Addis Ababa University
School of Graduate Studies

Determination of the Magnitude of Hepatitis B Viral Infection in Healthcare Workers in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia

By

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Abbreviations and Acronyms

Abs:	Antibodies
Ags:	Antigens
Anti-HBcAg:	Antibody for Hepatitis B core Antigen
Anti-HBeAg:	Antibody for Hepatitis B e Antigen
Anti-HBsAg:	Antibody for Hepatitis B surface antigen
CDC:	Center for Disease prevention and Control
CI:	Confidence interval
DNA:	Deoxyribonucleic Acid
ELISA:	Enzyme Linked Immunosorbent Assay
EPI:	Expanded program for immunization
HBcAg:	Hepatitis B core Antigen
HBIG:	Hepatitis B immunoglobulin
HBsAg:	Hepatitis B surface antigen
HBV:	Hepatitis B virus
HCC:	Hepatocellular carcinoma
HCWs:	Health care workers
PEP:	Post Exposure Prophylaxis
PPE:	Personal Protective Equipment
Ups:	Universal precautions
NSI:	Needle sticks injury
RNA:	Ribonucleic Acid
SPs:	Standard Precautions
SOP:	Standard Operation Procedure
SPSS:	Statistical Package for the Social Sciences
Vs:	Versus
WHO:	World Health Organization

Abstract

Background: Hepatitis B virus infection is a serious global health problem, with 2 billion people infected worldwide, and 350 million suffering from chronic HBV infection. About 3 million healthcare workers face occupational exposure to bloodborne viruses each year in which about 2 million to hepatitis B virus infections. This study was conducted to determine the magnitude and associated risk factors of Hepatitis B viral infections in healthcare workers.

Objective of the Study: This study was initiated to determine the magnitude and associated risk factors of Hepatitis B viral infections in healthcare workers in St Paul Hospital Millennium Medical College Addis Ababa, Ethiopia.

Methods: Data were obtained from a cross-sectional study conducted in St Paul Hospital Millennium Medical College, among healthcare workers from November 2013 – May 2014. A convenient sampling method was utilized to get the required sample size. A structured questionnaire was used to capture individual socio-demographic characteristics and associated risk factors. Five ml blood was collected, centrifuged and the serum was analyzed for the serologic markers of HBsAg, anti-HBc and anti-HBs using Chemiluminescent Microparticle Immunoassay. Descriptive and logistic regression models were used for analysis.

Results: Among the 313 healthcare workers, the seroprevalence of current hepatitis B viral infection was 2.6%; while prevalence of life time exposure was 25.6%. Prevalence of needle stick and sharp injuries were 33.9% and 35.5% respectively. While, exposure to blood and body fluids were 57.2% and 44.4% respectively. Consistent use of gloves was reported by 49.8% of HCWs. Doctors practiced 71.4% of consistent use of glove, while laboratorians were the least likely to consistently use gloves (40.0%). Only 1.6% of HCWs had completed scheduled vaccination against HBV and 73.8% of HCWs were susceptible to infection. Exposure to blood (COR: 9.351, 95% CI: 1.164 – 75.095, $p < 0.012$), jaundiced and diagnosed liver disease (COR: 3.096, 95% CI: 1.051 – 9.120, $p < 0.032$), and HBV vaccination ($\chi 2 = 11.145$, $p < 0.002$), were independent risk factors that were potentially associated with hepatitis B viral infections.

Conclusions: The prevalence of current hepatitis B virus infection and life time exposure to hepatitis B viral infection among health care workers was high. Exposure to potentially infectious body fluids, needle stick and sharp injuries was also high. Whereas a small proportion of healthcare workers are vaccinated against hepatitis B virus infection. Besides the doctors, nurses and medical laboratory professionals; cleaners, porters and general service providers were also at a comparably high or more risk of HBV infection as they interact with patients and clinical wastes. Emphasis to continuous medical education and training on infection prevention and safety precautions, vaccination package to HCWs, compliance with universal precautions, access to safer injection technologies and post-exposure management are strongly recommended to improve safety of HCWs and quality of patient care.

Keywords: Hepatitis B Virus, HBsAg, anti-HBc, anti-HBs Seroprevalence, Vaccination, Risk Factors, HealthCare Workers.

Chapter 1: Introduction

1.1.General Introduction

Viral hepatitis type B is a common, serious disease caused by the hepatitis B virus (HBV), a partially double stranded DNA virus of the Hepadnaviridae family. HBV is one of the main causes of hepatic decompensation, cirrhosis and hepatocellular carcinoma (HCC). Acute disease usually occurs when the immune response is well preserved, while patients with an immunodeficiency are more likely to develop a chronic disease, then becoming a source for new infections [1, 2].

