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Seroprevalence of Hepatitis B and C Virus and Occult Hepatitis B among Blood Donors at National Blood Bank, Addis Ababa, Ethiopia

By:

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This thesis prepared by Misgana Petros which is entitled ***"Seroprevalence of Hepatitis B and C Virus and Occult Hepatitis B among Blood Donors at National Blood Bank: Addis Ababa, Ethiopia"*** is my original work and submitted for the partial fulfillment of the requirements for the degree of Master of Sciences in Clinical Laboratory Sciences (Diagnostic & Public Health Microbiology). It complies with the regulations of the University and meets the accepted standards with respect to originality and quality. All sources of material used for the thesis has been duly acknowledged.

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List of Abbreviations

Anti-HBcTot:	Antibody to Hepatitis B Core total
Anti-HBs:	Antibody to HBsAg
CMIA:	Chemiluminecent Micro particle Immunoassay.
CCC:	Covalently closed Circular
DNA:	Deoxyribonucleic Acid
ELISA:	Enzyme linked Immunosorbent Assay
HBsAg:	Hepatitis B surface Antigen
HBV:	Hepatitis B virus
HCV:	Hepatitis C virus
ICL:	International Clinical Laboratory
IU:	International Unit
OBI:	Occult Blood infection
ORFs:	Open reading frames
OLT:	Orthotropic liver transplantation
PCR:	Polymerase Chain Reaction
RLUs:	Relative Light Units
RT:	Real time
S/CO:	Signal to cutoff
SP:	Sample preparation
SPSS:	Statistical Package for Social Sciences
TAHBV:	Transfusion associated hepatitis B viral infection
TTI's:	Transfusion transmittable infections

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ABSTRACT

Background: Transfusion associated hepatitis B and C viral infection continues to be a major problem in developing countries, even after the adoption of mandatory screening for HBsAg by ELISA method. This is mainly because of the blood from the donors with 'occult' HBV infection which is defined as viral DNA without detectable HBsAg observed after the initial period of primary infection and most of the time accompanied by the presence of anti-HBc.

Objective: To assess Seroprevalence and associated risk factors of Hepatitis B and C Virus and Screening of Occult Hepatitis B among Blood Donors at National Blood Bank, Addis Ababa, Ethiopia

Methods: A cross-sectional study was conducted on 2977 volunteer blood donors from April to Jun 2017. All donors' blood were screened for HBV and HCV with ELISA technique. For occult HBV, 391 blood samples which were found to be negative for all transfusion transmissible infections (TTIs) (for HIV, HCV, HBV and Syphilis) were taken and analyzed for anti-Hepatitis B core total (IgG and IgM) using Architect Chemiluminecent Micro particle Immunoassay (CMIA) technique. Socio demographic and associated risk factors were gathered using predesigned questions. Bivariate and multivariate logistic regression analyses were used to quantify the effect of different risk factors on hepatitis B and C infections. P values <0.05 was considered statistically significant. Data was analyzed using SPSS v.23.

Result: Of 2977 donors tested for HBV and HCV, 2.3 %(n=69/2977) and 1.2 %(36/2977) were found to be positive for HBsAg and anti-HCV respectively while 0.23 %(7/2977) of them showed dual infection. First time donation (AOR=0.21; 95% CI=0.15-1.21) (p<0.001) , Age between 36-45 (AOR=2.96; 95% CI=1.17-7.0) (p=0.021), contact to multiple sexual partners (AOR=, 0.07, CI= 0.02-0.29) (p<0.001) for Hepatitis B virus and sharing of sharp materials/scarification tattoos (AOR=0.13, CI=0.03-0.47,) (p=0.002) for HCV has shown statistical significant association. The prevalence of Anti-HBc (IgG and IgM) was found to be 14.6 %(n=57/391) and among this 22.8 %(n=13/57) were multiple time donors. History of transfusion (AOR=, 0.07, CI= (0.03-0.14) (p<0.001) and previous surgical procedures (AOR=, 0.08, CI= (0.01-0.52) (p=0.003) have shown statistical significant association with Anti-HBc positivity.

Conclusion: In this study, intermediate prevalence rate of HBV and low rate of HCV was found as per world health organization interpretation. Community based education should be planned regarding risk factors associated with these infections and modes of transmissions. Significant prevalence rate of anti HBC positivity was also determined. Since analyzing all HBsAg negative samples for nucleic acid test (PCR) is not practical economically in the country, incorporation of routine anti-HBc screening of blood as a surrogate marker of occult HBV is recommended to provide safe and healthy blood transfusion.

Key Words: Seroprevalence, Hepatitis B and C, Occult hepatitis B virus, National blood bank Addis Ababa Ethiopia

1. Introduction

1.1 Background

1.1.1 Biology and Pathogenesis of HBV and HCV

Hepatitis B virus has a small (3.2 kb), partially double-stranded, relaxed-circular DNA genome that encodes four overlapping ORFs. The largest ORF encodes the viral polymerase, which also has reverse transcriptase (RT) activity that generates the first strand of the DNA genome from an RNA intermediate. The second largest ORF encodes the three viral envelope proteins: large (L-), middle (M-), and small (S-) surface antigen (HBsAg) [1, 2]. Hepatitis C virus is a small, enveloped virus, a member of the flaviviridiae family and the lone example of the genus Hepacivirus. The genome consists of a single stranded positive sense RNA molecule approximately 9.6 kb long encoding a large ORF, which is flanked by highly structured 5′ and 3′ un translated regions [3].

Hepatitis B virus enters predominantly by the parental route, but its primary site of replication is unknown [4]. However, HBcAg is present in the nuclei of hepatocytes as early as 2 weeks. During the latter half of the incubation period 5-8 weeks after infection, the blood becomes infectious and it contains detectable HBsAg. The infection can cause acute and chronic liver diseases including cirrhosis and hepatocellular carcinoma (HCC). Hepatocellular injuries of HBV infection are predominantly immune mediated, and the natural history of chronic infection can be divided in to three phases based on virus-host interactions namely, immune tolerance, immune clearance, and viral integration phase [5, 6].

HCV enters its target cells in a highly coordinated process involving components of the virus particle and numerous cellular factors. Remarkably HCV entry is restricted to only human and chimpanzee cells, suggesting a block at the level of virus entry in other cell types [7]. Although replication of HCV RNA has been shown in mice, the virus cannot enter murine cells due to lack of specific receptors. So far several putative receptors have been demonstrated to be involved in HCV entry into the permissive human hepatocyte cell line Huh7.5. These include CD81, SR-B1 (the scavenger receptor class B type 1) and Claudin1 (CLDN1). Recently human Occluding (OCLN) has also been shown to be an essential entry factor that contributes to HCV susceptibility of human cells [8, 9].

1.1.2 Transmission and Associated Risk factors of HBV and HCV

Contact with infected blood, semen, or other body fluids; sexual contact with an infected person; sharing contaminated needles, syringes, or other drug injection equipment; and needle sticks or other sharp-instrument injuries from an infected person can transmit the HBV virus [10]. In addition, an infected woman can pass the virus to her newborn, but HBV vaccination of the baby at birth can prevent her newborn from becoming infected [11, 12]. HCV is mostly transmitted through exposure to infectious blood. This may happen through transfusions of HCV-infected blood and blood products, contaminated injections during medical procedures and sharing of needles [12, 13].

1.1.3 Occult Hepatitis B virus

Whenever HBV infection is mentioned, it is noticeable that Occult HBV will be raised as an important issue since it is the major cause of post transfusion hepatitis B, with higher risk of transmission than for HCV or HIV [14]. Occult HBV is defined as the presence of HBV DNA and the absence of HBV surface antigen (HBsAg encoded by the S gene) in plasma or serum of HBV infected individuals. It is mostly due to the indefinitely intrahepatic persistence of the viral genome of wild-type HBV (without any mutations) in the pre core and core promoter regions) [15 16]. Conversion of viral genome to a covalently closed circular DNA (cccDNA), which is formed in the nuclei of infected hepatocytes within the first 24 hr following virus inoculation and forming of a mini chromosome after binding to proteins, is the molecular basis of persistence [17].The stability and long-term persistence of viral cccDNA molecules, together with the long half-life of hepatocytes, imply that HBV infection, once it has occurred, may possibly continue for life [18].

Transfusion associated hepatitis B viral infection (TAHBV) continues to be a major problem in developing countries, even after the adoption of mandatory screening for HBsAg by ELISA method [19].Thus the absence of HBsAg in the blood of apparently healthy individuals may not be sufficient to ensure the lack of circulating HBV.Blood containing anti-hepatitis B core antibody (anti-HBc) without detectable presence of HBsAg might be infectious. In spite of the fact that implementation of nucleic acid testing (NAT) reduces the risk of residual infection it may not be economically feasible at every blood bank in developing countries like Ethiopia. Therefore if highly sensitive HBV DNA testing is not feasible, anti-HBc should be used as alternative marker for identifying potential seropositive OBI individuals in cases of blood donations [20, 21].

1.2 Statement of the problem

The World Health Organization (WHO) estimated that transfusion of unsafe blood accounted for 8-16 million hepatitis B virus infections and 2.3-4.7 million hepatitis C infections each year [22]. Approximately 1 000,000 people die each year (~2.7% of all deaths) from causes related to viral hepatitis most commonly liver disease including liver cancer [23].

An estimated 57% of cases of liver cirrhosis and 78% of cases of primary liver cancer result from HBV or HCV infection [24]. Overall, initial hepatitis B infections will progress to chronic in approximately 5-10% of unimmunized older children and adults. For hepatitis C virus there is an overall 75-85% chance of an acute infection resulting in a chronic infection [25].

Regarding Occult HBV prevalence among blood donors across the world, it varies among the Unites States, Europe, and Asia. In Europe, despite improvements in screening for blood donation, occult HBV is detected in 0-1.6% of cases. It is also the major cause of post transfusion hepatitis B in western countries and in countries like India and Taiwan, with higher risk of transmission than for HCV or HIV [26]. In Japanese 19% cases were identified that confirmed hepatitis B viral infection transmission rate of occult carriers, with a peculiar set of anti-HBc and DNA screening protocols. Similar study in japan revealed that 10 HBV infections that occurred among 37 recipients (27%) of occult hepatitis B positive units that were negative for HBsAg [27, 28].

Donations carrying anti-HBc only and HBV DNA can be infectious and this is a threat where anti-HBc is not screened. A study conducted by Candotti and his friends confirmed that 91% of 77 donor samples of European origin were HBV DNA-positive/HBsAg-negative.[29] a study in India also showed that a considerable number of HBV-infected donors remain undetected, if only HBsAg is used for screening [30].

The problem of hepatitis B virus infection is highest in the developing world. World Health Organization estimates that the prevalence of hepatitis B virus infection in Africa is on average more than 10%. It is estimated that in sub-Saharan Africa, more than 45,000 hepatitis B viruses or hepatitis C virus infections are transmitted through contaminated transfusions annually [31].

The burden of occult hepatitis B virus in Africa varies from 5.4%-10% from the total donor population and it accounts 22%-90.5% among Anti-HBc total positive donor population showing that majority of HBsAg negative anti HBc positive individuals will have occult hepatitis B virus [32-36]

In Ethiopia studies done in different part of the county showed that the prevalence of HBV and HCV on blood donors varied ranging from 2.1% - 25% and 0.2%-13.3% respectively [37-43]. However, in Addis Ababa there is limitation of published data on Seroprevalence and associated risk factors of Hepatitis B and C Virus among blood donors. Regarding to occult hepatitis B, no studies have been conducted yet to screen HBsAg negative blood donors to Anti HBc total for the presence of occult Hepatitis B virus among Blood Donors.

