0.767
Male	1.10		2.05		2.56		0.53	
ABO Blood groups								
A	1.19	0.269	1.53	0.099	2.08	0.378	0.82	0.006*
B	0.92		1.59		2.19		0.46	
AB	0.81		1.90		2.55		0.20	

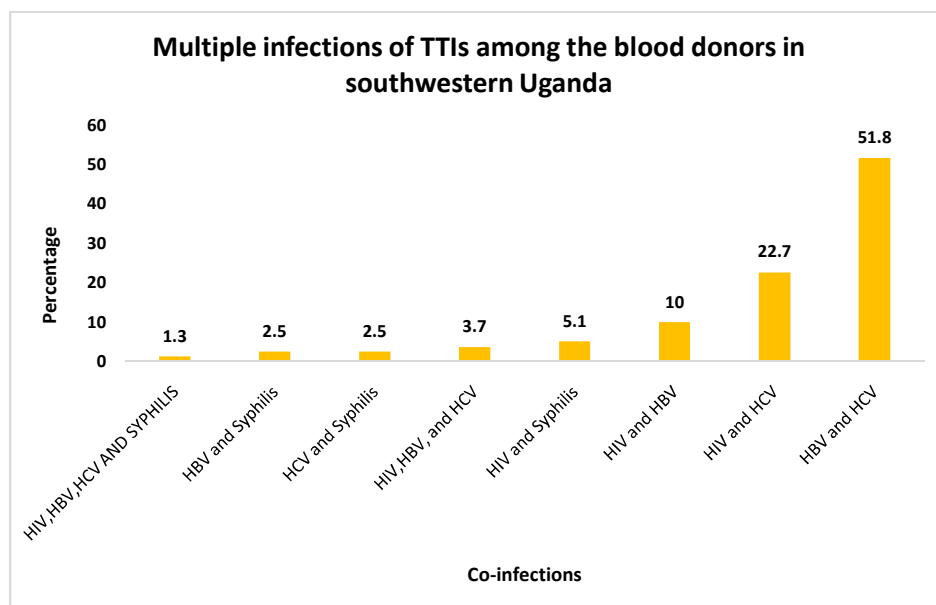
*significant at p-value less than 0.01

Comment [san4]: 47&above

Multiple infections of TTIs among the blood donors in southwestern Uganda

Results in Figure 1 below show 0.34% (n=79) voluntary blood donors had multiple infections. Of these, 51.8% (n=41) had co-infections of hepatitis B and hepatitis C viruses, 22.7% (n=18) were co-infected with HIV and hepatitis C Virus, 10.0% (n=8) were co-infected with HIV and hepatitis B, and meanwhile 2.5% (n=2) was the respective prevalence for the co-infections of hepatitis B and syphilis and as well as for hepatitis C and syphilis. The prevalence of triple infections was: 3.7% (n=3) for HIV, hepatitis B and hepatitis C and then 1.3% (n=1) for HIV, hepatitis C and syphilis. There were no other combinations of triple infections and the prevalence of all the four (quadruple) infection was zero.

Figure 1: Multiple infections of TTIs among the blood donors in southwestern Uganda



Discussions

Transfusion of blood and blood components is a life-saving measure and help people worldwide. Conversely blood transfusion can mean to be a potential significant route of infection although risk may be reduced by vigorous screening of donors or donated blood with laboratory screening tests. In the developed countries, the estimated incidence of transfusion induced transmitted HIV, HBVs Ag and HCV is very low due to improved screening methods of blood and blood products¹¹.

