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PREVALENCE OF TRANSFUSION TRANSMITTED INFECTIONS AMONG BLOOD DONORS AT YIRGALEM HOSPITAL AND HAWASSA BLOOD BANK CENTER, SIDAMA ZONE, SNNPRS, ETHIOPIA

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Abbreviations

AIDS	Acquired immune deficiency syndrome
ELISA	Enzyme Linked Immune Sorbent Assay
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
NRCS	National Red Cross society
RDT	Rapid diagnostic test
SNNPRS	South Nation National People Region State
SSA	Sub Sahara Africa
TTIs	Transfusion-transmitted infection
WHO	World Health Organization

Operational Definition

TTI: -Transfusion Transmitted Infection

In this paper TTI stands only for HIV, HBV, HCV, and Syphilis & Malaria.

Summary

Background

Blood is one of the integral components of body constituents. Blood transfusion is important therapy during maternal bleeding, surgery and also a lifesaving procedure for anemic patients. Transfusion reaction and transfusion transmitted infections (TTI) are major problems associated with blood transfusion. TTI is ranging from 8% up to 43%. The burden of transfusion transmitted infections in developed countries decline from time to time but it is still a challenge of developing countries like Ethiopia due to lack of adequate volunteer donors, testing material, good infrastructure and well trend personnel because of resource constraints. In addition to that there are insufficient data on seropostivity of TTI among blood donors in our setting.

Objective

To assess the prevalence of transfusion transmitted infections among blood donors at Yirgalem hospital blood bank and Hawassa blood bank center, Sidama zone, SNNPRS Ethiopia.

Methodology

Institutional based cross-sectional study was conducted, convenient sampling techniques used to address 384 donors and blood collection was done from health volunteers and replacement donors from April-May, 2015 G.C at Yirgalem Hospital and Hawassa regional Blood bank. Data was coded, cleaned, entered and analyzed by using SPSS version 21.Proportion, odds ratio and p-value were used for analyses. P-value <0.05 considered as statistically significant.

Result

A total of 384 blood donors were screened and the overall prevalence of TTI was 28 (7.29%). The Seroprevalence of HIV, HBV, HCV, Syphilis and Malaria was 6(1.6%), 16(4.2%), 2(0.5%), 3(0.8%) 1(0.3%) respectively. Two out of 384(0.5%) had multiple infections. The combinations were HIV-HBV 1/384(0.26%) and HIV-HCV1/384(0.26%). The prevalence of HBV was 13/258(5.0%) and 3/126(2.4%) in male and female respectively, p=0.001. When see the age of donors, 15/331(4.5%) and 1/41(2.4%) in age group18-24 and 25-34 years respectively. The prevalence of HIV was 1/25(4.0%) and 5/359(1.4%) in urban and rural respectively, p=0.002

Conclusion and Recommendation

The prevalence of TTI was 28(7.29%), the seroprevalence of HIV 6(1.6%), HBV 16(4.2%), HCV2 (0.5%), Syphilis 3(0.8%) and Malaria 1(0.3%). High prevalence of HBV, HCV and syphilis infection were found among family replacement blood donors when compared with voluntary blood donors. Students highly participated in voluntary blood donation program when compared with others. Creating awareness among the public is important to have voluntary donors and this helps to provide safe blood to blood transfusion and collection services.

1. Introduction

1.1Background

Blood is one of the integral components of body constituents, which flows throughout body and becomes a reason for survival. Blood transfusion is important therapy during maternal bleeding, surgery and also a lifesaving procedure for anemic patients. Transfusion reaction and transfusion transmitted infections are major problems associated with blood transfusion. Providing safe and adequate blood is recommended to be an integral part of every countries national health care policy and infrastructure. WHO suggests that all activities associated to blood collection, testing, processing, storage and distribution be matched at the national level through effective organization and a national blood policy. This is now supported by appropriate legislation to promote even implementation of standards and consistency in the quality and safety of blood and blood products (1, 2).

Over a million blood units are collected from donors every year, and many more millions still need to be collected to meet global demand and ensure sufficient and timely provision of blood. An unsafe blood transfusion is very costly from both economic and human point of view. Long-term morbidity and mortality, delayed viraemia and hidden states resulting from the transfusion of infected blood have far-reaching consequences, not only for the recipients themselves, but also for their families and their communities (3, 4). A number of viruses, bacteria and parasites can be transmitted through blood or blood products including hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), malaria and syphilis(5).

Globally, transfusion of contaminated blood causes up to 16 million new infections with hepatitis B, and 5 million new infections with hepatitis C every year. With each blood unit transfused, there is always a 1% likelihood of transfusion-linked risks including transfusion-transmitted infections. Prevention of transfusion transmitted infections (TTIs) in developed countries has been achieved by reducing unnecessary transfusions, using only regular voluntary donors, excluding donors with specific risk factors and systematic screening of all donated blood for infection. By contrast, in many developing countries none of these interventions is applied uniformly and the risk of transfusion-transmitted infections remains high (6-8).

WHO recommends that all blood donations should be screened for infection prior to use. Screening should be mandatory for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and syphilis (9).

Only continuous improvement and implementation of donor selection, sensitive screening tests, and effective inactivation procedures can ensure the elimination, or at least reduction of the risk of acquiring TTIs. There are several methods to screen transfusion transmissible infections; assays for HIV infection, Hepatitis B virus, Hepatitis C virus, Syphilis and malaria are screened by using 4th generation ELISA Vironostika HIV Uni-Form II AG/Ab, ELISA Hepanostika HBSAgUNi-Form II, using the Human anti-HCV 3rd generation ELISA, using Rapid Plasma-Reagnic and both malaria rapid test and malaria thick blood film examination respectively.there is no comprehensive study in the study area that is done on all major types of transfusion transmitted infections (HIV, HBV, HCV, Syphilis and Malaria), only there is fragmented study done on two or three type of transfusion transmitted infection. Therefore, the present study is intended to include mostly reported transfusion transmitted infections (10).

1.2 Statement of the Problem

Millions of lives are saved each year through blood transfusion but, a safe blood is a critical component of health care to prevent the spread of blood borne infectious diseases. Safe blood is a continuing concern especially in developing countries where 80% of the world population lives. General lack of quality systems, poor laboratory procedures, inadequate testing of donated blood, inappropriate use of blood and blood components may contribute for unsafe blood transfusion services (11).

Burden of the disease and loss of life resulting from unsafe blood transfusions is very serious problem for the communities in developing countries. The transfusion transmissible infections (TTI) like hepatitis B, hepatitis C, HIV, malaria and syphilis etc. were first observed in the process of blood transfusion in the late 1940s.Hepatitis B virus (HBV), hepatitis C virus (HCV), Human immune deficiency virus (HIV) and syphilis are the most important lethal agents in transfusion transmitted infections (TTIs) and it remains a large health care burden globally. The incidence rates across the world are difficult to calculate given the asymptomatic and often latent nature of the disease prior to clinical presentation. Every blood transfusion therefore carries a potential risk for transmissible diseases (11-13).

The picture is very different in developing countries compared with developed countries, where blood transfusions remain a major cause of the spread of TTI. In the period 2001–2002, more than six million blood units were not screened for major blood-borne infections, including HCV. Although this number was considerably less than during the 1998–1999, this reduction cannot be considered sufficient to limit the continuing HCV epidemic (14). Developing nations largely fail to meet the key requirements for a modern blood transfusion system: i.e. the collection of a safe and locally sufficient blood supply from non-remunerated donors within a formal organizational and legislative framework. The underlying problems are structural and attributable to various causes. Blood safety is threatened by a combination of factors due to poverty; including a lack of infrastructures, frequent electricity breakdowns, the unavailability of properly trained professionals, an inadequate supply of instruments and laboratory reagents, and also difficulties in mobilizing volunteer donors (14).

The risk of transmission of syphilis from unscreened donations is variable; the burdens of syphilis in different countries vary from place to place due to different factors. Some country like Ital 5 years reports showed 132/ 419,740 (0.031%) were positive for syphilis. Also in china prevalence of syphilis among a total of volunteer blood donors over the 5-year period of 2006–2010; 575/ 159, 902 (0.36%) samples showed positive. The percentage of samples testing positive for syphilis increased from 0.24% in 2006 to 0.51% in 2010. Pakistan's syphilis prevalence indicated 449/ 14,352 (3.1%) confirmed cases found to be syphilis positive. Therefore, the burden of transfusion transmitted syphilis among blood donors is critical (15-17).

Unsafe blood transfusions have contributed to the enormous burden of HIV infections in sub-Saharan Africa and still continue to add to this burden. The risk of HIV infection through unsafe blood and blood products is exceptionally high (95–100%) compared to other common routes of HIV exposure: for example, 11–32% for mother-to-child transmission and 0.1%–10% for sexual contact. Sub-Saharan Africa has a particularly high level of transfusion-associated HIV compared with other regions due to a higher risk of infected blood being transfused (18).