Hence our study targeted to assess the Seroprevalence and associated risk factors of Hepatitis B and C Viruses and Screening of Occult Hepatitis B virus among Blood donors at national blood bank, Addis Ababa, Ethiopia.

1.3 Significance of the study

There were different studies conducted in different parts of Ethiopia among blood donors on Seroprevalence of Hepatitis B and C Virus infections but there is limited study conducted in Addis Ababa blood donors. Screening of Anti-HBc total from samples ready to be given to recipients for highlighting the presence of Occult Hepatitis B Activity has not been studied yet. Henceforth,

- This study has generated information on the current prevalence of HBV and HCV among volunteer's blood donors in Addis Ababa which could be important to gain insight into the burden of these viruses in the general population of Addis Ababa.
- The findings of this study can be useful for further developing national intervention program specially in proposing blood samples to be pre-tested for Occult HBV prior to transfusion. This could support in providing safe blood donation, which is significant especially for immunocompromised recipients
- In addition it provides information on associated risk factors of HBV and HCV infections which can be helpful to responsible bodies for awareness creation or health education
- Finally these all information could also be used as a reference data for further similar studies.

2. Literature review

2.1 Prevalence and Associated Risk Factors of Hepatitis B and C Viruses

A study by Dhruva GA. et al. on Transfusion Transmitted Diseases/Infections among blood donors in a Tertiary Care Hospital in Gujarat, India indicates Seroprevalence of HBV and HCV was 0.68% and 0.08% respectively. The prevalence of both viruses was found to be higher in males as compared to females [44]. A similar study in India by Basavaraj B.et al. was conducted from January 2005 to December 2009 on Seroprevalence of hepatitis B and C viral infections among blood donors. From the total 19,413 screened participants, 2.12% and 0.1% was recorded respectively [45].

Another study in Brazil by Valois, R. et al. on HCV infection through perforating and cutting material among candidates for blood donation illustrates that 146 (1.1%) persons with anti-HCV antibodies were detected. Among risk factors, use of needles and syringes sterilized at home; shared use of razors at home, sharing of disposable razors had statically significant association with HCV infection. [46].

A cross-sectional study was conducted by Viet L.et al. on the Prevalence of hepatitis B and hepatitis C virus infections in potential blood donors in two rural communities in Quang Tri, Vietnam and the analyses showed that 11.4% of study samples (137/1200, 95% CI 9.6 - 13.2) were positive for HBsAg, 51.7% (620/1200, 95% CI 48.8 - 54.5) were anti-HBc-positive, while 9.5% (114/1200, 95% CI 7.9 - 11.3) were positive for both HBsAg and anti-HBc. Further, 1.9% (23/1200, 95% CI 1.2 -2.8) of the serum samples were positive for HBsAg and negative for anti HBc; and 42.2 %(506/1200, 95%CI 39.4- 45.0) negative for HBsAg and positive for anti-HBc. There were only two positive anti-HCV samples (0.17%) [47].

Seroprevalence and Determinants of Hepatitis-C Virus Infection in Blood Donors of Lahore, Pakistan by Majeed A et al. indicate that the Seroprevalence of HCV was estimated to be 17.78%. It was found that the occupational status (p=0.002, OR=3.789, 95% CI=1.515-9.473), place of surgical treatment (private sector) (p=0.035), history of blood transfusion (p=0.000, OR=6.364, 95% CI=2.38-17.02), ever pricked by sharps (p=0.045, OR=2.893, 95% CI=0.979-8.543), habit of injecting drugs (p=0.04, OR=2.45, 95% CI=0.995-6.062) and glass sharing (p=0.017, OR=2.327, 95% CI=1.1454.731) were significantly associated with occurrence of hepatitis C in blood donors [48]. Similarly study from Jordan by Al-Gani F. et al show that from 24173 blood donors, 370 were found to be positive for HBsAg, giving an overall prevalence of HBsAg of 1.4% and Infection with HCV was detected in 73 (0.9%) of 8190 donors [49].

In Europe: a study by Hajrullah F. et al. on Prevalence of HBV and HCV among blood donors in Kosovo shows HBV prevalence of 4.2%, which range Kosovo to the second zone according to the CDC classification .The HCV prevalence among the blood donors was 0.3%. Compared to the other European countries this level of prevalence is relatively low [50]. Another study in Morocco by Warda B.et al on Prevalence and risk factors of hepatitis B and C virus infections among blood donors and among the general population was conducted. Prevalence of anti-HCV and HBsAg among blood donors was 0.62% and 0.96% respectively [51].

A study conducted by Afolabi A. et al. on transfusion transmissible viral infections among potential blood donors at blood bank of the University College Hospital in Ibadan, Nigeria. In those study 507 sera tested for antibody using ELISA Out of this, 30(5.9%) were positive for HBsAg, 7(1.4%) for HCV [52]. Similar study in Nigeria conducted by shitu et al. on Seroprevalence of HBV Surface Antigen and HCV among Intending Blood Donors at Mother and Child Hospital, also indicates that the prevalence of Hepatitis infections among the intending blood donors was 290(9.9%). HBsAg and anti-HCV were reactive in 7.4% and 2.1% of the study population respectively while co-infection was recorded in 0.4% [53].

Another study in Mozambique conducted by stok x. et al. illustrates prevalence of HBV and HCV was lower in voluntary than in replacement donors. Seroprevalence of transfusion-transmissible infections and evaluation of the pre-donation screening performance was assessed and HBV and HCV infection was 10.6% (95% CI: 8.4-13.2%), 0.0% respectively [54]. Similar study conducted by Abou et al. on the Seroprevalence of Hepatitis B virus and Hepatitis C virus among blood donors in Sudan was conducted. A total of 400 male blood donors were tested for the detection of HBsAg and anti-HCV antibodies from these 6.25% were found reactive for HBsAg and 0.65% were reactive for anti-HCV antibodies. The highest percentage (30.8%) of HBV reacted samples were seen within the age group 19-24. On the other hand the highest percentage (50%) of HCV reacted samples were aged within the age group 31-36[55].

A retrospective analysis in 2011 from the National Blood Transfusion Service in Eritrea by Nahom F.et al was conducted. A total of 29,501 units of blood were collected from 23,385 voluntary blood donors. The overall prevalence of TTI's were 3.8% with 3.5% in voluntary blood donors. The sero-prevalence for HBV and HCV were 2.58% and 0.57% respectively [56].

A study by Gelaw B et al. in Amhara and Tigray regional states at different 4 blood bank sites was conducted regarding the prevalence of HBV, HCV and malaria parasites among blood donors. The overall prevalence of HBsAg, HCV among the blood donors in the 4 blood banks was 6.2% (37/600) and 1.7% 10/600) respectively. The prevalence of HBsAg was 4.7% for Gondar, 6% for Bahir Dar Hospital, 3%

(3/100) for Dessie and 14% for Mekele hospital blood banks. The prevalence of HCV anti-body was 2.3% and 3% for Gondar and Bahir Dar respectively, while 0% (0/200) for Dessie and Mekele Hospitals had been recorded [57].

Another study conducted by Dessie A et al on Seroprevalence of major blood-borne infections at Felege Hiwot referral hospital Northwest Ethiopia .In the study 324 blood donors were included in study. Seroprevalence of HBV and HCV was 25% and 13.3% respectively. Among the co-infections HBV and HCV infections were found to be significantly co-existing in the study ($p<0.05$) [58]. Similar study conducted by Tessema B. et al on Seroprevalence of HIV, HBV, HCV and syphilis infections among blood donors at Gondar University Teaching Hospital, Northwest Ethiopia shows that the overall Seroprevalence of HIV, HBV, HCV and syphilis was 3.8%, 4.7%, 0.7%, and 1.3% respectively. From the total of 6361 consecutive blood donors, 607 (9.5%) had serological evidence of infection with at least one pathogen and 50 (0.8%) had multiple infections. Among those with multiple infections, the most common combinations were HIV - syphilis 19 (38%) and HIV - HBV 17 (34%). Significantly increased HBV seropositivity was observed among age groups of 26 - 35 and 36 - 45 years [59].

In addition a study by Alemshet Y. et al. on Hepatitis B and C virus infections and their association with Human immunodeficiency in Jimma University specialized hospital Blood Bank found that the prevalence of HBV and HCV infection were 126 (2.1%) and 10 (0.2%) respectively. Highest prevalence in the age group 40-44 years and lowest among the age group of 45 years or more 13 of 509 (4.5%) and 3 of 509 (0.9%) respectively [60].

In 2016 a study by Habte Y.et al on Hepatitis B Virus Infection and Associated Factors among Blood Donors at Dire Dawa, Eastern Ethiopia was conducted in 2016 and out of 4,157 individuals who donated their blood 155 (3.73%) (95% Confidence Interval (CI)=3.15-4.31) confirmed having a sero-prevalence positive for HBV. HBV Seroprevalence has shown a statistical significance association with male sex (Adjusted Odds Ratio (AOR=1.93, 95% CI=1.10-3.55) (p=0.036) and age group 33-40 (AOR=3.7, 95% CI=1.19-9.56) (p=0.029) [61].

Also in 2016, a study was conducted regrinding the prevalence of transfusion transmitted infections at Hawassa blood bank center by Bonja F.et al. The overall prevalence of transfusion transmitted infections (TTI) was 28 out of 384 (7.29%) 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%) and 1 (0.3%) respectively. Two out of 384 (0.5%) had co-infections with HIV-HBV 1 (0.26%) and HBV-HCV 1 (0.26%). A 1:14 ratio (7.14%) of the blood collected was discarded only due to the presence of TTI. The highest discard rate was

recorded from HBV infected units 57.1%, followed by HIV 21.40%, syphilis 10.7%, HCV 7.10%, and malaria 3.60%. Overall, TTIs were found in males (7.8%), married (2.5%), rural (8%), private/NGO employed donors (28.6%), 45–54 age group (20%), and in replacement/family donors (16.1%) (p = 0.039) [62].

A recent study published in 2017 which is conducted by Bedada G.et al on Seroepidemiology of hepatitis B and C virus infections among blood donors in Ethiopia shows that 3.75% HBV and 0.5% HCV prevalence in Addis Ababa blood donors. The study also indicates HBV and HCV Seroprevalence among blood donors in Ethiopia is intermediate and low, respectively. A total of 56 885 sera were tested for HBsAg and anti-HCV antibodies. Of these, 3.9% were found HBsAg positive, 0.52% anti-HCV-positive, and 0.054% dual positive. HBV prevalence relatively higher in Adama (5.91%) than Gondar (4.05%), Jimma (3.87%), Addis Ababa (3.75%), and Tigray (3.7%); and in males (4.64%) than females (2.1%). Overall, HBV and HCV prevalence increased with age [63].