The overall prevalence of TTIs in this study when compared with similar studies conducted elsewhere in other parts of African countries was low, suggesting that blood donor population in southwestern Uganda are relatively healthier than other African donor populations with regard to the TTIs mentioned. However higher rates of TTIs were found in Burkina Faso 29.82%¹², southwest Nigeria 28.8%¹³, Tanzania with 15.9%¹⁴, Cameroon with 13.2%¹⁵ and Ethiopia with 9.5% but our overall prevalence was higher than those reported for Namibia with only 1.3%¹⁶ and Eritrea 3.8%¹⁷. Lower rates of TTIs was reported elsewhere in India among blood donors of 0.17%¹⁸. The prevalence of TTIs found among the donor population in this study may not be comparable with the general population in Uganda due to the blood donor selection procedures involved in selecting donors. Blood donors undergo pre-donation screening for risk factors, which is aimed at excluding donors potentially at risk of being infected with TTIs and probably be the reason for the low distribution of the four TTIs obtained among the study population.

Geographic differences in endemicity of HCV infection can be described on regional prevalence; high (>3%), moderate (prevalence 2-2.9%), low (prevalence 1.0-1.9%), and very low (prevalence <1.0%)^{19, 20}. The moderate prevalence of HCV (2.22%) observed in this study among blood donors in southwestern Uganda is higher than values ranging from 0.1% in Namibia¹⁷ and 1.3% in Douala-Cameroon¹⁵, while it is lower than 6.0% in south-west Nigeria¹³, 4.1% in Uganda²¹, 8.4% in a study of blood donors in Ghana²² and the highest prevalence of hepatitis C among^{5, 6}. Like many countries with moderate prevalence of HCV, unsafe therapeutic injections performed by both professionals and nonprofessionals appear to be the predominant mode of HCV infection worldwide with Uganda inclusive. The current prevalence of HCV found in the present study is in line with findings in other parts of Africa, showing a range of between 0.2% and 3.0%²³ and further agrees with the estimated worldwide prevalence of HCV infection of 2.2%²⁴. The finding of a moderate prevalence of anti-HCV antibodies among apparently healthy blood donors in southwestern Uganda further confirms the presence of hepatitis C infection in Uganda and highlights the necessity to adopt measures that will ensure safe blood transfusion.

The seroprevalence of hepatitis B (1.87%) in this study is lower than the 8.8% in Dar Es Salaam, Tanzania¹⁴, 10.4% in Nigeria²⁵, 4.7% in Ethiopia², and 15.0% in Ghana²². However, it is higher than the 1.3% in Namibia¹⁶ and 1.09% in India²⁶. Seroprevalence of hepatitis B infection among blood donors differs widely among donors. The major route of HBV transmission is parenteral and it is the most infective among bloodborne viruses and chronic carrier state is associated with chronic liver diseases, cirrhosis and hepatocellular carcinoma. There was a statistical association between male gender and having HBV and HCV infections ($p=0.001$ and $p<0.001$) respectively. There may be no apparent reason attributed to this association but other studies have reported similar prevalence differences among the gender²⁷.²⁸ The findings in this study found male donors being more positive for HBV and HCV than females

with ratio of 2.1%: 1.4% for HBV, and 2.6%:1.31% for HCV. This finding was in concordance with similar findings obtained from Tanzania¹⁴.

The seropositivity of syphilis in our study (0.56%) when compared with similar studies done elsewhere on some African countries is low. For instance in southwestern Nigeria it was 1.1%¹³, 8.1% in Doula, Cameroon¹⁵, 1.3% in Ethiopia², 4.5% in Dar es Salaam, Tanzania¹⁴ and 7.5% in Accra, Ghana²⁹ but our prevalence of 0.56% for syphilis among the blood donors was higher than those reported by Ejele et al³⁰ in Port Harcourt 0.1% and 0.31% reported by TEO et al³¹ in Brunei Darussalam. In other similar studies done on blood donors in India, even much lower prevalence of syphilis were reported; 0.1% and 0.17%¹⁸. The reason for the low rate of seroprevalence in our study when compared with the higher prevalence obtained elsewhere from other African countries mentioned above (Nigeria, Cameroon, Ethiopia, Tanzania and Ghana) could not be discerned.