The transmission of malaria by blood transfusion was one of the first recorded incidents of transfusion-transmitted infections (TTIs). Although the World Health Organization (WHO) recommends that blood for transfusion should be screened for TTIs, malaria screening is not performed in most malaria-endemic countries in sub-Saharan Africa (SSA). The prevalence of malaria in blood donors is variable but can reach 55% in some parts of West Africa. The transfusion of infected red blood cells may lead to severe post-transfusion clinical manifestations of malaria, which could be rapidly fatal (19).

The prevalence of malaria parasitaemia in African donors depends on the local endemicity and transmission season varies from 0.67% in Nairobi, Kenya, a non-endemic area, to over 55% in highly endemic northern Nigeria (with a median prevalence of 10.2%) (20). Plasmodium species are the most prevalent transfusion transmissible pathogens especially in Sub-Saharan Africa (SSA) (16). In fact, among SSA blood donors, the prevalence of human immunodeficiency virus, hepatitis C virus and hepatitis B virus ranges from 0.5-16%, 0.5%-12.3% and 2.5-20% respectively. The overall prevalence of TTI ranging from 8% up to 43%(21).

In Ethiopia, the transfusion transmitted infection burden varies in different places. When see the prevalence of TTI among blood donors at FelegeHiwot referral hospital the overall prevalence was 43.2%; out of this 25% HBsAg, 13.3% HCV, 11.7% HIV and 1.2 Syphilis. Another northwest Ethiopia data showed prevalence of HBV 3.8%, HCV 4.7% and syphilis 1.3%. In addition to that, HBV 6.2%, HCV 1.7% and Malaria 1% in Gonder , Bahirdar, Dessie and Mekele among health blood donors. The difference may be due to type of infection disease that was screened and characteristics of the population (22, 23, and 24).

1.3 Significance of the Study

The burden of transfusion transmitted infections in developed countries decline from time to time due to implementation of different advanced sensitive diagnostic tests, avoiding unwanted transfusion and early detection by using advanced laboratory assays of the infectious agents from donated blood; but TTI is still a challenge of developing countries like Ethiopia due to lack of adequate volunteer donors, testing material, good infrastructure and well trained personnel because of resource constraints. In addition to that, there are insufficient data on seropostivity of TTI among blood donors in our country. Therefore, the present study is intended to find out the actual magnitude of TTI among blood donors, contributing factors and also based on research findings to recommend remedy action for further planning to improve blood service delivery and recommend possible interventions to save lives risked due to unsafe transfusions.

2. Literature Review

Among United State (US) America voluntary blood donations, the prevalence rates of infection markers for hepatitis C virus (HCV) and hepatitis B virus (HBV) have decreased over time, although rates for markers of human immune deficiency virus (HIV) did not. The incidence (/100 000 person-years) of HIV and HCV among repeat donors showed apparent increases from 1.55 and 1.89 in 2000 through 2001 to 2.16 and 2.98 in 2007 through 2008. These observed fluctuations confirm the need for continuous monitoring and evaluation (25).

Indian institutional based studies during the various blood donation camps in Mangalore on Prevalence of HIV, HCV, HBV and Syphilis in Blood donors from 2008 to 2010 indicated that the seroprevalence HBV, HCV, HIV and Syphilis infection was to be 0.53%, 0.098%, 0.08% and 0.09% respectively. Another Indian hospital study's done from January 2008 to December 2011 showed that 210/25633 (0.82%) positive for TTI; among this HIV, HBsAg, HCV and syphilis was 0.1%, 0.5%, 0.08% and 0.07% respectively. The incidences of seroposetivity high between 18-33 age group than older age (26). Another cross-sectional study was conducted from January 2013 to December 2013 at Indian blood bank in Rajkot hospital results showed Seroprevalence of HIV, HBV (HBsAg), HCV, Syphilis and Malaria were observed to be 0.074%, 0.68%, 0.074%, 0.065% and 0.037% respectively. TTI were dominant among male blood donors compared to female blood donors. Higher HIV and HCV seroprevalence was observed among males compared to females ($p<0.05$) (27).

WHO is trying to improve blood safety in sub Sahara Africa by establishing different systems. when we see the burden of TTI, it remains the greatest threats to blood safety in Burkina Faso from the total of 4,520 blood donors in 2009, 1,348 (29.82%) were infected with at least one pathogen and 149 (3.30%) had serological evidence of multiple infections. The overall seroprevalence rate of HIV, HBV, HCV and syphilis was 2.21%, 14.96%, 8.69% and 3.96%, respectively (28).

Another Retrospective Single Centre Cross Sectional Study done in Pakistan on prevalence of transfusion transmitted infections among blood donors showed prevalence for HCV, HBV, HIV, Syphilis and Malaria were found to be 8.34%, 3.91%, 0%, 0.89% and 1.20% respectively(29).The same country research done from Jan 2002 to Dec 2006 in RAWAIPindi general hospital

indicated prevalence of HIV,HBV and HCV in healthy blood donors was found 0%,2.31%(=n 3241) ,and 4.87%(n=6834). HCV2.1 times more prevalent than HBV also the report showed the HBV and HCV prevalence declined in 2004 compared to 2002 (30). Another Pakistan study done on trend of transfusion transmitted infections frequency in blood donors: provide a road map for its prevention and control showed out of 127,828 sample of blood donors, recorded mean prevalence for HBs Ag, anti-HCV, anti-HIV and syphilis was 2.68%, 2.46%, 0.06% and 0.43%, respectively, with an increasing trend in frequencies of TTIs (31).

A Nigerian study showed that 54 (19.3%) donors had serological evidence of infection with at least one pathogen, while 8 (2.9%) had multiple infections. The seroprevalences of syphilis, HBsAg, HCV, and HIV were found to be 7.5%, 11.1%, 1.8%, and 1.4%, respectively (3).And also prevalence of HBsAg and HIV among blood donors in Nigeria indicated that 124 donors (19.9%) were positive for HBsAg; 19.6% males and 21.0% females. 21 (3%) donors were positive for HIV-1 antibody; 3.4% males and 3.2% females and 3 donors (0.5%) were positive for combined HBsAg and HIV-1 antibodies, 0.4% males and 3.8% females. HIV-2 antibody was not detected in any of the sample. The result of this study showed that the prevalence of hepatitis B and HIV infections is high among apparently healthy blood donors of all gender and age groups and therefore the need to mandate all organization involved in blood banking to ensure proper screening of blood units prior to transfusion in order to reduce the risk of HBV and HIV infections among recipients of donated blood and the community at large (32).

Egypt university students blood donation indicated that the overall prevalence of HBsAg, anti-HCV, anti-HIV-1 , anti-HIV-2, and syphilis were 4.3%, 2.7%, 0.0% and 0.05% respectively (33). In Eritrea, a retrospective study done on transfusion transmitted infections –analysis from the National Blood Transfusion Service from January 2006 to November 2009 indicated that the overall prevalence of TTI's were 3.8% with 3.5% in voluntary blood donors and 5.1% in family replacement donors. The sero-prevalence for TTI markers were 0.18% HIV, 2.58% HBV, 0.57% HCV and 0.49% Syphilis (34).

In Ethiopia, cross-sectional study was conducted to determine the seroprevalence of major transfusion transmissible infections among blood donors at FelegeHiwot referral hospital which showed that the overall seroprevalence of major blood borne pathogens among blood donors was 43.2%, of which HBsAg, anti-HCV IgG, HIV seropositivity and syphilis accounted for 25%,

13.3%, 11.7% and 1.2% respectively. Crude seroprevalence of major blood borne pathogens was significantly higher in commercial blood donors (56.6%) compared to voluntary (17.6%) and replacement blood donors (53.6%) (p=0.0001)(22).

Another study done in Northwest Ethiopia indicated the overall seroprevalence of HIV, HBV, HCV and syphilis was 3.8%, 4.7%, 0.7%, and 1.3% respectively. Among those with multiple infections, the most common combinations were HIV - syphilis 19 (38%) and HIV - HBV 17 (34%). The seropositivity of HIV was significantly increased among female blood donors, first time donors, housewives, merchants, soldiers, drivers and construction workers. Significantly increased HBV seropositivity was observed among farmers, first time donors and age groups of 26 - 35 and 36 - 45 years. Similarly, the seroprevalence of syphilis was significantly increased among daily labourers and construction workers. Statistically significant association was observed between syphilis and HIV infections, and HCV and HIV infections. Moreover, significantly declining trends of HIV, HCV and syphilis seropositivity were observed over the study period (23). Another research that conducted to determine the prevalence of HBV, HCV and malaria parasites among healthy adult blood-donors in Gondar, Bahirdar, Dessie and Mekele blood banks showed the overall prevalence of HBV, HCV and malaria parasites were 6.2%, 1.7% and 1% respectively. The possible reason for the discrepancy between reports in Ethiopia; the donor types, type of infection disease that was screened and study design (24).