Hepatitis B virus occurs worldwide and constitutes a serious public health problem. Globally, more than 2 billion people have been infected with HBV at some time in their lives. Of these, about 350 million people remain infected chronically and become carriers of the virus, and 1.5 million deaths occur from HBV related liver diseases, including end stage cirrhosis and hepatocellular carcinoma each year. It was estimated that liver cancer represents approximately 4% of all new cancer cases diagnosed worldwide and that more than 50% of liver cancers were attributable to HBV [3-5].

The global prevalence of HBV infection varies widely; and its endemicity ranges from high (≥8%) to intermediate (2-7%) and low (<2%) [6]. Regions like South East Asia, Sub Saharan Africa, the Eastern Mediterranean countries, South and Western Pacific Islands, the Amazon basin and Certain parts of the Caribbean are high endemic areas for HBV. Regions with moderate prevalence includes; South Central and South West Asia, Eastern and Southern Europe, the Russian Federation and most of Central and South America. Regions with low prevalence include; Australia, New Zealand, Northern & Western Europe, and North America [7]. Ethiopia, being part of the Sub Saharan Africa region, is ranked as an area with high endemicity for HBV infection, based on the previous Sero-epidemiological population based study [8].

HBV is a DNA, partially double stranded (with single stranded regions), enveloped hepadnavirus. The virus often referred to as a Dane particle, carries hepatitis B core antigen (HBcAg), surface antigen (HBsAg), secreted protein antigen (HBeAg), and viral DNA. During infection, Dane particles are released in to the bloodstream together with small spherical and rod-like particles of envelope origin. The spherical and rod-like particles carry HBsAg and are non-

infective. Hepatitis B e antigen (HBeAg) is part of the core protein of Dane particles and its presence in the blood is associated with infectivity. It is secreted from infected liver cells during the acute stage of the disease and in some carriers, when there is active virus replication [3]. Hepatitis B virus is carried in blood and other body fluids, including saliva, tears, semen and vaginal secretions. Depending on the epidemiological pattern within a geographic area, the main routes of transmission are sexual intercourse, parenteral contact and perinatal infection of the baby from an infected mother [1, 3]. Hepatitis B virus is the most commonly transmitted bloodborne virus in the healthcare settings, via contaminated instruments or accidental needle stick or sharps injuries. Hepatitis B virus has been shown to survive in dried blood on surfaces at room temperature for at least a week. The risk of HBV infection among healthcare workers is 3 to 5 times higher than in the general population, particularly in surgeons, pathologists, physicians, laboratory technicians; housekeeping staff and nurses. Research findings have indicated that 10%–30% of health care workers show serologic evidence of past or present HBV infection [9, 10].

Worldwide annual proportion of health care workers (HCWs) exposed to HBV infection were about 5.9%. In developing countries, 40-60% of HBV infection in HCWs was attributed to professional hazard while in developed countries the attributed fraction was less than 10% due to vaccination coverage [11, 12]. Being the instruments of health care system, their interaction with patients is likely to pose unavoidable safety risks for the HCW's [13, 14]. The chances of contracting HBV after an HBV contaminated accidental needle stick average 1 in 20 [10, 15]. Hepatitis B is not only the most transmissible infection, but also is preventable by vaccination [16]. In developing countries, Hepatitis B vaccination coverage among healthcare workers is very low for various reasons, including awareness, risk assessment, and low priority given by the health managements of both government and private hospitals [17]. Occupational risks associated with exposure affects the quality of care delivered as well as health care workers safety and well being. As a result exposed workers experience significant fear, anxiety and emotional distress that can result in occupational and behavioral changes [18]. Considering the importance of health care workers and lack of any significant report in HCWs from different regions of Ethiopia, this study is initiated to determine the prevalence and associated risk factors of HBV infections in HCWs.

Chapter 2: Literature Review

2.1. Global Epidemiology and magnitude of Hepatitis B Viral Infections

Hepatitis B virus is classified by the World Health Organization (WHO) as the world's second greatest carcinogen after tobacco [19]. The prevalence of chronic HBV; defined as being HBsAg positive for more than 6 months is markedly different geographically throughout the world and ranges from 0.2% to 20% [20].

Of the 2 billion people or one-third of the world's population that has been infected with HBV, it is estimated that 350 million people are chronic carriers [19]. Hepatitis B virus infection is estimated to be the cause of 30% of cirrhosis and 53% of liver cancer in the world. Approximately 15-40% of patients with chronic HBV will develop cirrhosis, end-stage liver failure or hepatocellular carcinoma (HCC) in their lifetime [21]. Mathematical modeling for the year 2000 estimated the annual number of HBV-related deaths to be more than 600,000 worldwide, and most of the deaths (94%) were attributed to complications of chronic infection, such as cirrhosis and HCC, and only 6% were attributed directly to acute hepatitis B [22]. Hepatocellular carcinoma is the sixth most common cancer and the third most common cause of cancer death in the world [23]. Chronic HBV infection is the most common cause of HCC, accounting for 50% of HCC cases worldwide and up to 80% of cases in high HBV endemic regions [24, 25].