2.2 Anti-HBc Screening for occult HBV

Hepatitis B virus (HBV) surface antigen (HBsAg) screening in blood banks reduced the risk of HBV transmission through transfusion. However, the detection of occult HBV infection among blood donors is vital for improving blood safety. An overview of different literatures on occult hepatitis B virus by Zeinab N.et al in the year 2011 indicates that occult blood was reported for the first time almost 30 years ago in a case report of HBV infection through blood transfusion by an antibody to hepatitis B core antigen (anti-HBc) positive donor [64].

A study conducted in North American population by Garland Y. et al on occult hepatitis B virus infection show that prevalence of occult hepatitis B virus was 18% in those with serologic evidence of previous hepatitis B virus infection and 8.1% in hepatitis seronegative individuals [65]. A similar study on the rate of occult HBV infection among HBsAg negative and anti- HBc positive blood donors of Rafsanjani blood transfusion center, Iran was determined and HBV- DNA found in the majority of HBsAg negative and anti-HBc-positive donors. Sera from 270 healthy blood donors, who were negative for both HBsAg and anti-HCV, were tested for anti-HBc antibodies by use of ELISA technique. The samples that were negative for HBsAg but positive for anti-HBc markers also examined for the presence of HBV-DNA by polymerase chain reaction (PCR). HBV-DNA was detected in 4/14 (28.57%) of HBsAg negative and anti-HBc positive samples [66].

Another study in the year 2011 by S. Shastry et al was conducted on prevention of post-transfusion hepatitis by screening of antibody to hepatitis B Core antigen in healthy blood donors, at tertiary care

referral hospital, India has shown 15(0.1%) anti HBc positive donors from the total 12232 volunteers after passing through the stringent criteria were selected for blood donation [67]. Also another similar study in India by Lavantia V et al on Prevalence of hepatitis B virus infection among blood donors with antibodies to hepatitis B core antigen was conducted and recommended that anti-HBc testing should be added to pre-transfusion screening to improve blood safety. 10.9% blood samples were found to be reactive for anti-HBc. History of transfusion OR 17.43 (3.99-76.05) and previous surgery have shown significant association [68].

A study conducted by Oluyinka OO et al on 429 Nigerian blood donors were confirmed, 72(17%) as occult blood infection by DNA detection in different reference labs. Of the 72 OBI samples, 48/70 (67%) or48/429 (11.2%) were positive for anti-HBc, 25(35%) positive for anti-HBs, and 2(3%) positive for HBeAg. Of the 72 OBI samples, 31(43%) were seropositive for either anti-HBc, anti-HBs [32].Similar study also conducted in Nigeria south-eastern state on the prevalence of OBI among blood donors. Out of 113 informed consented repeat blood donors there were100 HbsAg negative donors, 8.0% (eight donors) were positive for anti-HBc total by ELISA and from this donors, all of them (100%) were HBV DNA positive [69].

Descriptive cross sectional study was conducted by Said Z. among 3167 Egyptian blood donors negative for hepatitis B surface antigen (HBsAg), hepatitis C antibody (HCV Ab) and human immunodeficiency virus Ab. 525/3167 (16.6%) of blood units were positive for total anti-HBc, 64% of those were anti- HBs positive. An overall prevalence of 451/3167 (14.2%) for total anti-HBc was determined. From these 17.2% were confirmed as occult. Univarate and multivariate logistic analysis for identifying risk factors associated with anti-HBc and HBV-DNA positivity among blood donors showed that age above thirty and marriage were the most significant risk factors for prediction of anti-HBc positivity with AOR 1.8 (1.4-2.4) and 1.4 (1.0-1.9) respectively[70].

A study was carried out in Sudan by Mahmoud OA.et al during the period between 2011 and 2012. It included 100 HBsAg-negative blood donors who attended the Central Blood Bank in Sudan. Sera collected from all donors were tested for HBsAg. Anti-HBc-positive patients were tested for hepatitis B virus (HBV)-DNA. The anti-HBc was detected in 42% of the blood donors, among whom 90.5% were positive for HBV-DNA indicating that when HBsAg is used as the sole detection marker for HBV, there could be a danger of HBV transmission through blood transfusion. [71]

3. Objectives of the study

3.1. General objective

- To assess the Seroprevalence and associated risk factors of Hepatitis B and C Virus and screening Occult Hepatitis B virus among Blood Donors at National Blood Bank: Addis Ababa, Ethiopia

3.2. Specific Objectives

- To determine the Seroprevalence of hepatitis B and C viruses among blood donors
- To assess Occult Hepatitis B virus among HBsAg negative blood donors
- To assess the risk factors associated with hepatitis B and C virus infections

4. Hypothesis

First Hypothesis

Null Hypothesis

The overall HBV and HCV Prevalence in this study group is the same as found in the previous study in Addis Ababa

Second Hypothesis

Null Hypothesis

The Prevalence rate of anti HBc total in this study group is the same as found in the previous studies in Africa

5. Materials, Methods, and Procedures

5.1 Study design and period

A cross sectional study was conducted on volunteer blood donors at national blood bank, Addis Ababa, Ethiopia; from April to Jun 2017.

5.2 Study Site

This study was conducted at National Blood Bank, Addis Ababa Ethiopia. The National Blood Bank was first established in 1969 by Ethiopian Red Cross society. Since 2004, it has been transferred to Ethiopian Federal Ministry of Health , and assigned with the responsibility of managing the blood donors, collection, testing and distribution of blood and blood products in Ethiopia. On average annually, the national blood bank collects forty to sixty thousand unit of blood and distribute for both governmental and private health facilities in and nearby Addis Ababa city.

5.3 Source population

All volunteers who came at Ethiopian national blood bank to donate blood during the study period.

5.4 Study population

All volunteer donors who donated blood according to the eligibility criteria of the national blood bank during the study period.

5.5 Sample size and sampling procedure

5.5.1 Sample size determination

The Sample size was calculated using a single population proportion formula by taking a prevalence value of 3.75% (P==0.0375) from a previous study in Addis Ababa, Ethiopia by Gelaw B etal.

n = z2 P (1-P)⁄d2 n = z^2 P (1-P)⁄d2

Where n = sample size,

Z = Z statistic for a level of confidence (95% level of confidence; z=1.96),

d = precision (if p= 0.0375 %, d =0.0187).

P = expected prevalence or proportion (p = 0.0375% taken from previous study by Gelaw B et al)

n = 1.96^2 0.0375(1-0.375)/ $(0.02)^2$

n = 346

Taking 10% contingency; the total sample size was 346+35= **381**

For occult HBV, since there was no study done previously in Ethiopia, the minimum sample size was **384** after calculation using the same formula by taking the value of P=0.5.

During the study period, 2977 eligible blood donors donated blood and all of them were included for HBV and HCV prevalence and associated risk factors study. For occult hepatitis B virus screening, keeping the minimum sample size calculated (which was 384), 391 donor's samples which were negative for all TTIs (HIV, HCV, HBV and Syphilis) were taken and analyzed for Anti HBc (IgM IgG).

5.5.2 Sampling technique

A convenient sampling technique was applied for HBV and HCV prevalence study to select the study participants during the study period. For occult HBV screening, systematic random sampling technique was applied by taking a K value of 7 (K=2819/384) from the total 2819 study participants who were negative for all TTIs during the period. The value of P for occult HBV was taken as 0.5 since there was no study done previously in Ethiopia.

5.6 Inclusion criteria

All blood donors who were eligible to donate blood according to the National Blood Bank Standards were included.

5.7 Study variables

5.7.1 Dependent variables

- Hepatitis B and C virus
- Anti HBc antibody status

5.7.2 Independent variables

- Age
- Sex
- Donor status
- Marital status
- Occupation
- History of multiple sexual Contact
- Sharing of sharps, Scarification marks/Tattoos
- History of any medical surgery
- History of blood transfusion
- Syphilis infection
- Blood group

5.8 Data collection and laboratory Measurement

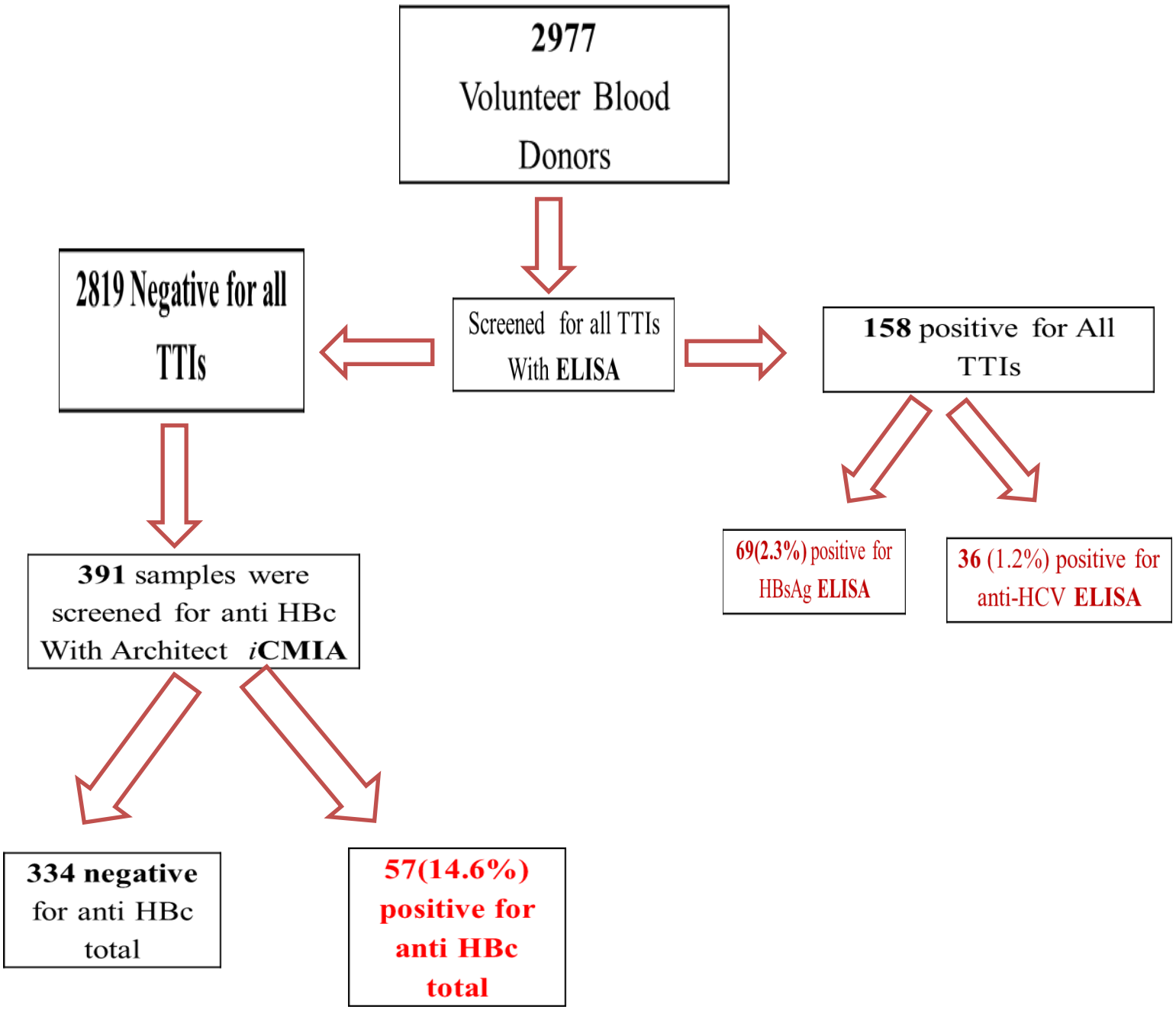
5.8.1 Data collection

With the aid of a structured questionnaire, relevant socio demographic and risk factors information were gathered from each volunteer blood donors. This includes donor status, age, sex, and exposure to multiple sexual partners, sharing of sharp materials or scarification tattoos. The questionnaires were self-administered by participants and any participant who needed help in filling the questionnaires was assisted by health professionals at the national blood bank.