3. Objective

3.1 General objective

- To assess the prevalence of transfusion transmitted infections among blood donors at Yirgalem hospital and Hawassa blood bank center, Sidama zone, SNNPRG.

3.2 Specific Objective

- To determine the prevalence of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) among blood donors.
- To determine the prevalence of syphilis among blood donors.
- To determine the prevalence of malaria among blood donors.
- To assess co-infection among blood donors.

4. Material and Methods

4.1 Study Design

Institutional based cross-sectional study was conducted and blood collection was done from healthy volunteers and replacement donors from April, 2015 to May, 2015 G.C at Yirgalem Hospital and Hawassa regional Blood bank.

4.2 Study Area

The study was conducted in SNNPRS, Ethiopia, at Yirglem Hospital and Hawassa regional blood bank service center. Yirgalem hospital was established In 1968 G.C. 42 km far from Hawassa city. Hawassa regional blood bank which is located in "Hawassa city" was established in May 2013 G.C and 275 km south of Addis Ababa the capital city of Ethiopia along the main road to Nairobi Kenya. Both blood banks recently working jointly in the service center and campaign and also screening was done in Hawassa blood bank center. Around 500 bags of blood are collected in monthly base by both blood banks. The study area screens only HIV, HBV, HCV and syphilis. The blood bank mainly provides services for those hospitals that are found under Sidama zone, Gedio zone and Halaba special woreda. Annual blood distribution is above 5000 bags of whole blood.

4.3 Study period

The study was conducted from April-May, 2015 G.C.

4.4 Population

4.4.1Source population

All potential donors who visited Yirgalem Hospital and Hawassa blood bank center.

4.4.2 Study population

All blood donors who attended the blood bank during the study period and who were eligible to donate blood.

4.5Inclusion and exclusion criteria

4.5.1 Inclusion criterion

All individuals who fulfilled the national and regional blood bank criteria were included(Annex).

4.5.2 Exclusion criterion

All consecutively recruited blood donors who did not meet the inclusion criteria (<18 years, > 65 years, history of long-term medication use and unwillingness to give oral informed consent) were excluded from study.

4.6 Study variable

4.6.1 Dependent variable

The prevalence of HIV, HBV, HCV, Syphilis and Malaria.

4.6.2 Independent variable

- Age
- Sex
- Marital status
- Blood group
- Occupation
- Educational status
- Type of donation

4.7 Sample size determination

Sample size was calculated based on single population proportion formula by using the following assumption: the proportion (P) of transfusion to be 50% because there was no comprehensive study done in the study area, only some of transfusion transmitted infections done in Ethiopia. So that I will try to increase sample size to achieve actual representation. With 95% confidence interval (CI) and marginal error (d) of 5% the sample size will be:

$$\underline{n=z^2\alpha/2\ p(1-p)}= 384$$
$$d^2$$

Where:

- Proportion of TTI (p) = 50%

- Confidence level of 95 % chosen
- Margin of error (d) = 5%
- Total sample is equal to 384.

4.8 Sampling method

All donors prospectively recruited in the study and convenient sampling techniques are employed to take the first 384 blood donors which are eligible to donation, interviewed and collected blood for screening of TTI.

4.9 Data Collection procedure

Prior to blood collection, the donors were requested to answer a questionnaire to determine whether they are eligible for donation per the criteria set by NRCS and by study area blood bank. Blood collected from each donor and separated serum after clotted the whole blood for examination of HIV, HBV, HCV, Syphilis and malaria. In addition to that whole blood for malaria thick blood film preparation and detection. Pre-tested semi structured Standard data collection tool was prepared for both independent and dependent variables. Data was collected by two oriented blood bank workers and by principal investigator.

4.10 Laboratory testing

Blood samples tested by using serological assays for HIV infection were screened by using 4thgeneration ELISA, Vironostika HIV Uni-Form II AG/Ab, Hepatitis B virus were screened by using an immunoassay ELISA Hepanostika HBSAgUNi-Form II, Hepatitis C virus using the Human anti-HCV 3rd generation ELISA, Syphilis using ELISA and malaria were screened by using malaria rapid tests and blood film examination by microscopic.

4.11 Quality Assurance

All positive test and 10% negative testes retested by regional and national laboratory to ensure the quality. Following Sops and QC materials was used for all serological testes. In addition to that quality was assured by well trained professionals, training, and supervision during sample collection, on site correction for sub standard practice, check completeness and accuracy of data collected, random checking of the data collection tools and check the standard practice of handling sample.

4.12 Data analysis

Data was entered into excel and exported to SPSS version 21 for analysis. Both dependent and independent variables and also proportion was used to determine the prevalence of TTI, Odds ratio and p-values were used to assess the strength of the association. P-value less than 0.05 was considered as statistical significance and 95% confidence interval was used.

4.13 Ethical consideration

Ethical clearance was obtained from ethical review committee of Addis Ababa University College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences and from SNNPRS health bureau. During data collection, brief explanation were given about the objective and significance of the study to each participant in order to obtain verbal consent and signed to avoid misunderstanding. All documents were kept confidential. Respondents were not identified by their name and the participant had the right to discontinue the participation any time.

4.14Dissemination of results

The research findings will be presented to AAU, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences, different conferences, manuscript will be submitted for publication and shared to South Regional Health Bureau.

5. Result

A total of 384 blood donors were screened during the study period among this 67.2 %(258) were male and 32.8% (126) were female with ratio 2:1. The minimum participant's age was 18 and maximum was 54 years old. The majority of donors 331 (86.2%) were in the age group between 18 to 24 years. From total donors, 91.9% (353) were voluntary donors and 31(8.1%) family replacement donors (Figure 1). The ABO and RH profile of the participants were 159 (41.4%) O, 121 (31.5%) A, 84 (21.9%) B, 20 (5.2%) AB. 357 (93.0%) were RH positive and 27(7.0%) were RH negative (Table 1). Socio-demographic characteristics the donors; majority were above12 grade 291(75.8%), student 330(85.9%), government workers 24(6.3%), Farmer 15 (3.9%), Private/NGO 7(1.8%), unemployed/jobless 1(0.3%). Ethnic group; 81(21.1%) were Sidama, 105(27.3%) were Amhara, 83(21.6%) were Oromo, 32(8.3 %) were Gurage, 22(5.7%) were Tigre and 61(15.9%) were others (Table 1).

Table 1 Socio—demographic characteristics of blood donors at Yirgalem hospital and Hawassa blood bank, Sidama zone, South Ethiopia April-May 2015

Characteristics		Frequency	Percent
AGE GROUP	18-24	331	86.2
	25-34	41	10.7
	35-44	7	1.8
	45-54	5	1.3
	Total	384	100.0
Sex	Male	258	67.2
	Female	126	32.8
	Total	384	100.0
Marital status	Unmaried	352	91.7
	Maried	32	8.3
	Total	384	100.0
Occupation	Goverement	24	6.3
	Merchant	7	1.8
	Farmer	15	3.9
	Student	330	85.9
	Uneploy/Jobless	1	.3
	Private/Ngo	7	1.8
	Total	384	100.0
Educational background	Illiterates	4	1.0
	1-4	5	1.3
	5-8	9	2.3
	9-12	75	19.5
	Above12grade	291	75.8
	Total	384	100.0
Donation type	Volunteers	353	91.9
	Replacements	31	8.1
	Total	384	100.0
ABO	A	121	31.5
	B	84	21.9
	AB	20	5.2
	O	159	41.4
	Total	384	100.0
RH	Postive	357	93.0
	Negative	27	7.0
	Total	384	100.0

Table-2 Prevalence of HIV, HBsAg, HCV, Syphilis and Malaria among blood donors at Yirgalem hospital and Hawassa blood bank service center, Sidama zone, SNNPRS, Ethiopia from April, 2015 to May, 2015.