Prenatal screening and treatment of pregnant women for syphilis is cost effective, even in areas of prevalence as low as 0.1%. In South Africa for instance, perinatal death was 19.4 times more likely if incomplete treatment or not at all was received³². No matter how low the incidence of syphilis is among the population of blood donors, incidence of syphilis has been associated with risk factor for mother to child transmission of HIV³³ and furthermore a systematic review of coinfection of syphilis and HIV in developed and developing countries predicts 9.8% of HIV positive persons to be coinfecting with syphilis³⁴. Since syphilis is a major public health problem worldwide, it is imperative to screen all blood donors for circulating antibodies to syphilis infection, at least as a surrogate marker. There was a statistically significant association observed between blood group A donors and having syphilitic infection ($p=0.006$). The reason (s) for this association is difficult to discern although other previous studies had found no association between any of the ABO and Rhesus blood group and syphilis infection³⁵.

This study highlights low (1.03%) seroprevalence of HIV infection among the voluntary blood donors in southwestern Uganda which is in concordance with other studies conducted in some parts of African continent such as Namibia with 1.3%¹⁶ but is lower than some of the prevalence of HIV found in other studies elsewhere in Africa such as in Ethiopia 3.8%², Mozambique in 2011 the prevalence was 8.5%³⁶, Tanzania in 2006 and 2009 was 3.8% and 0.01%^{14, 31}, in Burkina Faso 2.21%¹², in Abeokuta-Nigeria 6.2%³⁰ and in Nigeria 3.1%¹³. In Kampala- Uganda, Carswell in 1987²¹ obtained a rate of 15.86% among the healthy population. There was no statistical difference between the female and male donors as far as HIV seropositivity was concerned. This finding was in concordance with the finding in Burkina Faso¹² where HIV seropositivity rate was also identical among female and male donors. The seropositivity of HIV in our study was even lower than the current prevalence of HIV infection found in the general population of Uganda 7.3%⁹.

Apart from the rigorous blood donor selection procedures involved in selecting donors in Uganda, the low prevalence of HIV found among the blood donors could be due to the reduction in risk behavior which provides the most consistent explanation for this decline of HIV-1 in Uganda³⁷. There are a number of strategies being implemented in this country towards the reduction of HIV transmission and prevention of HIV infection through the Uganda HIV prevention programme evolved along the interventions of promoting abstinence, being faithful, and condom use (the ABC approach)³⁸. Other strategies being used are treatment of opportunistic infections, prevention of mother to child transmission of HIV (PMTCT), use of antiretroviral therapy and HIV counseling and testing³⁸. The research finding revealed high prevalence 2.7% of HIV infection among the age group above 47 years ($p=0.001$) and is in

concordance with the findings of 3.70% of subjects above 40 years among blood donors in Burkina Faso¹². This finding contrasts with a recently reported reduction of HIV prevalence among young people in sub-Saharan Africa³⁹.

In this study, high prevalence rate of HIV, HBV, HCV, and syphilis dual and triple co-infections was revealed among the blood donors. The significant prevalence of TTIs among the blood donors in this study compares very well with similar study conducted by Walana et al²⁰ and found the highest number of dual co-infection cases being HBV-HCV (45.5% 10/22), followed by HIV-HCV (27.3% 6/22) and then HIV-HBV (18.2% 4/22). Very high dual co-infections were obtained by Buseri et al¹³ in their study on prevalence of TTIs among blood donors in Osogbo, southwest Nigeria. The most common dual co-infections got by Tessema et al² at Gondar University Teaching hospital was HIV-syphilis 38% (n=19) and HIV-HBV 34% (n=17), a finding which compares very well with our study. The high rate of coinfection and the statistically significant relationship between HBV and HCV, and HIV and HCV coinfection might be due to the fact that these pathogens share common modes of transmission and risk groups⁴⁰. Hepatitis B virus and HCV coinfection is relatively common as an estimated 7 to 20 million people suffer this condition globally⁴¹. The shared modes of transmission have been reported as the reason for most HBV-HCV co-infection. However, super-infection seems to be the commonest cause of HBV-HCV co-infection⁴². Persons with either HIV-HBV or HIV-HCV co-infection stand a greater risk of proceeding at a faster rate to developing hepatocellular carcinoma as the immune system deteriorates rapidly^{43,44}.