5.8.2 Laboratory Measurement

All donors' blood was screened for hepatitis B surface antigen and hepatitis C antibody with ELISA kit. For occult hepatitis B (anti-HBc screening) samples found to be negative for HBsAg [in our case 391] were taken and anti-Hepatitis B core total was analyzed using Architect Chemiluminecent Micro particle Immunoassay (CMIA). [For Detailed Instruction Refer Annex IV]

5.8.3 Overall work flow



5.8.2.1 HBV, ELISA principle

This is a Sandwich Enzyme linked Immunosorbent assay method in which polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Donor's serum or plasma sample is added to the microwells together with a secondary antibody conjugated with horseradish peroxidase (HRP) and directed against a different epitope of HBsAg. During incubation, the specific immune-complex formed in the case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum protein and unbound HPR-conjugate, chromogen solution containing tetramethyl benzidine (TMB) and urea peroxidase are added to the walls. In the presence of the antibody-antigen-antibody (HRP) sandwich immune-complex, the colorless chromogens are hydrolyzed by the bound HPR conjugate a blue colored product. The blue color turns to yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antigen in the sample.

5.8.2.2 HCV, ELISA principle

Polystyrene microwell stripes are pre-coated with recombinant, highly immune-reactive antigens corresponding to the core and non-structural regions of HCV. Durining the first incubation step, anti-HCV specific antibodies, if present, will be bound to the phase precoated HCV antigens. The wells are washed to remove unbound serum proteins, and rabbit antihuman IgG antibodies (anti-IgG) conjugated to HRP is added. During the second incubation step, these HRPconjugated antibodies will be bound to any antigen- antibodies complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing TMB and urea peroxidase are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immune-complex, the colorless chromogens are hydrolyzed by the bound HRP-conjugated to a blue colored product. The blue color turns to yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antibody in the sample.

5.8.2.3 Anti-HBc, CMIA principle

The Anti-HBc assay is a two-step immunoassay for the qualitative determination of anti-HBc in human serum or plasma using chemiluminescent micro particle immunoassay (CMIA) technology. In the first step, sample, assay diluent, specimen diluent, and rHBcAg coated paramagnetic microparticles are combined. Anti-HBc present in the sample binds to the rHBcAg coated microparticles and the reaction mixture is washed. In the second step, anti-human acridinium-labeled conjugate is added. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting Chemiluminecent reaction is measured as relative light units (RLUs). A direct relationship exists between

the amount of anti-HBc in the sample and the RLUs detected by the ARCHITECT *i* System optics.The presence or absence of anti-HBc in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active ARCHITECT Anti-HBc II calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, then the sample is considered reactive for anti-HBc.

5.9 Data entry and analysis

Data was entered into excel sheet and exported to IBM SPSS Software Version 23.0 for analysis. Proportions and actual number of cases were used to describe frequency outputs for categorical variables. Cross-tabulations was used to explore and display the relation between two categorical variables. Bivariate and multivariate logistic regression analysis was used to identify the strength of association of the various potential determinants. P-value of less than 0.05 was considered to be statistically significant

5.10 Quality assurance and quality control

The collected data was checked for its completeness, accuracy and clarity on a regular base by the primary investigator during the data collection period. All specimens were collected according to the standard operating procedure of specimen collection and the quality of the specimen was checked and all laboratory procedure is done based on the manufactures instructions. A Negative Control and a Positive Control was used for every run and external quality assessment samples were also run together with our samples for more verification of our result.

5.11 Ethical considerations

Ethical clearance paper was obtained from the department of research and ethical review committee, (DRERC), Department of Medical Laboratory Sciences, School of Allied Health Sciences, college of Health Sciences; Addis Ababa University. Written permission letter was also obtained from Ethiopian national blood Bank. The study participants were involved in the study only after they read and signed the consent form. All study participants had a unique code number so that the findings of the study were kept confidential.

5.12 Operational definitions

- **High prevalence of Hepatitis B and C**: prevalence rate of greater or equal to 8% and 3.5% respectively
- **Intermediate prevalence of Hepatitis B and C**: prevalence rate of 2% up to 7% and 1.5% up to 3.5% respectively
- **Low prevalence of Hepatitis B and C**: prevalence rate of less than or equal to 2% and 1.5% respectively

6. Results

6.1 Socio-Demographic Characteristics

In this study, during data collection period 2989 voluntary blood donors were approached to participate and fill the questionnaire but, 12 were rejected because of incomplete data, which gives a 99.5% response rate. Of the 2977 voluntary blood donors, 62.1% (n=1848/2977) were males and 37.9% (n=1129/2977) were females. 79 % (n=2352/2977) of the participants were first time donors. The mean age of the study participants with standard deviation was 25.9 ±8.26 years (range 18-65 years). Most study subjects 59.8 % (n=1781/2977) were found within 18-25 years of age. Regarding marital status, 78.7 %(n=2342/2977) of the participants were never married and majority 41.7 %(n= 1242/2977) of the study participants were students. (Table 1).

Table 1: Socio-demographic characteristics of voluntary blood donors at national blood bank Addis Ababa, Ethiopia, April to June, 2017

Variables	Frequency (n=2977)	Percentage (%)
Gender		
Male	1848	62.1
Female	1129	37.9
Age Group		
18-25	1781	59.8
26-35	829	27.8
36-45	254	8.5
46-55	95	3.2
56-65	17	0.6
Marital Status		
Single	2342	78.7
Married	629	21.1
Divorced	6	0.2
Occupation		
Student	1242	41.7
Private company employee	879	29.5
Self employed	566	19
Government employed	290	9.7
Type of donor		
First time donors	2352	79
Multiple donors	625	21

6.2 Prevalence of HBV and HCV

Prevalence of HBV and HCV among volunteer blood donors was 2.3 % (n=69/2977) and 1.2 % (n=36/2977) respectively. From 69 participants who were positive for HBV, 64% (n=44/69) were males and from 36 participants who were positive for HCV, 58% (n=21/36) were males (Table 2). The overall HBV/HCV co infection rate was 0.23 %(n=7/2977) and from the total positive TTIs (HIV, HBV, HCV, syphilis) which was 5.3% (n=158/2977), HBV/HCV co infection was 4.4% (n=7/158) and was found to be significantly co-existing in our study population ($p<0.05$). (Figure 1)

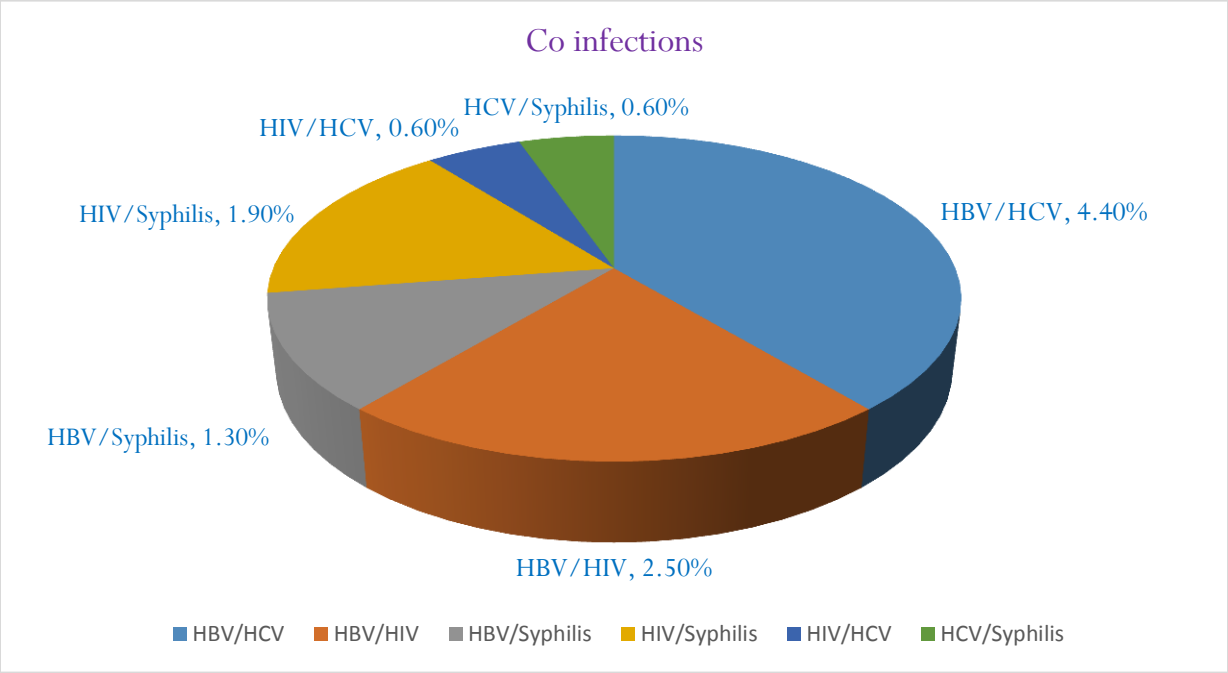


Figure 1. Co infections from total TTIS positives; national blood bank Addis Ababa, Ethiopia, April to June, 2017

6.3 Prevalence of anti-HBc total antibody

Among the total 2977 volunteer blood donors who screened for TTIs, 94.6 %(n= 2819/2977) were negative for all TTIs (HIV, HBV HCV, syphilis). Among this seronegative donors whose samples were ready to be given for the recipients, 391 participants' sample were taken and screened further for anti-HBc total positivity. Among 391 further screened donors 14.6 %(57/391) were positive for anti HBc total antibody. From this 8.9% (n=34/391) were males and 5.7% (n=23/391) were female donors and age group of 26 to 30 years showed maximum seropositivity (7%; n=27/391) (Table 2)

Table 2: HBV, HCV and anti HBC prevalence among Donor type, gender and different age groups of blood donors at national blood bank Addis Ababa, Ethiopia, April to June, 2017

Variables	Frequency N(%)	HBsAg(n=2977)		Anti-HCV status(n=2977)		Anti-HBc total status (n=391)	
		Negative N (%)	**Positive N (%)**	**Negative N (%)**	**Positive N (%)**	**Negative N (%)**	**Positive N (%)**
Gender							
Male	1848[62.1]	1804[97.6]	44[2.4]	1826[98.7]	22[1.3]	215[86.4]	34[13.6]
Female	1129[37.9]	1104[97.8]	25[2.2]	1115[98.8]	14[1.2]	119[83.8]	23[16.2]
Age Group							
18-25	1781[59.8]	1745[98.0]	36[2]	1756[98.6]	25[1.4]	223[92.2]	19[7.8]
26-35	829[27.8]	805[97.1]	24[2.9]	823[99.3]	6[0.7]	76[74]	27[26]
36-45	254[8.5]	248[98.8]	6[2.4]	252[99.2]	2[0.8]	22[70.9]	9[29.1]
46-55	95[3.2]	92[96.8]	3[3.2]	92[96.8]	3[3.2]	10[83.3]	2[16.7]
56-65	17[0.6]	17[100]	0[0]	17[100]	0[0]	3[100]	0[0]
Donor type							
First time	2352[79]	2286[97]	65[3]	2318[98.5]	35[1.5]	283[87]	44[13]
Multiple	625[21]	622[99.4]	4[0.6]	624[99.8]	1[0.2]	51[79.6]	13[20.4]
Total	2977[100]	2908[97.7]	69[2.3]	2941[98.8]	36[1.2]	334[85.4]	57[14.6]

6.4 Risk Factors associated with HBV and HCV

Among 2977 study participants 2.3%(n=69/2977) were positive for Hepatitis B surface Antigen and after bivariate and multivariate analysis of possible risk factors first time donation ,age , multiple sexual exposure and HCV infection indicated statistically significant association with HBV infection.($p<0.05$) (Table 3)

When positivity was compared among study participants based on donor status, first time donors were 0.21 times as less likely to be negative for HBV (AOR 0.21; 95% CI=0.15-1.21, $p<0.001$) compared to people give blood more than one times. Regarding age category, participants aged between 36-45 were 2.9 times as likely to be positive for HBV (AOR=2.96; 95% CI=1.17-7.48, $p=0.021$) compared to age range 18-25.