Type of TTI		Frequency	Percent
HIV	positive	6	1.6
	Negative	378	98.4
	Total	384	100.0
HBV	positive	16	4.2
	Negative	368	95.8
	Total	384	100.0
HCV	positive	2	0.5
	Negative	382	99.5
	Total	384	100.0
SYHPILIS	positive	3	0.8
	Negative	381	99.2
	Total	384	100.0
MALARIA	positive	1	0.3
	Negative	383	99.7
	Total	384	100.0

The overall prevalence of TTI was 28(7.29 %) out of 384 apparently healthy donors. The Seroprevalence of HIV, HBV, HCV, Syphilis and Malaria was 6(1.6%), 16(4.2%), 2(0.5%), 3(0.8%) 1 (0.3%) respectively (Table 2). Two out of 384 (0.5%) had multiple infections. Among those with multiple infections, the most common combinations were HIV- HBV 1 (0.26%), p-value=0.162 and HBV - HCV 1 (0.26%) p-value=0.026 (Table 3).

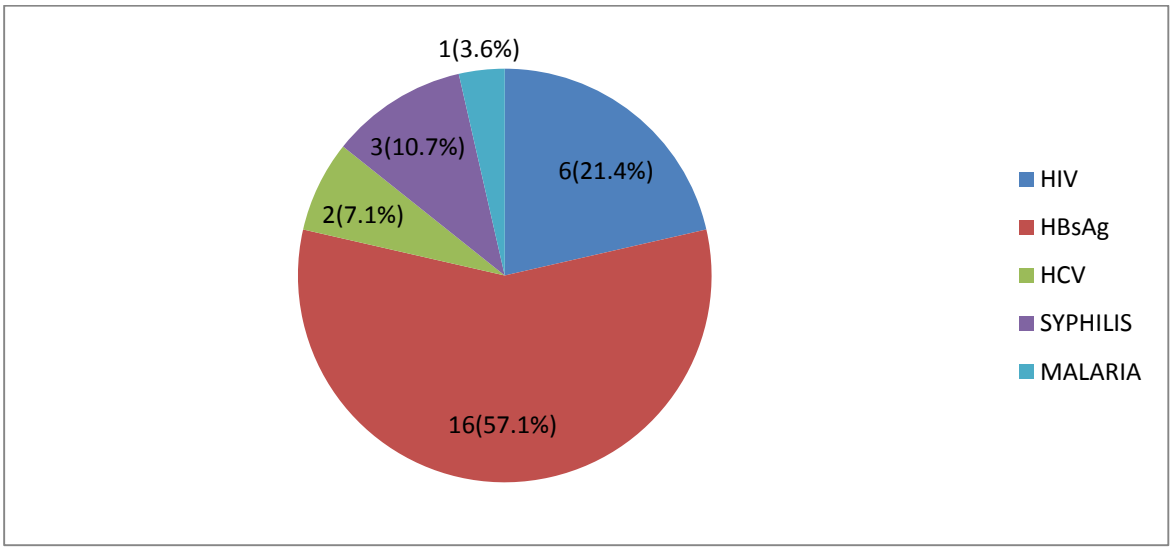


Figure -1 Pie chart showing proportion of transfusion transmitted infection donors among health donors from April 2015 to May 2015 in Yirgalem Hospital and Hawassa blood bank service Sidama zone, SNNPRG, Ethiopia

Table 3 Prevalence of co-infections of HIV, HBV and HCV among blood donors at Hawassa blood bank and Yirgalem hospital in Sidama, SNNPRG, Ethiopia April-May 2015.

Co-infection	Number	percent	OR(CI)	P-value
HIV-HBV	1	0.26%	0.207(0.023-1.88)	0.162
HBV-HCV	1	0.26%	0.041(0.002-0.685)	0.026
Total	2	0.52%		

The prevalence of HBV was 13/258(5.0%) and 3/126(2.4%) in male and female respectively, p=0.001. HCV 2/258(0.8%) and 0/126(0.0%) in male and female respectively, p=0.813.Malaria 1/257(0.4%) and 0/127(0.0%) in male and female respectively, p=0.672 (Table 4). In addition to that, the prevalence of HIV was 5/331(1.5%) and 1/41(2.4%) in age group 18-24 and 25-34 years respectively. The prevalence of HBV was high in18-24 age group15/331(4.5%) compare with 25-34 years 1/41(2.4%). The prevalence of Malaria was 1(0.3%) in age group 18 to 24 and HCV 2/331(0.6%) was in age group 18 to 24 years. The prevalence of syphilis was 1/331(0.30%) and 1/7(14.3%) in age group 18-24 and 45-54years respectively. Urban residents were HCV1/25(4.0%) and rural residents 1/359(0.3%) p=0.002. The prevalence of HIV was 1/25(4.0%) and 5/359(1.4%) in urban and rural respectively, p=0.002 (Table 4-6).The prevalence of HBV was 3/31 (9.7%), HCV 1/31(3.2%) and Syphilis 2/31(6.5%) in family replacement donors. Based on occupational

status the prevalence of HIV was 1/24(4.2) and 5/330(1.5%) in government workers and students respectively, p=0.002. HCV was 1/24(4.2) and 1/330(0.3%) in government workers and students respectively, p=0.002 (Table 4-6).

Table 4 Prevalence, Odds ratio and P-value of HBV and HCV among voluntary & family replacement donors at Yirgalem hospital & Hawassa blood bank service in Sidama, SNNPRS, Ethiopia April-May, 2015.

Characteristics		HCV POSTIVE (%)	OR(95% CI)	P-Values	HBV POSTIVE N (%)	OR (95% CI)	P-Values
Sex	Male	2/258(0.8%)	0.992(.982-1.003)	0.813	13/258(5.0%)	18 (10-32)	0.001
	Female(constant)	0/126(0.0%)	1	-	3/126(2.4%)	1.00	-
Type of donation	Voluntary	1/353(0.3%)	1	-	13/353 (3.7%)	1	-
	Family replacement	1/31(3.2%)	0.085(.005-1.397)	0.084	3/31 (9.7%)	9 (2-30)	0.001
Marital status	UNMARIED	2/352(0.6%)	0.994(0.986-1.002)	1.000	14/352(4.0%)	1.610(.349-7.418)	0.542
	MARIED	0/32(0.0%)	1	-	2/32(6.3%)	1.00	-
Number of donation	First	2/298(0.7%)	148(36-594)	0.001	12/298(4%)	-	-
	Second	0/43(0.0%)	-	-	3/43(7%)	-	-
	Third	0/18(0.0%)	-	-	1/18(5.6%)	-	-
	Greater than three	0/25(0.0%)	1	-	0/25(0.0%)	-	-
Education	Illiterates	0/4(0.0%)	1	-	0/4(0.0%)	1	-
	9-12	1/75(1.3%)	74(10-532)	0.001	6/75(8%)	0.409(0.144-1.165)	0.094
	Above12grade	1/291(0.3%)	290(40-2065)	0.001	10/291(3.4%)	1	-
Occupations	GOVEREMENT	1/24(4.2%)	23(3-170)	0.002	1/24(4.2%)	3.833(.208-70.635)	0.366
	STUDENT	1/330(0.3%)	329(46-2342)	0.001	14/330(4.2%)	3.762(0.424-33.400)	0.234
	PRIVATE/NGO	0/7(0.0%)	1	-	(14.3%)	1	-
Residences	Urban	1/25(4.0%)	24(3-177)	0.002	1/25(4%)	1	-
	Rural	1/359(0.3%)	1	-	15/359(4.2%)	1.047(0133-8.261)	0.966
HBV	Positive	1/16(6.3%)	0.041(0.002-.685)	0.026	1/6(16.7%)	.207(.023-1.880)	0.162
	Negative	1/368(0.3%)	1	-	15/ 378 (4.0%)	1	-

Table 5 Prevalence, Odds ratio and P-value of Human immune deficiency virus (HIV) among voluntary and family replacement donors at Yirgalem hospital & Hawassa blood bank service in Sidama, SNNPRS, Ethiopia April-May, 2015.

Characteristics		HIV POSTIVE N (%)	OR (95% CI)	P-Values
Sex	Male	4/258(1.55%)	1	-
	Female(constant)	2/126(1.59%)	1.024(0.18-5)	0.640
Marital status	UNMARIED	6/352(1.7%)	57 (25-129)	0.001
	MARIED	0/32(0.0%)	1	-
Number of donation	First	6/298(2.0%)	48(21-109)	0.001
	Greater than three	0/25(0.0%)	1	-
Education	Illiterates	0/4(0.0%)	1	-
	Above12grade	6/291(2.1%)	47(21-106)	0.001
Occupations	GOVEREMENT	1/24(4.2%)	23 (3-170)	0.002
	STUDENT	5/330(1.5%)	65(26-157)	.001
	PRIVATE/NGO	0/7(0.0%)	1	-
Residences	Urban	1/25(4.0%)	324(3-177)	.002
	Rural	5/359(1.4%)	1	-
HBV	Positive	1/16(6.3%)	15 (1.9-.113)	0.009
	Negative	5/368(1.4%)	1	-

Table 6- Prevalence, Odds ratio and P-value of syphilis among voluntary and family replacement donors at Yirgalem hospital & Hawassa blood bank service in Sidama, SNNPRS, Ethiopia April-May, 2015.