Triple co-infection permutations of HIV, HBV and HCV 3.61% (n=3) and HIV, HBV and syphilis 1.2% (n=1) were observed in our study. Similar triple coinfection permutations were observed in other similar studies conducted among blood donors in Africa¹²⁻¹⁴. No quadruple

permutationsofco-infections of HIV, HBV, HCV, and syphilis was also observed among the blood donors in our study. There is paucity of data on quadruple permutation of TTIs infection and only one report on an inmate was cited for having a quadruple co-infection with HIV-HBV-HCV and syphilis in a study conducted by Adjei et al²⁹ in Accra Ghana.

Conclusion and Recommendation

A significant percentage (5.67%) of seroprevalence of transfusion transmissible infections with at least one pathogen and 0.35% with multiple infections among our blood donors in southwestern Uganda is alarming and calls for a comprehensive screening of donor's blood and blood products for HIV, HBV, HCV, and syphilis using standard sensitive methods are highly recommended to ensure the safety of blood for recipients. The practice of strict selection of donors with emphasis of getting young voluntary non remunerated donors in all the regions in Uganda should be adhered to. Introducing nucleic acid testing (NAT) for HIV, HBsAg and HCV is recommended to detect the infection during the window period. We recommend similar research to be conducted in all other Blood Transfusion Services stations in Uganda in order to establish the current burden of TTIs among the blood donors and guarantee the safety of blood for recipient in Uganda.

Limitations

This study was a retrospective study so it was not possible to follow up donors for repeat analysis in cases of indeterminate results obtained during the initial run. Even though the sample size was large (23,504 samples), a substantial data was lost due to incompleteness of some of the data on donors. Some needed variables like that on sociodemographic issues were missing, which if collected could have greatly improved our understanding of the dynamics of the TTI infections among the blood donors. The extrapolation of research findings to the general population is difficult because of the bias sampling method of donors who were mainly drawn from the young school going age children mostly at the ages of 18 years and below. The serology of the blood samples were carried out from

the blood bank using the Architect i 2000 immunoassay system and all the positive samples were re-tested using an ELSA system. Like many immunoassays Architect/2000 system is prone to producing nonspecific reactions due to some reasons especially when testing in low prevalence populations. Repeatedly reactive specimens should be investigated further with sensitive supplemental tests such as recombinant immunoblots, antigen tests and nucleic acid tests. Nucleic acid test (NAT) is not being performed in the blood bank as part of the routine testing and yet the NAT results would capture clients who are infected with the viruses (HIV, HBV and HCV) and are in the window period. Architect HIV Ag/Ab Combo results does not distinguish between the detection of HIV p24 antigen, HIV-1 antibody or HIV-2 antibody. Testing for hepatitis B core antibodies (anti-HBc) and HBV DNA are not being done on the samples from the blood bank and therefore individuals with HBsAg negative with isolated anti-HBc and occult HBV infection could have been missed through this method of screening.

Competing interests

The author(s) declare that they have no competing interest

Author's contributions

All authors read and approved the final copy of the manuscript. ROA participated in the conception, design, data collection, analysis, and drafting and approval of the manuscript; EMM participated in the conception, drafting and approval of the manuscript; FB participated in the conception, design, data collection, analysis, and drafting and approval of the manuscript. AB participated in the conception, drafting and approval of the manuscript. YB2 participated in the drafting and approval of the manuscript, FBB participated in the drafting and approval of the manuscript. JB participated in the drafting and approval of the manuscript.

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