While observing at the association of HBV infection and multiple sexual exposure, participants who were not exposed to multiple sexual partnership were 93% less likely to be positive for HBV (AOR=, 0.07, CI= 0.02-0.29) compared to those who were exposed.

Table 3 Risk factors association with prevalence of Hepatitis B virus, Bivariate and multivariate analysis; volunteer Blood Donors at national blood bank Addis Ababa, Ethiopia, April to June, 2017

Variables	Response category	No[%]	HBsAg positive No[%]	Crude OR[95%CI]	P value	AOR[95%CI]	P value
Sex	Male	1848[62.1]	44[63.8]	1			
	Female	1129[37.9]	25[36.2]	0.928[0.56,1.52]	0.769		
Age	18-25	1781[59.8]	36[52.2]	1			
	26-35	829[27.9]	24[34.8]	1.44[0.85,2.43]	0.168		
	36-45	254[8.5]	6[8.7]	3.26[1.32,7.9]	0.009	2.96[1.17,7.48]	0.021**
	46-55	95[3.2]	3[4.3]	0.57[0.17,1.89]	0.367		
	56-65	17[0.6]	0[0]				
Marital status	Single	2342[78.7]	55[79.7]	1			
	Married	629[21.1]	14[20.3]	0.94[0.523,1.713]	0.856		
	Divorced	6[0.2]	0[0]				
Occupation	Student	1242[41.7]	24[34.8]	0.55[0.26,1.16]	0.120		
	Private company employee	879[29.5]	21[30.4]	0.68[0.31,1.47]	0.333		
	Self employed	566[19.0]	14[20.3]	0.71[0.31,1.6]	0.416		
	Government employed	290[9.7]	10[14.5]	1			
History of transfusion	Yes	100[3.4]	1[1.4]	1			
	No	2877[96.6]	68[98.6]	2.39[0.32,17.43]	0.38		
Sharing sharp materials	Yes	34[1.1]	4[5.8]	1			
	No	2943[98.9]	65[94.2]	0.16[0.05,0.49]	0.001	0.29[0.20,1.10]	0.070
Ever had surgical procedure	Yes	7[0.2]	1[1.4]	1			
	No	2970[99.8]	68[98.6]	0.14[0.01,1.18]	0.071		
Multiple Sexual exposure	Yes	15[0.5]	4[5.8]	1			
	No	1962[99.5]	65[94.2]	0.06[0.02,0.19]	0.000	0.07[0.02,0.29]	<0.001**
Syphilis status	Positive	34[1.1]	2[2.9]	1			
	Negative	2943[98.9]	67[97.1]	0.37[0.88,1.58]	0.182		
HCV status	Positive	36[1.2]	7[10.1]	1			
	Negative	2941[98.8]	62[89.9]	0.08[0.03,0.21]	0.000	0.10[0.04,0.23]	<0.001**
Donor type	First time	2352[79]	65[94.2]	0.15[0.04,0.32]	0.002	0.21[0.15,1.21]	<0.001**
	Multiple	625[21]	4[5.8]	1			

** There is statistical significant association between the variables and HBV prevalence

From the total volunteer donors, 1.1% (n=34/2977) had experience of sharing sharps or Scarification marks/tattoos and from these 5.8 %(n=4/34) were positive for HBV. Even though it was not statistically significant, when compared to volunteers who had experience of sharing sharps or Scarification marks/tattoos, donors who had no experience were 0.40 times less likely to be infected with HBV. (AOR=0.40, CI=0.36-1.10).

HBV test positivity was somewhat higher among males 62.1 %(n=1848/2977) than females 37.9 %(n=1129/2977) (OR=, 0.93, CI= 0.56-1.52). Similarly, considering marital status of participants tested positive for HBV; test positivity was higher among unmarried (79.7%) than married (20.3%).

Among the total 2977 study participants 1.2% (n=36/2977) were positive for Hepatitis C surface Antigen and after bivariate and multivariate analysis of possible risk factors sharing sharps or Scarification marks/tattoos and being infected with HBV exhibited statistically significant association with HCV infection.($p<0.05$)(table 5). From the total participants, 34(1.1%) had experience of sharing sharps or Scarification marks/tattoos and from these 5(13.9 %) were positive for HCV. When compared to volunteers who had experience of sharing sharps or Scarification marks/tattoos, donors who had no experience were 0.13 times less likely to be infected with HCV. (AOR=0.13, CI=0.03-0.47,)

When HCV positivity was compared among participants who were exposed and not exposed to multiple sexual contact, participants who were not exposed to multiple sexual partnership were 0.06 times less likely to be positive for HCV (AOR=, 0.06, CI= (0.01-0.23) when compared to those who were exposed but this was not statistically significant. The likely hood of being infected with HCV among HBV negative individuals was 0.13 times less likely to be infected with HCV than those who were positive for HBV (AOR=0.13, CI 0.05-0.36) ($p<0.001$).

Considering marital status of participants tested positive for HCV, test positivity was higher among unmarried (80.6%)than married and also test positivity was higher among males 24(66.7%) than females 12(33.3%).

Being positive for syphilis, occupation, marital status, sex, history of transfusion, age category and ever exposed to surgical procedures didn’t show statistical significant association.

Table 4 Risk factors association with prevalence of Hepatitis C virus, Bivariate and multivariate analysis; volunteer Blood Donors at national blood bank Addis Ababa, Ethiopia, April to June, 2017

Variables	Response category	No[%]	HCV positive No[%]	Crude OR[95%CI]	P value	AOR[95%CI]	P value
Sex	Male	1848[62.1]	24[66.7]	1			
	Female	1129[37.9]	12[33.3]	0.81[0.40,1.63]	0.56		
Age	18-25	1781[59.8]	36[52.2]	1			
	26-35	829[27.9]	24[34.8]	0.49[0.20,1.20]	0.119		
	36-45	254[8.5]	6[8.7]	0.53[0.12,2.27]	0.391		
	46-55	95[3.2]	3[4.3]	1.45[0.33,6.20]]	0.367		
	56-65	17[0.6]	0[0]				
Marital status	Single	2342[78.7]	29[80.6]	1			
	Married	629[21.1]	7[19.4]	0.89[0.39,2.05]	0.791		
	Divorced	6[0.2]	0[0]				
Occupation	Student	1242[41.7]	16[44.4]	1.24[0.36,4.31]	0.720		
	Private company employee	879[29.5]	12[33.3]	1.32[0.37,4.72]	0.661		
	Self employed	566[19.0]	5[13.9]	0.85[0.20,3.59]	0.828		
	Government employed	290[9.7]	3[8.3]	1			
History of transfusion	Yes	100[3.4]	3[8.3]	1			
	No	2877[96.6]	33[91.7]	0.37[0.11,1.24]	0.10		
Sharing sharp materials	Yes	34[1.1]	5[13.9]	Ref*			
	No	2943[98.9]	31[86.1]	0.06[0.02,0.17]	0.000	0.13[0.03,0.47]	0.002**
Ever had surgical procedure	Yes	7[0.2]	2[5.6]	1			
	No	2970[99.8]	34[94.4]	0.03[0.01,0.15]	0.010	0.042[0.005,0.367]	0.062
Multiple Sexual contact	Yes	15[0.5]	2[6.06]	1			
	No	1962[99.5]	31[93.93]	0.02[0.01,0.4]	0.080	0.06[0.01,0.23]	0.071
Syphilis status	Positive	34[1.1]	1[2.8]	1			
	Negative	2943[98.9]	35[97.2]	0.39[0.05,2.98]	0.37		
HBV status	Positive	69[2.3]	7[19.4]]	1			
	Negative	2908[97.7]	29[80.6]	0.08[0.03,0.21]	0.000	0.13[0.05,0.36]	<0.001**
Donor type	First time	2352[79]	35[97.2]	1			
	Multiple	625[21]	1[2.8]	0.28[0.04,2.65]	0.34		

Regarding the association of anti HBc positivity with different socio demographic characteristics and associate risk factors, statistical significant association was not observed for Sex, Age, Marital status, Sharing sharp materials or Scarification marks/tattoos and exposure to multiple Sexual partners.

On the other hand statistical significant association was shown with history of transfusion and ever had surgical procedure. Individuals who have no history of blood transfusion were 0.07 times less likely to be positive for anti HBc than those who had not (AOR=, 0.07, CI= (0.03-0.14) and also individuals who had not go through any surgical procedures were 0.08 times less likely to be positive for anti HBc than those who did not . (AOR=, 0.08, CI= (0.01-0.52) (table 4)

Table 5 Risk factors association with prevalence of anti-HBc , Bivariate and multivariate analysis;

Variables	Response category	Anti-HBc positive No[%]	Crude OR[95%CI]	P value	AOR[95%CI]	P value
Sex	**Male**	**34[59.6]**	**1**			
	Female	**23[40.4]**	**0.47[0.25,0.89]**	**0.020**	**0.58[0.30,1.12]**	**0.106**
Age	**18-25**	**19[33.3]**	**1**			
	26-35	**27[47.4]**	**3.12[1.72,5.6]**	**0.000**	**1.56[0.73,3.32]**	**0.245**
	36-45	**9[15.8]**	**3.40[1.52,7.61]**	**0.003**	**1.45[0.55,3.83]**	**0.451**
	46-55	**2[3.5]**	**1.99[0.45,8.69]**	**0.358**	**0.53[0.10,2.69]**	**0.450**
	56-65	**0[0]**				
Marital status	**Single**	**41[71.9]**	**1**			
	Married	**16[28.1]**	**1.46[0.81,2.62]**	**0.201**		
	Divorced	**0[0]**				
Occupation	**Student**	**11[19.3]**	**0.25[0.10,0.59]**	**0.002**	**0.57[0.20,1.65]**	**0.304**
	Private company employee	**22[38.6]**	**0.71[0.33,1.53]**	**0.394**		
	Self employed	**14[24.6]**	**0.71[0.31,1.61]**	**0.416**		
	Government employed	**10[17.5]**	**1**			
History of transfusion	**Yes**	**18[31.6]**	**1**			
	No	**39[68.4]**	**0.13[0.03,0.18]**	**0.003**	**0.07[0.03,0.14]**	**<0.001****
Sharing sharp materials	**Yes**	**2[3.5]**	**Ref***			
	No	**55[96.5]**	**0.30[0.07,1.30]**	**0.109**		
Ever had surgical procedure	**Yes**	**3[5.3]**	**1**			
	No	**54[94.7]**	**0.06[0.013,0.325]**	**0.001**	**0.08[0.01,0.52]**	**<0.001****
Multiple Sexual exposure	**Yes**	**1[1.7]**	**1**			
	No	**56[98.3]**	**0.28[0.03,2.22]**	**0.234**		
Donor type	**First time**	**44[77.2]**	**1**			
	Multiple	**13[22.8]**	**1.52[0.71,2.73]**	**0.241**		

** There is statistical significant association between the variables and HBV prevalence volunteer Blood Donors at national blood bank Addis Ababa, Ethiopia, April to June, 2017

6.5 Distribution of transfusion transmission infections (TTIs) with blood group

All blood donors' in this study were tested for their ABO and RH blood group type using forward and reverse grouping techniques. Based on this, 1205(40.5%) had blood group O and 2717 (91.3%) of the participants were Rh positive. (Figure 2).