Characteristics		Syphilis POSTIVE N (%)	OR (95% CI)	P-Values
Sex	Male	2/258(0.8%)	1.024(0.092-11.0)	0.985
	Female(constant)	1/126(0.8%)	1	-
Type of donation	Voluntary	1/353(0.3%)	1	-
	Family replacement	1/31(6.5%)	0.041(0.004-0.468)	0.010
Marital status	UNMARIED	1/352(0.3%)	1	
	MARIED	2/32(6.3%)	0.043(0.004-0.485)	0.011
Occupations	GOVEREMENT	1/24(4.2%)	3.833(.208-70.6)	0.366
	MERCHANT	0/7(0.0%)	-	-
	FARMER	0/15(0.0%)	-	-
	STUDENT	0/330(0.0%)	-	-
	PRIVATE/NGO	1/7(14.3%)	1	-
Residences	Urban	0/25(0.0%)	1	-
	Rural	3/359(0.8%)	1.008(0.999-1.018)	0.817

6. Discussion

In this study, the overall prevalence of TTI was 28(7.29 %). This number had low prevalence compared with the overall prevalence of the study done by Nwankwo E *et al* in Nigeria 54 (19.3%), Waheed U *et al* in Islamabad, Pakistan out of 1,454 donations (14.34%) were HIV, HBV, HCV, Syphilis and Malaria positive(3,29). A possible reason for the discrepancy may be due to characteristics of the donor types, around 62(22.1%) were commercial donors in Nigeria and 9573(94%) in Pakistan.

However, the present study showed the overall prevalence of TTI was high compared with study done by Shrestha AC *et al* Nepal which showed 1.7%, Bommanahalli BP *et.al* in India study was showed the overall TTI was 2.22% , Dhruva GA *et al*. in Gujrat, India 101(0.93%) (10, 11, 27).

The present study showed seroprvalence of Human Immunodeficiency Virus 6(1.6%), Hepatitis B Virus (HBV) infection 16(4.2%), Hepatitis C Virus (HCV) infection 2(0. 5%), syphilis 3(0.7%), and Malaria 1(0.2%). The overall prevalence in males 22/258(8.5%) was higher than in females 6/126(4.8%); this result is consistent with previous study done by Zulfikar A *et. al* from studies conducted in Pakistan the male 202 (0.86%) and female 8 (0.38%). The possible reason for discrepancy may be due to low number (<10%) of female participation. Another study conducted by Dhruva GA *et. al* in India was higher among the male donors 0.95% compared to female donors 0.85% (26, 27).The prevalence of family replacement donors were 6(19.4%) high when compared with voluntary donors 22(6.2%), the finding was consistent with the study done by Bommanahalli BP in India family replacement donors 2.3% compare with voluntary donors 1.5%(11).

Our study finding indicated high prevalence of HIV when compared with the study done by Lathamani K *et al* HIV 0.08% in India(1), Nwankwo E 1.4% in Nigeria(3). Shrestha AC *et al* 0.12%, in Nepal (10). Drago F et al HIV 0.005% in Italy (15). Chen Yin et *al* 0.03% in China (16). Zulfikar A et al 0.12% in Pakistan (26). Dhruva GA *et al* 0.074% in India(27) Khan ZT et al 0.0% in Rawalpindi, Pakistan (30). Attaullah S *et al* 0.06 % in Pakistan (31). Even if the finding of our study was higher than other countries findings, but low compare with the prevalence of HIV that

was done by Waheed U *et al* in Islamabad, Pakistan 846 (8.34%)(29).The possible reason of discrepancy study design, duration of the study and type of the study participant characteristics.

The seroprevalence of Hepatitis B Virus (HBV) infection was 16(4.2%) among blood donors in our study participants. This study finding showed high prevalence compared with the study done by Nada HA *et al* which was 3440/149,381 (2.3%) in Egypt(4). Shrestha AC *et al* 0.47%, in Nepal(10). Bommanahalli BP *et al* 2.12%in India (11). Zulfikar A et al 139/25,633(0.5%) in Pakistan (26) and Dhruva GA *et al* 74/10,788 (0.68%) in India (27).Waheed U *et al* 397/10,145(3.91%) in Islamabad Pakistan (29). and Khan ZT *et al* 3241(2.31%) in Rawalpindi, Pakistan(30). In other way the seroprevalence of Hepatitis B Virus (HBV) infection in our study findings showed low prevalence when compared with the study done by Nwankwo E *et al* in Nigeria 31/280(11.1 %) (3). the possible reason of discrepancy may be study design, duration of the study, donor type and type of the study participant characteristics.

The prevalence of syphilis among blood donors in our study was 0.8%, this finding showed low prevalence when compared with the study done by Nwankwo E *et al* 7.5% in Nigeria(3). And also agreed with the study done by Khan ZT *et al* 90(0.89%) in Islamabad, Pakistan (26). But the present study findings was higher when compared with study done by Lathamani K *et al* 0.09% in India(2), Shrestha AC *et al* 0.48% in Nepal(10). Drago F *et al* 0.031% in Italy (15). Zulfikar A et *al* 0.07 % (26). and Attaullah S *et al* 0.43% in Pakistan (31).

The prevalence of syphilis among blood donors according to the present study in both sexes had equal proportion, male donors was 2/258(0.8%) and the females was 1/126(0.8%), this finding was the same with the study done by Shrestha AC et al in male 90 (0.48%) and female 16 (0.48%) (10). But quite different when compared with another study done by Nazir S in pakistan, the prevalence among the males donors was high 446 (3.1%) when compared with female donors, 3 (1.6%) (17).

The seroprevalence of Hepatitis C Virus (HCV) infection in the present study was 3(0.5%), and this finding was low compared with the previous study done by Nwankwo E *et al* indicated 1.8%, in Nigeria(3). Nada HA *et al* 7.2% in Egypt(4), by Shrestha AC *et al* 0.64 % in Nepal(10) Khan S et al 2.46% in Pakistan(13) and , Waheed U *et al* 846 (8.34%) in Islamabad Pakistan(28).Khan ZT *et al* 6,834(4.87%) in Rawalpindi, Pakistan (30).But high prevalence was

observed in study done by Bommanahalli BP et al 0.1%in india(11). Zulfikar A *et al* 18(0.07%) in pakistan (26).and Dhruva GA *et al* 0.074% in India (27).

The prevalence of malaria among blood donors in this study was 1/384 (0.3%) this is high proportion when compared with the study done by Dhruva GA *et al* on prevalence of malaria in India 4/10,788(0.037%) (25). But low compared with another study done by Waheed U et al 121/10,145(1.20%) in Islamabad, Pakistan (29). and Gelaw B *et al* 6/600(1%) in Northern part of Ethiopia (Gondar, Bahirdar Dessie and Mekele)(24).The discrepancy may be due to study design, study duration, seasonal variation and Geographical difference of the study populations.

The present study prevalence was low compared with study done by Dessie A *et al* at FelegeHiwot referral hospital, Ethiopia the overall prevalence of TTI was 43.2%; HBV 25%, HCV 11.7% and Syphilis 1.2%. And also study was done by Gelaw B *et al* the overall prevalence of Gonder, Bahirdar, Dessie and Mekele were showed high HBV 6.2%, HCV 1.7% and malaria 1% compared with the present study finding(22,24).the possible discrepancy may be due to study design ,population characteristic and study duration.

7. Strength and limitation of the study

7.1 Strength

The strength of the study is mostly reported transfusion transmitted infections like HIV, HBV, HCV, Syphilis and Malaria are included. Also our study was prospective; it avoids incompleteness and increase data accuracy.

7.2 Limitation

The limitation of the study was short study period, budget and season of study conducted. Because some transfusion transmitted infection prevalence varies according to season such as malaria infection. In this study, donors come from different places therefore it may not represent the population around the study area.

8. Conclusion and Recommendation

8.1 Conclusion

The prevalence of TTI was 28(7.29%), the seroprevalence of HIV 6(1.6%), HBV 16(4.2%), HCV 2(0.5%), Syphilis 3(0.8%) and Malaria 1(0.3%). High prevalence of HBV infection were found among family replacement blood donors when compared with voluntary blood donors. Students highly participated in voluntary blood donation program. In this study low percentage of multiple infections 2/384(0.5%) was observed compare with other studies.

8.2 Recommendation

The national blood bank should develop malaria screening policy because; still there is a significant prevalence of malaria infections among blood donors in Ethiopia. And also voluntary blood donation should be encouraged to provide safe blood. The present study duration is short therefore further study should be use wide study duration to avoid seasonal variation interference and also to include the representative populations' parts.

10. References

1. Screening donated blood for transfusion. 1211 Geneva 27, Switzerland, World Health Organization.2010; 1-73.

2. Lathamani K, Bhaktha G, Nayak S, Kotigadde S. Prevalence of HIV, HCV, HBV and Syphilis in Blood donors among the Dakshina Kannada District, India**.***Int.J.Curr.Microbiol.App.Sci*2013; 2(10): 249-252.

3. Nwankwo E, Momodu I, Umar I, Musa B, Adeleke S.Seroprevalence of major blood-borne infections among blood donors in Kano, Nigeria.*Turk J Med Sci.* 2012; 42 (2): 337-341.
4. Nada HA, Atwa M. Seroprevalence of HBV, HCV, HIV and Syphilis markers among Blood Donors at Suez Canal University Hospital Blood Bank in Egypt. *J Blood Disorders Transf*. 2013; 5: 177-181.
5. Kaur P, Basu S Transfusion-transmitted infections: existing and emerging pathogens. *J Postgrad Med.* 2000; 551: 146-151.
6. Glynn SA, Kleinman SH, Schreiber GB, Busch MP, Wright DJ. Trends in incidence and prevalence of major transfusion-transmissible viral infections in US blood donors, 1991 to 1996. Retrovirus Epidemiology Donor Study (REDS). *JAMA* 2000; 284: 229-235.
7. Arora D, Arora B, Khetarpal A. Seroprevalence of HIV, HBV, HCV and syphilis in blood donors in Southern Haryana. *Indian J PatholMicrobiol*. 2010; 53:308-309.
8. Hutin Y, Kitler ME, Dore GJ, Perz JF, Armstrong,GL, Dusheiko G et al. Global burden of disease (GBD) for hepatitis C. *J Clin Pharmacol.*2004; 44:20–29.
9. Qasmi SA, Aqeel S, Ahmad M, Alam IS and Ahmad A. Frequency of seropositive blood donors for HBV, HCV & HIV. *JCPSP*. 2000; 10(12):467-469.
10. Shrestha AC, Ghimre P, Tiwari BR, Rajkarnikar M. Transfusion-transmissible infections among blood donors in Kathmandu. *J Infect Dev Ctries.*2009; 3(10):794-797.
11. Bommanahalli B.P, Javali R, MallikarjunaSwamy CM, Gouda KU, Siddartha K, Shashikala KP.Seroprevalence of Hepatitis B and Hepatitis C viral infections among blood donors of central Karnataka, India *Int J Med Sci and Public Health.*2014; 3:272-275.
12. Bhawani Y, Rao PR, Sudhakar V. Seroprevalence of transfusion transmissible infections among blood donors in a tertiary care hospital of Andhra Pradesh. *Biol Med.* 2010, 2(4):45-48.

13. Khan S, Attaullah S, Ayaz S, Khan SN, Shams S, Ali I. Molecular epidemiology of HCV among the health care workers of Khyber Pakhtunkhwa. *Virol J* .2011; 8(1):105-107.
14. Prati D. Transmission of hepatitis C virus by blood transfusions and other medical procedures: A global review. *J Hepato*. 2006; 45: 607–616.
15. Drago F, Cogorno L, Ciccarese G , Strada P , Tognoni M, Rebora A, and et al .Prevalence of syphilis among voluntary blood donors in Liguria region (Italy) from 2009 to 2013 .*International Journal of Infectious Diseases*. 2014;28: 45–46. RNs
16. Chen Y , Liu Z , Zhang Q , Chen J , Sun W , Yi J , et al. Trend in prevalence of syphilis among voluntary blood donors in Xi'an, China from 2006 to 2010. *International Journal of Infectious Diseases.*2014; 19: 98–99.
17. Nazir S, Khan A, Nazar A, Fayyaz A, Khan M S, Ahmed S. Prevalence of syphilis in Pakistani blood donors. *Int J Advancement in life sciences*. 2013; 1(1):27-30.JOURNs
18. Dhingra N. blood transfusion safety world health organization: making safe blood available in Africa .WHO report.2006; 1-7.
19. Nansseu JR, Noubiap N, Ndoula ST, M Zeh AF and Monamele CG. What is the best strategy for the prevention of transfusion-transmitted malaria in sub-Saharan African countries where malaria is endemic? *Malaria J*. 2013, 12:465-474.
20. Owusu-Ofori AK, Parry C, Bates I: Transfusion-transmitted malaria in countries where malaria is endemic: a review of the literature from sub-Saharan Africa. *Clin Infect Dis.* 2010, 51:1192-1198.
21. Allain JP, Owusu-Ofori S, Bates I. Blood transfusion in sub-Saharan Africa. *Transfusion Alternatives Transfusion Medicine*. 2004; 6:16–23.
22. Dessie A, Abera B and Wale F.Seroprevalence of major blood-borne infections among blood donors at FelegeHiwot referral hospital, northwest Ethiopia.*Ethiop.J.Health Dev.* 2007; 21(1):68-69
23. Tessema B, Yismaw G, Kassu A, Amsalu A, MuluA.Seroprevalence of HIV, HBV, HCV and syphilis infections among blood donors at Gondar University Teaching Hospital, Northwest Ethiopia: declining trends over a period of five years. *BMC Infectious Diseases* 2010, 10:111-118.
24. Gelaw B, Mengistu Y. The prevalence of HBV, HCV and malaria parasites among blood donors in Amhara and Tigray regional states .*Ethiop.J.Health Dev.* 2008; 22(1):1-5s

25. Zou S, Stramer SL, Dodd R Y. Donor Testing and Risk: Current Prevalence, Incidence, and Residual Risk of Transfusion-Transmissible Agents in US Allogeneic Donations. *Transfusion Medicine Reviews*. 2012; 26(2):119-128
26. Zulfikar A, Umaru N, Shreesha K. sero prevalence of transfusion transmitted infection among blood donors in mangalore. *J medica innovatica*. 2012; 1(2): 24-27.
27. Dhruva GA, Agravat AH, Dalsania JD, Katara AA and Dave RG.Transfusion Transmitted Diseases/Infections among Blood donors in a Tertiary Care Hospital at Rajkot, Gujrat, India.*Int Res J. Medical Sci.*2014; 2(4):16-19.
28. Nagalo MB, Sanou M, Bisseye C, Kaboré MI, Nebie YK, Kienou K, et al. Seroprevalence of human immunodeficiency virus, hepatitis B and C viruses and syphilis among blood donors in Koudougou (Burkina Faso). *Blood Transfus.* 2011;9:419-424.
29. Waheed U, Khan H, Satti HS, Ansari MA,Malik MA ,Zaheer HA. Prevalence of Transfusion Transmitted Infections among Blood Donors of a Teaching Hospital in Islamabad.Ann. *Pak Inst Med Sci*. 2012; 8(4): 236-239.
30. Khan ZT, Asim S, Tariq Z, Ehsan IM, Malik RA, Ashfaq B, et al. prevalence of transfusion transmitted infection in health blood donors RawaIpindi distrect pakistan a five year study. *int J path*. 2007;5(1):21-25
31. Attaullah S, Khan S and Khan J. Trend of transfusion transmitted infections frequency in blood donors: provide a road map for its prevention and control. *J Translational Med* 2012; 10:20-22.
32. Oladele OO, Adesina TB, Omolola ZT and Sunday TS.Prevalence of HBsAg and HIV among blood donors in Osogbo, Osun State, Nigeria. *Int Res J Med Sci*. 2013; 1(3): 68-71.
33. El-Gilany A-H and El-Fedawy S. Blood borne infections among student voluntary blood donors in Mansoura University, Egypt. *Eastern Mediterranean Health Journal*. 2006; 12(6):742-748.
34. Fessehaye N, Naik D &Fessehaye T. Transfusion transmitted infections – A retrospective analysis from the National Blood Transfusion Service in Eritrea. *Pan Afr Med J*. 2011; 9:40-46.

Annex I consent form

Subject Information Sheet (for participants, English)

Title of the Research Project: Prevalence of Transfusion Transmitted Infection among blood donors at Yirgaleme Hospital blood bank and Hawassa Blood Bank Center, Sidama Zone, SNNPRG, Ethiopia**.**

Name of Investigator: Fisseha Bonja (BSc)

Name of the Organization: Addis Ababa University, College of Health Science, Department of Clinical Laboratory Science.

Greetings!

You are invited to participate in a study to be conducted by MSC student at Addis Ababa University, College of health sciences, School of Allied Health Science, Department of Medical Laboratory Sciences. It is aimed at determining the Prevalence of Transfusion Transmitted Infection among blood donors at Yirgalem Hospital blood bank and Hawassa blood bank Center, Sidama Zone, SNNPRG, Ethiopia

This study aims to establish blood safety and to give quality blood service in different blood bank service center in Ethiopia. Please read the following statements and ask any unclear points before you agree to participate.