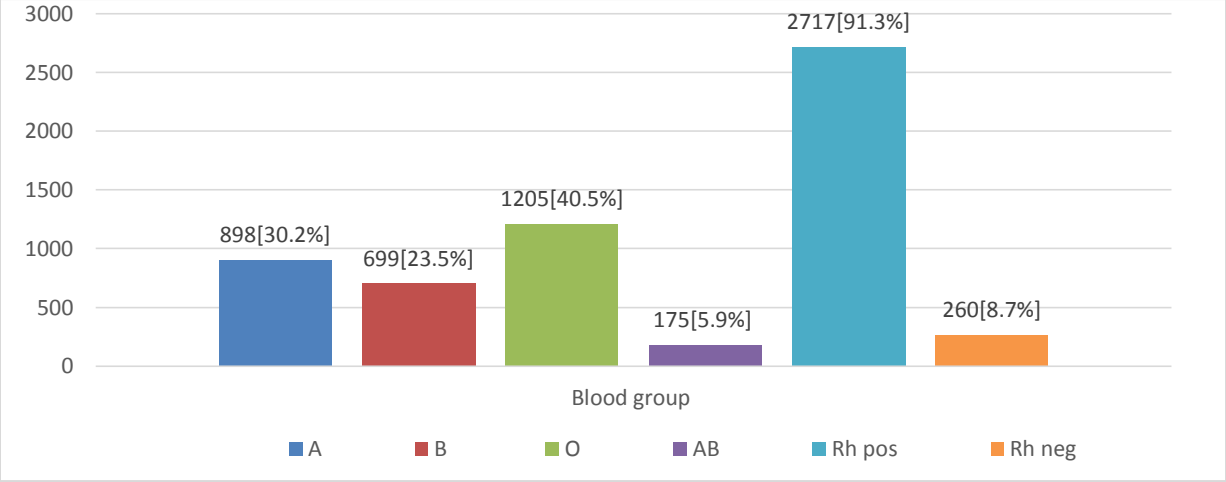


Figure 2: Distribution of voluntary blood group among blood donors' at national blood bank Addis Ababa, Ethiopia, April to June, 2017

Bivariate analysis was performed to assess the association between blood group and transfusing transmitted infections, based on this analysis, both ABO and RH blood types have no significant association with any of the transfusion transmitted infections (Table 6)

Table 6 Bivariate analysis for the association of HBV, HCV, HIV and Syphilis with blood group at national blood bank, Addis Ababa, Ethiopia, April to June, 2017

BG Rh	HBV + N(%)	OR[95%CI]	P-V	HCV+ N(%)	OR[95%CI]	P-V	HIV+ N(%)	OR[95%CI]	P-V	Syphilis + N(%)	OR[95%CI]	P-V
A	25(36.2)	0.97[0.36,2.57]	0.95	7(19.4)	0.45[0.11,1.75]	0.25	3(15.8)	0.29[0.04,1.74]	0.17	9(26.5)	0.87[0.18,4.08]	0.86
B	13(18.8)	0.64[0.22,1.83]	0.41	7(19.4)	0.58[0.14,2.26]	0.43	3(15.8)	0.37[0.06,2.24]	0.28	6(17.6)	0.74[0.15,3.74]	0.72
0	26(37.7)	0.75[0.28,1.97]	0.56	19(52.8)	0.91[0.26,3.13]	0.89	11(57.9)	0.79[0.17,3.62]	0.76	17(50)	1.23[0.28,5.4]	0.77
AB	5(7.2)	1		3(8.30	1		2(10.5)	1		2(5.9)	1	
Rh-	6(8.7)	0.99[0.42,2.3]	0.99	3(8.3)	0.94[0.28,3.11]	0.93	1(5.3)	0.57[0.07,4.35]	0.59	6(17.6)	2.26[0.93,5.3]	0.07
Rh +	63(91.3)	Ref*		33(91.7)	Ref*		18(94.7)	Ref*		28(82.4)	Ref*	

7. Discussion

The Sero-prevalence of HBV in this study was 2.3%. This finding was lower as compared to previous studies in Addis Ababa (3.7%), (4.0%)Hawassa (4.2%),Dire Dawa(3.73%) Jigjiga (10.9%), [60, 61, 62, 42]. This finding was also a lower finding compared to studies done in different parts of Africa such as, 6.25% in Sudan [54], 5.9% Nigeria [52], 10.6% in Mozambique [53] and 4.2% in Kosovo [50] .This might be because of difference in donor status. Our study populations are all volunteers and most of them could be confident of their sero status. Difference in the study subjects and sample size, might also be the cause of such differences. For example in Dire Dawa and Jijiga studies 76% and 98% of participants were replacement donors respectively, In Sudan study (n=400) males were the only study participants.

On the other hand, the prevalence of HBV in the current study was higher than studies in Iraq (0.7 %), Jordan (1.4%) Morocco (0.68%), India (0.68%) [48, 49, 51, 44] and possible factors for the difference could be due to geographical location, Socio cultural difference and viral genotype differences, but also our study was comparable with different studies like a study at Jimma blood bank by Alemshet Y. et al, 2.1% [60] at Dessie blood bank, by Gelaw B. et al, 3% [57] and at neighboring Eritrea national blood bank by Nahom F.et al, 2.5% of HBV prevalence [56].

Significantly increased in Seroprevalence of HBV was observed in the age group 36-45 and it was 2.96 times as likely to be HBsAg positive (AOR=2.96; 95% CI=1.17-7.48, p=0.021) compared with age group 18-25.This was in correspondence with previous reports in Ethiopia by Tessema B. et al [59] (OR=2.17, 95% CI=1.23-3.81) (p=0.007) and Habte Y. et al [61] (AOR=3.7, 95% CI=1.19-9.56) (p=0.029) in which higher prevalence was observed among productive and economically viable age group. Similar studies in Ethiopia and other neighboring countries like Sudan have also shown that age to be one of the independent predictors of acquiring HBV infection [39, 55]. The significant association of HBsAg positivity and age group 36-45 in our study would probably due to lack of immunization against HBV in their times and lower risk for HBV infection among donors above 45 years might have resulted from a spontaneous clearance of HBsAg over time.

In this study another statistical significant association was observed between HBV infection and exposure to multiple sexual partners (AOR=, 0.07, CI= 0.02-0.29, p<0.001). In spite of the fact that there were no sufficient studies conducted in Ethiopia regarding wide range of risk factors of HBV on donor population, this stdudy finding is in agreement with similar findings done by Balew M. et al (AOR=8.1, 95%CI=1.8-33.97) [72] and by *Weldemhret L et al* (AOR = 4.2, 95 % CI 1.3–13.1) [73] which had reported a strong statistically significant association between HBV infection and having multiple sexual partners.

This finding supports the fact that people who have exposure to multiple sexual partners will be prone to HBV infections as well as for other sexually transmitted diseases.

Significantly increased in Seroprevalence of HBV was observed in first time donors(AOR=0.21; 95% CI=0.15-1.21) compared with multiple donor population. This study finding is in agreement with similar findings done by Tessema B. et al at Gonder [59]. This significantly increased HBV Seroprevalence among first time donors might be due to the fact that people who regularly donate blood usually have a profile of low-risk HBV infection because they were eligible to give blood more than one times.

Concerning HCV frequency, the overall prevalence in the present study was 1.2% and our finding is in line with HCV prevalence among blood donors done in Morocco (0.9%), Sudan (0.7%), Nigeria (1.4%) and Eritrea (0.6%) [49, 55, 56, 52].Our finding was also comparable with different studies in Ethiopia like a study by *Gelaw B* et al at Amhara and Tigray Regional states [57] and *Assefa A* et al at Bahir Dar city [59] which had reported HCV prevalence rate 1.7 % and 0.6% respectively.

This study finding on HCV prevalence was a little bit higher than the prevalence previous studies from Addis Ababa 0.5% [63], Jimma 0.2% [60], Hawassa 0.5% [62], also in different European countries like Kosovo 0.3%[50]. But this study result was lower than different studies in Gonder, Bahir Dar and Felege Hiwot referral hospital; recorded as 2.3%, 3%, 13.3% respectively [57, 58] and this difference might be due to difference in type of study subjects. Case in point, among donors at Felege Hiwot referral hospital more than half (56.6%) of the study participants were commercial donors whereas our study subjects are volunteer blood donors.

After bivariate and multivariate analysis exposure to sharing sharps or Scarification marks/tattoos has been found to be having statistically significant association with HCV infection. Donors who had no experience were 0.13 times less likely to be infected with HCV (AOR=0.13, CI=0.03-0.47,) this was concordant with studies in Brazil (p=0.01, OR=8.3, 95% CI=4.6 -12.2) and Pakistan (p=0.045, OR=2.893, 95% CI=0.979-8.543) blood donors [46, 49]. These findings could serve to alert health authorities in Ethiopia about the risk that these may represent for the maintenance of HCV transmission.

It is notable that HBV/HCV confection is not rare in endemic areas because of the shared modes of transmission. In our study from the total positive TTIs (HBV, HCV, syphilis) transfusion transmitted infections HBV/HCV confection was 0.23% (n=7/2977) and after multivariate logistic analysis, the likely hood of being not infected with HCV among HBV negative individuals was 0.13 times more likely to be infected with HCV than those who were positive for HBV($p<0.05$). This finding is concordance with a studies in Ethiopia by Bonja F et al at Hawassa at which 0.5 % had HBV/HCV co-infections [62] and also in Nigeria by shitu et al in which 0.4% co-infection was recorded among blood donors [53].

This finding 0.23% (n=7/2977) was somewhat higher than Bedada's G.et al recent study report of 0.065 %(n= 29/46052) HBV/HCV co infection in Addis Ababa [63].This could be due to large difference in sample size. Various studies have reported HBV/HCV confection prevalence rates, ranging from 0.7 to 15%, [25] but our result indicates lower prevalence rate than thes studies. This may perhaps be due to differences in study subjects as HBV/HCV confection is more frequently found in high-risk populations, comprising injecting drug users, patients of hemodialysis organ transplant or ß-thalassemia [29].