Participation in the study is voluntary. If you are not willing to participate in the study or if you want to withdraw even after deciding to participate, there will be no consequences. If you decide to participate, you have to sign the consent form.

Introduction and Informed consent

Hello. My name is ---------- I am one of the data collectors working with Mr Fisseha Bonja in the study of the above topic. We are assessing Prevalence of transfusion transmitted infection among Blood donors at yirgalem hospital and Hawassa Regional Blood Bank. We would very much appreciate your participation in this study. The information will help to improve the quality of blood bank service . The survey usually takes between 15 to 20 minutes to complete. Whatever information you provide will be kept strictly confidential and will not be shown to other person. Participation in this study is voluntary and you can choose not to answer any individual question or all of the questions. However, I hope that

you will participate freely in this survey since your views are important.

At this time, do you have any questions about the survey?

May I begin the interview now?

Yes =>start interviewing] No => end interview

Name of interviewer_____

Start time_____ End time_____

I certify that I filled this questionnaire in accordance with the training I was given and instruction started in it. I have confirmed that information in it is correct.

Signed_____ Date_____

Please forward any question or problems you may encounter during this study to

Fisseha Bonja

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Email: fisseha.bonja@aau.et.edu

Annex II Questioner

A, Donor's Demographic data Questioner

Question number	Question	Respondents answer	remark
A001	Age	In year	
A002	Sex	Male ☐ Female ☐	
A003	ID No		
A004	Marital status		
A005	City		
A006	Sub city Zone /region		
A007	Ethnicity	1.Sidama ☐ 2.Amahara ☐ 3. Tigre ☐ 4.Gurage ☐ 5.Oromo ☐ 6.Others_____	
A008	What is your religion?	1. Protestant ☐ 2. Catholic ☐ 3. Orthodox ☐ Others specify_____	
A009	Residence	Rural ☐ Urban ☐	

A010	Occupation	1. Employed ☐ 2. House wife/home activities ☐ 3. Daily laborer ☐ 4. Merchant ☐ 5. Farmer Student ☐ 6. Jobless ☐ 7. Other Specify_____	

B, Donor's medical history and risk related history Questioner

Question number	Question	Respondents answer	Remark
B001	Are you feeling well and health today?	Yes No	
B002	Have you had an operation?	Yes No	
B003	Do you get blood transfusion in the last one year?		
B004	How many times		
B005	Have you ever suffered from yellowing of the eye / jaundice in the last one year?		
B006	Are there any swellings on your neck or in the armpit?		
B007	Have you lost more than 10kg weight in the last 6 months?		

B008	Has any part of your body every procedure been pierced with unsterile instruments?		
B009	Have you had a tattoo or any scarification done in the last one year?		
B010	Have you had sex with more than one partner in the last 6 months?		
B011	Do you Know about transfusion transmitted infections?		

This is the end of the questionnaire. Thank you very much for taking time to answer these questions. We appreciate your help.

Annex III Questionnaire Amharic Version

Annex IV: Laboratory procedure

Vironostika HIV Ag/Ab procedure

1. Fit the strip holder with the required number of microelisa strips.
   Note: the microwells can be visually inspected to check for the addition of specimen diluent, samples and controls. upon addition of specimen diluents the purple conjugate sphere will dissolve to give a green solution. This solution becomes blue/purple after addition of either sample or control.
2. Pipette 100 µl specimen diluent into all wells, i.e. including control wells.
3. Pipette 50 µl sample or control into assigned wells. Include three negative control, one anti-HIV-1 positive control, one ani-HIV-2postive control and one HIV-1 antigen positive control in each strip holder. Always pipette the controls after pipetting the samples. Fill any unused wells with specimen diluents in order to dissolve the conjugate sphere and prevent malfunction of the aspiration /wash system.
4. Mix well (e.g., using a micro shaker, speed approximately 15 Hz.=900 revolution per minute for 15 seconds ,or equivalent) and incubate the strips at 37^0c for 60±5 minutes.
5. Wash and soak each well six times with phosphate buffer.
6. Pipette 100µl TBM substrate into each well. Do not mix or shake.
7. Incubate the strips at 15 to 30 for 30±2 minutes in the dark.
8. Stop the reaction by adding 100 µl 1mol/l sulfuric acid to each well; use the same pipetting sequence and time intervals used for TBM substrate addition. Ensure thorough mixing. e.g., by tapping the side of the strip holder. Plates should be read within 15 minutes.
9. Blank the reader on air, i.e. without strip holder and strip, and read the absorbance of the solution in each well at 450 nm and 620 to 700 nm as reference (dual wavelength).
   Result
   Calculate cut-off value:

- If the test run is valid, calculate the cut-off NCx+0.100
- A test sample reactive if sample absorbance is ≥ cut-off value.
- A test sample non reactive if sample absorbance is < cut-off value.

Enzime Linked Immuno Sorbent Assay for HCV Ab Procedure

1. reagent preparation: allow the reagents and samples to reach room temperature for at least 15-30 minutes .check the wash buffer concentrate for the presence of salt crystal.if crystals have formed in the solution, resolubilize by warming at 37^0c until crystals have formed dissolve. Dilute the stock wash buffer 1:20 with distelled or deionized water. Use only clean vessels to dilute the wash buffer.
2. Numbering the wells: set the strips needed in strip-holder and number sufficient number of wells including three negative controls, two positive controls and one blank.
3. Adding diluents: add 100 µl specimen diluents into each well except the blank.
4. Adding sample: add 100 µl of positive control, negative control and specimen into their respective wells.
5. Incubating (1): cover the plate with the plate cover and incubate for 30minutes at 37^0c.
6. Washing (1): after the end of incubation, remove and discard the plate cover. Wash each well 5 times with diluted wash buffer. Each time, allow the micro wells to soak for 30-60seconds.fter the final washing cycle, turn the strips plate onto blotting paper or clean towel, and it tap it to remove any remainders.
7. Adding HRP-conjugate: add 100 µl HRP-conjugate to each well except the blank.
8. HRP-conjugate incubating (2): cover the plate with the plate cover and incubate for 30minues.
9. Washing (2): at the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted wash buffer as step 6.
10. Coloring: dispense 50 µl of substrate solution B and after that 50 µl of substrate solution A in to each well including the blank and mix by tapping the plate gently. Incubate the plate at 37^oc for 15 minutes avoiding light .the enzymatic reaction between the chromogen A/B solutions produces blue color in positive control and anti-HCV positive sample wells.
11. Stopping reaction by adding 50 µl stop solution into each well.
12. Measuring the absorbance: calibrate the plate reader with the blank well and read the absorbance at 450nm.

    Result interpretation

    Calculation of <u>cut-off value (c.o.)=the mean of negative control+0.12</u>

    Negative result sample giving absorbance less than cut-off value are negative for this assay.

    Positive result sample giving absorbance greater than or equal to the cut-off value are considered initially reactive for this assay. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive sample can be considered positive.

PRINCIPLE OF THE ASSAY MICRO ELISA HCV

- The 3rd generation HCV Microlisa is based on a highly sensitive technique, Enzyme Linked
- Immunosorbent Assay which detects antibodies against HCV in human serum and plasma. The
- HCV proteins are present in serum at levels well below the limits of detection. Thus,
- immunodiagnosis of HCV infection is based on detection of host generated antibodies (anti-HCVs) to viral proteins.
- The 3rd generation HCV Microlisa utilises a combination of antigen with the sequence of both HCV structural and non-structural antigen i.e. CORE, E1, E2, NS3, NS4 and NS5. It has an obvious advantage over the available 2nd generation and 1st generation ELISA with improved sensitivity and specificity.
- The combination of antigens for the structural and non-structural HCV proteins are coated onto the micro wells.
- Diluted sample and controls are then incubated. Antibodies to HCV, if present, bind to the immobilized HCV antigens on the microwell during this incubation period.
- The microwells are then thoroughly washed with the diluted wash buffer to remove excess of unbound anti-HCV or other human IgGs which may interfere with the test. An enzyme conjugate, anti-human IgG conjugated with HRPO is added. The excess of enzyme conjugate is again removed with diluted wash buffer. At this stage the microwells hold only the bound antigen-anti HCV-enzyme conjugate complex.
- In the next step, the freshly prepared substrate solution is incubated with the complex in the microwells. The enzyme substrate reaction leads to development of a blue colour which is indicative of the Ag-Ab reaction which has occurred in the microwell. In the final step the
- Stop Solution is added and the optical density of the developed color is read photometrically.