The use of nucleic acid testing for the detection of HBV DNA has not been adopted in most developing countries, including Ethiopia this is because it requires high cost and modern technology. However, several studies have shown varying proportions of those positive for other markers of HBV infection aside from HBsAg and HBV DNA to be capable of transmitting the virus and despite non-detection of HBV DNA in serum there have been reports of post transfusion HBV infection in recipients of blood positive for anti HBc [27-36]

Therefore Anti-HBc is very important in screening blood donors as a way of reducing the residual risk of post transfusion HBV infection and has been variously investigated with varying prevalence without the detection of HBV DNA in the serum [74] In our study, the overall prevalence of total anti-HBc antibodies among HBsAg negative blood donors was 14.6% (n=57/391). This was comparable to 14.2% in Egypt [70] from which 17.2% confirmed as occult and a study in Nigeria 11.2% [69] from which 17% were confirmed as occult blood infection by DNA detection in different reference lab.

In areas of higher HBV infection prevalence about 20%-70% of subjects are positive for anti-HBc antibody [70]. The prevalence of anti-HBc in our blood donor population was higher than a study in USA [66]8.1% and India 8 % [67] but relatively low when we compare it studies in Iraq 28% [66] Sudan 42% from which 90.5% confirmed as occult [71].This anti HBc positivity difference might be due to difference in sample size and regional differences in the prevalence of HBV infection and also usage of different equipment's having different sensitivity and specificity.

Regarding to anti HBc positivity, statistical significant association was shown with history of transfusion. (AOR=, 0.07, CI= 0.03-0.14) and ever had surgical procedure (AOR=, 0.08, CI= (0.01-0.52). This finding was concordant with a study done in India which showed both risk factors, history of transfusion and ever had surgical procedure have shown statistical significant association with anti HBc positivity [68]

The important thing in our study regarding screening of anti HBc for highlighting the presence of occult HBV is that since the burden of occult hepatitis B virus in Africa varies from 5.4%-10% from the total donor population and 8-42 % of anti HBC positivity has been reported in different countries [67,70 ,71] and also from this anti HBc positive individuals 22%-90.5% were positive viral DNA testing showing that significant number of HBsAg negative anti HBc positive individuals will have occult hepatitis B virus[32-36]. Henceforth, our 14.6% (n=57/391) anti HBc positivity finding should be taken as essential information for the sake of safe blood transfusion.

8. Strength and Limitation of the Study

- The study has tried to assess occult hepatitis B virus among blood donors from HBsAg negative blood donors which has not been addressed before in the country.
- The study was restricted on screening of occult hepatitis B virus using anti HBc total [IgG and IgM] antibody testing

9. Conclusion and Recommendation

9. 1 Conclusion

As WHO interpretative criteria, the prevalence rate of HBV 2.3 %(n=69/2977) was intermediate and that of HCV 1.2 %(n=36/2977) was low among blood donors. After bivariate and multi variate statistical analysis of different variables first time donation, age and contact to multiple sexual partners shows statistical significant association with HBV prevalence and for that of HCV prevalence sharing of sharp materials/scarification tattoos shows statistically significant association. Screening of anti HBc for occult hepatitis B yielded a 14.6% (n=57/391) prevalence rate and among this 22.8 %(n=13/57) of them were multiple blood donors. This finding could support the implementation of anti-HBc screening in the donor selection criteria to rule out and identify the HBV infections and emphasizes on the need for establishing safe and healthy blood transfusion. History of transfusion and ever had surgical procedures have shown statistical significant association with ant-HBC positivity.

9.2 Recommendations

- This study recommends that incorporation of routine anti-HBc screening of blood as a surrogate marker of occult HBV infection to prevent some transfusion-transmitted HBV infections.
- A molecular based study is recommended to be conducted countrywide to find out the true picture of occult hepatitis B virus.
- A national study, including a statistically significant number of blood donors from different blood donation centers across the country, should be carried out to determine the screening for anti-HBc in addition to HBsAg detection
- Since donors are generally selected from the general populations, there is a need for community based HBV and HCV infection study in order to further investigate associated risk factors which are crucial to implement effective prevention program

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11. Annexes

Annex-I Information sheet

Seroprevalence of Hepatitis B and C Virus and Occult Hepatitis B among Blood Donors at National Blood Bank, Addis Ababa, Ethiopia

Introduction

First of all I would like to express my great thankfulness for your participation in this study. You are invited to participate as a study participant voluntarily. The research team includes principal investigator and advisors.

Purpose

The main objective of the study is to determine prevalence and associated risk factors of hepatitis B and C virus and Screening Anti HBc for occult HBV among voluntary blood donors at National Blood Bank Ababa, Ethiopia

Risk associated with the study

You will not be at any physical or psychological risk and should experience no discomfort resulting from the research procedures.

Benefits of the study

Your participation will contribute to our country by providing crucial information about occult hepatitis B virus and its associated risk factors and also in improving the quality of blood and blood products that are used in transfusion. There will not be any compensation or payment for your involvement in this study.

Confidentiality of your information

I would like to indicate that your name will not be written in the form and I assure you all the information you give will be kept strictly confidential. The researchers conducting this study will review your records and follow the progress of the research, but nothing that can be used to identify you will be used in reports of this study.

The study proposal is approved by the departmental research and ethics review committee(DRERC) of the medical laboratory sciences, school of allied health sciences, College of Health Sciences; Addis Ababa University, to make sure that participants are protected from any harm.

Person to contact: If you have any question you can contact the principal investigator and you may ask at any time you want. Principal investigator: Misgana Petros, Cell phone 0947382252

Annex – II. Consent Form

Here the undersigned agree on terms and the conditions and give my consent to participate in the study to assess Seroprevalence of Hepatitis B and C Virus and Occult Hepatitis B among Blood Donors at National Blood Bank, Addis Ababa, Ethiopia. I understand that my participation is voluntary and there is no serious procedure that harms.

Do you agree to answer the following questions to the best of your ability?

Yes_____ No_____

Thank you for participation in the study!

Participant's signature: _____ Date: _____

Participant's Code _____

Annex- III. Questionnaire

Code No.: _____

Date: _____

1. Sex: M ☐ F ☐

2. Age: _____ Years _____

3. Marital status:

Single ☐ Married ☐ Divorced ☐ widowed ☐

4. Occupation:

Student ☐ private☐ Government ☐ self employed ☐

5. Have you ever been transfused?

Yes ☐ No ☐

If yes when? In the past one year ☐ before a year ☐

6. Have you had sexual contact with more than one person?

Yes☐ No☐

7. Have you experienced sharing sharps or Scarification marks/Tattoos?

Yes ☐ No ☐

8. Have you ever had medical procedures (dental procedure, surgery and others?)

Yes ☐ No ☐

9. Have you had donated blood?

Yes ☐ No ☐ if yes how many times? _____

Annex IV principle and procedure of tests

1. Test Principle and interpretation for HBsAg- ELISA

For detection of HBsAg, the kit (AiD wantai HBSAg ELISA) uses antibody sandwich ELISA method in which polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Donor's serum or plasma sample is added to the microwells together with a secondary antibody conjugated with horseradish peroxidase (HRP) and directed against a different epitope of HBsAg. During incubation, the specific immune-complex formed in the case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum protein and unbound HPR-conjugate, chromogen solution containing tetramethyl benzidine (TMB) and urea peroxidase are added to the walls. In the presence of the antibody-antigen-antibody (HRP) sandwich immune-complex, the colorless chromogens are hydrolyzed by the bound HPRconjugate a blue colored product. The blue color turns to yellow after stopping the reaction with sulfuric acid.

The amount of color can be measured and is proportional to the amount of antigen in the sample. Wells containing sample negative for HBsAg remain colorless. Every detail procedure will be followed according to manufacture instruction. Interpretation and calculation of cut-off value: The result will be calculated by relating each sample optical density (OD) value to the cut-off value (CO) of the plate. If the cut-off reading is based on single filter plate reader, the result must be calculated by subtracting the blank OD value from the print report value of the sample control. In case the reading is based on dual filter plate reader, do not subtract the blank well OD from the print repot values of the sample and control. Cut-off value (CO) = NCx2.1 (NC=the mean absorbance value for three negative control)

Negative result = S/CO <1

Positive result =S/CO $\geq$1

Borderline sample with absorbance to cut-off ratio between 0.9-1.0 needs to be retested.

The test results are valid if the quality control criteria are fulfilled.

- The A value of the blank well, which contains only chromogen and stop solution, is <0.080 at 450nm
- The A value of the positive control must be$\geq$0.800 at 450/600 or at 450 after blanking
- The A value of the negative control must be <0.100at 450/600 or at 450 after blanking

2. Test Principle and interpretation for anti HCV- ELISA

The kit (AiD wantai anti-HCV ELISA) uses a two-step incubation enzyme immunoassay at wich polystyrene microwell stripes are pre-coated with recombinant, highly immune-reactive antigens corresponding to the core and non-structural regions of HCV. Darning the first incubation step, anti-HCV specific antibodies, if present, will be bound to the phase precoated HCV antigens. The wells are washed to remove unbound serum proteins, and rabbit antihuman IgG antibodies (anti-IgG) conjugated to HRP is added. During the second incubation step, these HRPconjugated antibodies will be bound to any antigen-antibodies complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing TMB and urea peroxidase are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immune-complex, the colorless chromogens are hydrolyzed by the bound HRP-conjugated to a blue colored product. The blue color turns to yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antibody in the sample. Wells containing samples negative for anti-HCV remain colorless.