Enzime Linked Immunosorbent Assay for Anti-Syphilis Ab Procedure.

1. Reagent preparation: Allow the reagents to reach room temperature. check the wash buffer concentrate for the presence of salt crystal.if crystals have formed in the solution, resolubilize by warming at 37^0c until crystals have formed dissolve. Dilute the stock wash buffer 1:20 with distelled or deionized water. Use only clean vessels to dilute the wash buffer.
2. Numbering wells: set the strips needed in strip-holder and number sufficient number of wells including three negative controls, two positive controls and one blank.
3. Adding diluents: add 100 µl specimen diluents into each well except the blank.
4. Adding sample: add 20 µl of positive control, Negative control and specimen into respective wells.
5. Incubating : mix by taping the plate gently.cover the plate with the plate cover and incubate for 60minutes at 37^oc.
6. Washing: At the end of the incubation ,remove and discard the plate cove. Wash each well 6 times with diluted wash buffer.
7. Coloring: Dispense dispense 50 µl of substrate solution B and after that 50 µl of substrate solution A in to each well including the blank, cover the plate with plate cover and mix by tapping the plate gently. Incubate the plate at 37^oc for 15 minutes avoiding light .the enzymatic reaction between the chromogen A/B solutions produces blue color in positive control and anti-TP positive sample wells.
8. Stopping reaction by adding 50 µl stop solution into each well.
9. Measuring the Absorbance: calibrate the plate reader with the blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm.

   Results

   Calculate of cut-off value (c.o.)=negative control mean + 0.18

   Negative result sample giving absorbance less than cut-off value are negative for this assay.

   Positive result sample giving absorbance greater than or equal to the cut-off value are considered initially reactive for this assay. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive sample can be considered positive.

TEST PROCEDURE (Malaria)

1. Bring the **RAPID TEST FOR MALARIA Pan / Pf** kit components to room temperature before testing.
2. Open the pouch and retrieve the device, sample loop and desiccant. Check the color of the desiccant. It should be blue. If it has turned colorless or pink, discard the device and use another device. *Once opened, the device must be used immediately.*
3. Tighten the vial cap of the clearing buffer provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.
4. Evenly mix the anti coagulated blood sample by gentle swirling. Dip the sample loop into the sample. Ensuring that a loop full of blood is retrieved, blot the blood so collected on to the sample pad in the sample port „A". (This delivers approximately 5 µl of the whole blood specimen)

OR

In case finger prick blood is being used, touch the sample loop to the blood on the finger prick. Ensuring that a loop full of blood is retrieved, immediately blot the specimen on to the sample pad in the sample port „A" (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate).

OR

Alternatively, 5 µl of the anticoagulated or finger prick specimen may be delivered to the sample pad in the sample port „A" using a micropipette.

NOTE: Ensure the blood from the sample loop has been completely taken up at the sample port „A".

5. Dispense **two** drops of the clearing buffer into port „B", by holding the plastic dropper bottle vertically.
6. Read the results at the end of **20 minu**tes as follows:

NEGATIVE for malaria:

Only one pink-purple band appears in the control window „C".

POSITIVE for malaria:

***P. falciparum* or mixed infection:** In addition to the control band, two pink-purple bands appear at regions „Pf" and „Pan" in the test window „T".

Other species (non falciparum): In addition to the control band, one pink-purple band appears only at region „Pan" in the test window „T".

8 The test should be considered invalid if no bands appear on the device. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

PRINCIPLE RAPID TEST FOR MALARIA

RAPID TEST FOR MALARIA Pan / Pf utilizes the principle of immunochromatography. As the test sample flows through the membrane assembly of the device after addition of the clearing buffer, the colored monoclonal anti-Pf. HRP-2 (IgG) specific / monoclonal anti-Pan specific colloidal gold conjugate antibodies complexes the proteins in the lysed sample. This complex moves further on the membrane to the test region where it is immobilized by the monoclonal anti-HRP-2 (IgM) / monoclonal anti-Pan specific antibody coated on the membrane leading to formation of pink-purple colored band/s which confirms a positive test result. While both the bands will appear at the test region in falciparum positive samples, only one band would appear in non-falciparum malaria positive samples. Absence of this colored band/s in the test region indicates a negative test result.

The un-reacted conjugate along with the rabbit globulin colloidal gold conjugate and unbound complex if any, move further on the membrane and are subsequently immobilized by anti-rabbit antibodies coated on the membrane at the control region, forming a pink-purple band. The control band formation is based on the „Rabbit / anti-Rabbit globulin" system. Since it is completely independent of the analyte detection system, it facilitates formation of consistent control band signal independent of the analyte concentration. This control band serves to validate the test performance.

Microscopic Tests

The microscopic tests involve staining and direct visualization of the parasite under the microscope. For more than hundred years, the direct microscopic visualization of the parasite on the thick and/or thin blood smears has been the accepted method for the diagnosis of malaria in most settings, from the clinical laboratory to the field surveys. The careful examination of a well-prepared and well-stained blood film currently remains the "gold standard" for malaria diagnosis. The most commonly used microscopic tests include the peripheral smear study and the Quantitative Buffy Coat (QBC) test.

The simplest and surest test is the time-honoured peripheral smear study for malarial parasites. None of the other newer tests have surpassed the 'gold standard' peripheral smear study.

Remember this:

- Ask for MP test in all cases of fever and related symptoms and also whenever there is high level of suspicion
- MP test can be done at any time. Do not wait for typical symptoms and signs or for chills
- A negative test DOES NOT rule out malaria. Repeated tests may have to be done in all doubtful cases. Duration of the illness, level of parasitemia, expertise of the technician and the method of examination may all have a bearing on the result of the M.P. test

Peripheral smear study for malarial parasites – The MP test

Light microscopy of thick and thin stained blood smears remains the standard method for diagnosing malaria. It involves collection of a blood smear, its staining with Romanowsky stains and examination of the Red Blood Cells for intracellular malarial parasites. Thick smears are 20–40 times more sensitive than thin smears for screening of Plasmodium parasites, with a detection limit of 10–50 trophozoites/μl. Thin smears allow one to identify malaria species (including the diagnosis of mixed infections), quantify parasitemia, and assess for the presence of schizonts, gametocytes, and malarial pigment in neutrophils and monocytes.

The peripheral blood smear provides comprehensive information on the species, the stages, and the density of parasitemia. The efficiency of the test depends on the quality of the equipment and reagents, the type and quality of the smear, skill of the technician, the parasite density, and the time spent on reading the smear. The test takes about 20 to 60 minutes depending on the proximity of the laboratory and other factors mentioned above. It is estimated to cost about 12 to 40 US cents per slide in the endemic countries.

Before reporting a negative result, at least 200 oil immersion visual fields at a magnification of 1000× should be examined on both thick and thin smears, which have a sensitivity of 90%. The level of parasitemia may be expressed either as a percentage of parasitized erythrocytes or as the number of parasites per microliter of blood. In nonfalciparum malaria, parasitemia rarely exceeds 2%, whereas it can be considerably higher (>50%) in falciparum malaria. In nonimmune individuals, hyperparasitemia (>5% parasitemia or >250 000 parasites/μl) is generally associated with severe disease.

The smear can be prepared from blood collected by venipuncture, finger prick and ear lobe stab. In obstetric practice, cord blood and placental impression smears can be used. In fatal cases, post-mortem smears of cerebral grey matter obtained by needle necropsy through the foramen magnum, superior orbital fissure, ethmoid sinus via the nose or through fontanelle in young children can be used.

Preparation of the smear: Use universal precautions while preparing the smears for malarial parasites – use gloves; use only disposable needles/lancets; wash hands; handle and dispose the sharp instruments and other materials contaminated with blood carefully to avoid injury.

Slides should be clean and dry. It is better to use neutral distilled water.

Thick films: The thick film is first de-hemoglobinised in water and then stained with Giemsa.

Rapid Giemsa: Prepare a 10% Giemsa in buffered water at pH 7.1. Immerse the slide in the stain for 5 minutes. Rinse gently for 1 or 2 seconds in a jar of tap water. Drain, dry and examine.

Problems: The exacting needs of the blood smear examination are often not met in certain remote and poor parts of the world. Detection of low levels of parasitemia, sequestered parasites of *P. falciparum* and past infections in aspiring blood donors; ascertaining viability of the detected parasites; difficulties in maintaining the required technical skills and resultant misdiagnosis due to poor familiarity and problems in accessing and activating the facility in emergencies are some of the deficiencies with the blood smear examination.

Annex V: Declaration

I the undersigned declare that this my original work and has not been presented for Msc degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Name: FISSEHA BONJA GELETO
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Date:
Signature:

This thesis has been submitted with our approval as university advisors the above named student.

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