Interpretation and calculation of cut-off value: The result will be calculated by relating each sample optical density (OD) value to the cut-off value (CO) of the plate. If the cut-off reading is based on single filter plate reader, the result must be calculated by subtracting the blank OD value from the print report value of the sample control. In case the reading is based on dual filter plate reader, do not subtract the blank well OD from the print repot values of the sample and control. Cut-off value (CO) = NC+2.1 (NC=the mean absorbance value for three negative control)

Negative result = S/CO <1

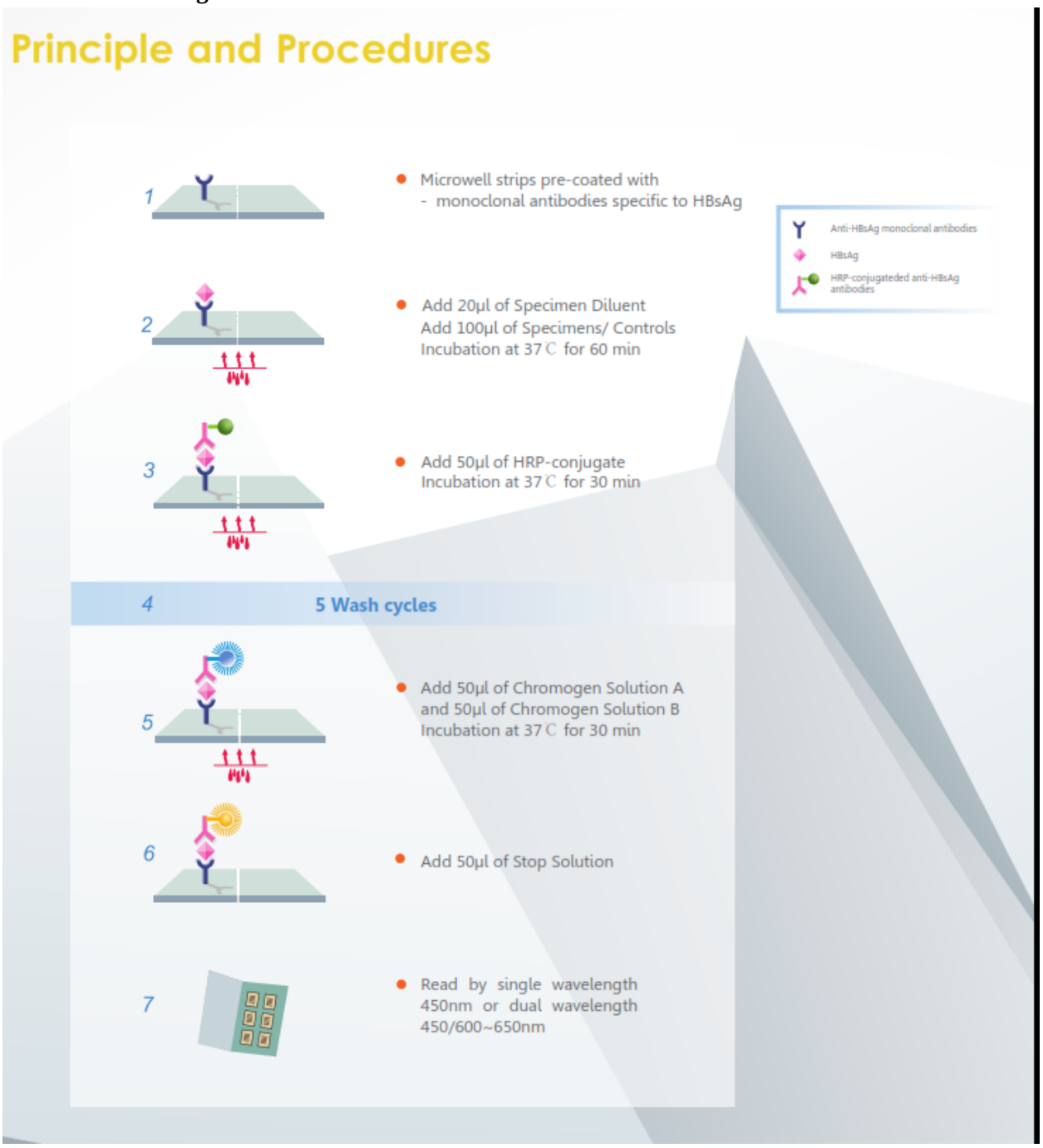
Positive result =S/CO $\geq$1

Borderline sample with absorbance to cut-off ratio between 0.9-1.0 needs to be retested.

The test results are valid if the quality control criteria are fulfilled.

- The A value of the blank well, which contains only chromogen and stop solution, is <0.080 at 450nm
- The A value of the positive control must be$\geq$0.800 at 450/600 or at 450 after blanking
- The A value of the negative control must be <0.100at 450/600 or at 450 after blanking

3. Guide for HBsAg ELISA



4. Anti-HBc testing using CMIA principle

The Anti-HBc assay is a two-step immunoassay for the qualitative determination of anti-HBc in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. In the first step, sample, assay diluent, specimen diluent, and rHBcAg coated paramagnetic microparticles are combined. Anti-HBc present in the sample binds to the rHBcAg coated microparticles and the reaction mixture is washed. In the second step, anti-human acridinium-labeled conjugate is added. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting Chemiluminecent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti-HBc in the sample and the RLUs detected by the ARCHITECT *i* System optics.

The presence or absence of anti-HBc in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active ARCHITECT Anti-HBc II calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, then the sample is considered reactive for anti-HBc.

Ordering procedure

- Before loading the Anti-HBcTot reagent kit on the system for the first time, the Microparticle bottle requires mixing to resuspended microparticles that have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - ✓ Invert the Microparticle bottle 30 times.
  - ✓ Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue inverting the bottle until the microparticles have been completely resuspended.
  - ✓ Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Carefully snap the septum onto the top of the bottle.

- Load the ARCHITECT Anti-HBcTotReagent Kit on the ARCHITECT *i* System.
- Select appropriate sample and check integrity of sample.
- Check volume of sample, minimum volume 500µl.
- Check the calibration status of the test. If calibration necessary refers calibration sections of this SOP.
- Run control according to the schedule; refer to control section of this SOP (how to run control.
- Enter the control to the poly tech system.
- Run the test as follows:-

- Select orders from the menu bar and select patient order.
- Select the carrier button.
- Enter a carrier ID in the C data entry box.
- Enter a position in the P data entry box.
- Enter the SID in the SID date entry box.
- Select an assay (Anti-HBc Total) from the assay list.
- Put sample tube /cup on the assigned position.
- Select F3 to add order.

Put the carrier on the carrier position, and then the machine automatically processes the testing Serum/plasma is stored at 2-8°C for 7 days.

Quality control

The recommended control requirement for the ARCHITECT Anti-HBc total assay is that a single sample of each control be tested once every 24 hours each day of use. If laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures. The ARCHITECT Anti-HBc II control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and samples must be retested. Recalibration may be indicated. Verification of Assay Claims For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual,

Interpretation of results

< 1.00 NONREACTIVE

≥ 1.00 REACTIVE

Annex V Amharic version of information sheet

<u>የጥናቱ ማብራሪያ</u>

የጥናቱ ርዕስ

የጉበት በሽታ ቫይረስ ስርጭት ጥናት በፈቃደኛ ደም ለጋሾች ላይ ብሔራዊ ደም ባንክ አዲስ አበባ

<u>መግቢያ</u>

በመጀመሪያ በጥናቱ ላይ ለመሳተፍ ፈቃደኛ ስለሆኑ ልባዊ ምስጋናዬን አቀርባለሁ ፡፡ እርስዎ በዚህ ጥናት ውስጥ ተሳታፊ እንዲሆኑ ሲጠየቁ ተሳታፊ የሚሆኑት ፈቃደኛ ከሆኑ ብቻ ነው፡፡ ይህንን ጥናት የሚያካሂዱት ሰዎች የተዋቀሩት በዋና ተመራማሪ እና በጥናቱ አማካሪዎች ናቸው፡፡

<u>ዓላማ</u>

የዚህ ጥናት ዋናው አላማ የጉበት በሽታ ቫይረስ ስርጭት በፈቃደኛ ደም ለጋሾች ላይ ለማወቅ ነው፡፡

ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት

በዚህ ጥናት ዝርዝር አሰራር ሂደት ውስጥ ምንም አይነት አካላዊም ሆነ አእምሮዊ ጉዳት የለም፡፡

<u>የጥናቱ ጥቅም</u>

ጤንነቱ የተጠበቀ ደም እና የደም ተዋፅኦ ለተጠቃሚው ማቅረብ ነው፡፡

ለተሳትፎ የሚሰጥ ማካካሻ

በዚህ ጥናት ውስጥ ለተሳታፊዎች የሚሰጥ ምንም አይነት የማስማሚያ ሆነ የማካካሻ የገንዘብ ክፍያ የለም፡፡

የጥናቱ ምስጢራዊነት

የተሳታፊዎችን መረጃ ሚስጢራዊነት ለመጠበቅ ይረዳ ዘንድ የጥናቱ ተሳታፊዎች ስም በጥናቱ ላይ አይገለፅም ፡፡ በስም ፋንታ መረጃዎቹ በሚስጢራዊ ቁጥር (ኮድ) ይመዘገባሉ፡፡

<u>ለጥናቱ ተጠሪ</u>

በማንኛውም ግዜ ስለ ጥናቱ መጠየቅ የሚፈልጉት ጥያቄ ካለ መጠየቅ ይችላሉ፡፡

ተመራማሪ ምስጋና ጴጥሮስ ስልክ 0947382252

Annex VI Amharic version of consent form

የስምምነት ውል

እኔ ከዚህ በታች የፈረምኩት የጥናቱ ተሳታፊ አከልት በሽታ ቫይረስ ስርጭት በፈቃደኛ ደም ለጋሾች ላይ የሚደረገው ጥናት አላማውና ጥቅሙ በሚገባ ተረድቻለሁ፡፡ በጥናቱ ላይ መሳተፍም ሆነ አለመሳተፍም በራሴ ፍቃድ የሚወሰን መሆኑንም ተገልጾልኛል፡፡ እንዲሁም ጥናቱ ምንም አይነት ጉዳት እንደማያደርስብኝ ተረድቻለሁ፡፡

በጥናቱ ለመሳተፍ ፈቃደኛ ነዎት?

ሀ. አዎ ነኝ ለ. አይደለሁም

በዚህ ጠቃሚ ጥናት ስለረዱኝ ከልብ አመሰግናለሁ፡፡

የጥናቱ ተሳታፊ መለያ ቁጥር _____

ፊርማ_____

ቀን_____

Annex VII Amharic version of data collection sheet

የጉበት በሽታ ቫይረስ ስርጭት በፈቃደኛ ደም ለጋሾች ላይ ለማጥናት የተዘጋጀ መጠይቅ፡-

የሚስጢር ቁጥር .....

ቀን .....

1.ፆታ

ወንድ ☐ ሴት ☐

2. ዕድሜ _____

3. የጋብቻ ሁኔታ

ያላገባ ☐ያገባ ☐የተፋታ ☐ በሞት የተለየ ☐

4. የሥራ ዘርፍ

ተማሪ ☐ የግል መስሪያ ቤት ሠራተኛ ☐

የመንግሥት ሠራተኛ ☐ የግል ሥራ ☐

5.ከዚህ ቀደም ደም ከሌላ ሰው ወስደው ያቃሉ?

አውቃለሁ ☐ አላውቅም ☐

6. ከአንድ በላይ ኛደኛ ጋር የግብረ ሰጋ ግንኙነት ፈፅመው ያውቃሉ?

አውቃለሁ ☐አላውቅም ☐

7.ሳለታ ነገሮችና በጋራ ተጠቅመው ያውቃሉ?

አውቃለሁ ☐ አላውቅም ☐

8.የህክምና አገልግሎቶች (የጥርስ ማስነቀል የቀዶጥገና እና የመሳሰሉ) አድርገው ያውቃሉ?

አውቃለሁ ☐ አላውቅም ☐

9. ከዚህ ቀደም ደም ለሌላ ሰው ደም ስጥተዉ ያቃሉ?

አውቃለሁ ☐ አላውቅም ☐ ሰጥተዉ ከሆነ ስንት ግዚ _____

Annex VIII Declaration

I the undersigned agree to accept all responsibilities for the scientific and ethical conduct of thesis. I will provide timely progress report to my advisor and seek the necessary advice and approval from my primary advisors in the course of the research. I will communicate timely to my advisors and all stakeholders involved in the study.

Name of the principal investigator:

Misgana Petros (BSc)

Name _____Signature_____ Date

Name of the Advisors:

1. Dr. Wendatir Nigatu (PhD, Associate professor)
   Signature _____Date_____
2. Mr. Melese Hailu (MSc. PhD fellow)
   Signature _____Date_____
3. Dr. Mesfin Nigussie (MD., Pathologist)
   Signature _____Date _____