In the United States, the National Health and Nutrition Examination Study found the prevalence of past and present infection to be 4.8% from data collected between 1996 and 2006 [26]. In Canada, the prevalence of hepatitis B varies greatly depending on the population. The overall prevalence in the general population is about 0.5-1.0%; however, assessments based on distinct populations can range from as low as 0.1% in people born in Canada to 6.9% in the Inuit population and 7.4% in immigrants from highly endemic countries [27]. In Mexico, Central America and South America; Hepatitis B is considered to be highly prevalent, but there is variability among and within each country. The estimated prevalence ranges from 0.5-8.0%, with the total number of carriers approaching 11 million [28].

The prevalence of HBV in Europe is heterogeneous with rates ranging from < 0.1% to as high as 12% [29]. The Northern Europe; Scandinavian countries and the United Kingdom generally characterized by a low HBsAg carrier rate of < 0.1%, most Western European countries, where the carrier rate ranges between 0.1% and 0.5%, and Southern Europe; countries bordering the Mediterranean Sea and Eastern Europe where the carrier rates in some parts can be greater than 8% [25, 30].

The Eastern Mediterranean region extends from the countries of North Africa through the Middle East to Pakistan. The WHO estimates that the HBsAg prevalence in this region ranges from 1-10%, making it a region of intermediate to high endemicity [25]. In this region, study of village populations in Egypt, revealed an overall HBV prevalence rate of 11.7% and a higher prevalence rate of 20.8% in young adults between the ages of 14-18 years, similarly, a study in the obstetric and gynecological population in Pakistan found 4. 6% HBV prevalence in women of child bearing age [31]. The South East Asia region; Bangladesh, Bhutan, North Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand and Timor-Leste, it is considered to be of intermediate to high endemicity with prevalence rates ranging from 1-10% [25].

Figure 2.1: Magnitude of hepatitis B viral infection as shown in HBsAg prevalence in the world by country, adapted from the World Health Organization with courtesy.

The prevalence of HBsAg in the Western Pacific region; Australia, Brunei Darussalam, Cambodia, China, Cook Islands, Fiji, Japan, Kiribati, Lao People's Democratic Republic, Malaysia, Marshall Islands, Mongolia, Nauru, New Zealand, Niue, Palau, Papua New Guinea, Philippines, South Korea, Samoa, Singapore, Solomon Islands, Tonga, Tuvalu, Vanuatu and Vietnam ranges from < 1% to 30%. The prevalence is lowest, about 0.1%, in the non-Aboriginal populations of Australia and New Zealand. Rates are highest in the small South Pacific island nations, reaching up to 30% [32]. The Northern and Central Asian countries have HBsAg prevalence rate ranging between 7 and 12% [25].

Figure 2.2: The six regions of the World Health Organization, adapted from World Health Organization with courtesy.

The WHO Africa region covers all of Sub-Saharan Africa and Algeria, has the second largest number of chronic carriers after Asia and is considered a region of high endemicity with 70 and 95% of the adult population show evidence of past exposure to HBV infection and the estimated HBsAg seroprevalence ranges from 6-20% [25]. Western Africa has the highest rates of endemicity within Africa with as many as 95% of the adult population displaying markers of past HBV exposure. The prevalence rate in Gambia and Senegal are about 15%, with age specific prevalence as high as 20% in 10 – 20 year olds. Not surprisingly, this region also has one of the world's highest rates of HCC. In Gambia, HCC is the most common cancer among men and the second most common cancer among women [33]. In Ethiopia a community based and nationwide seroepidemiological studies showed an overall prevalence varies from 4.7 – 10.8% for HBsAg and 70-76.38% for at least one marker positive [8, 34-36].

2.2. The Magnitude of Hepatitis B Viral Infections in HCWs

The risks for occupational infection with HBV have been a source of concern among health professionals because of their frequent and often substantial exposures to patient blood and body fluids and all of which are associated with significant morbidity and mortality. The prevalence of HBV infection in HCWs, a high risk group for acquiring infection with blood borne pathogens due to occupational contact with infected body fluids, depends upon HBV prevalence in the general population. A review of studies on HBV done in the USA among HCWs found high prevalence rates of 13 to 18% in some categories of HCWs such as surgeons, and up to 27% prevalence rates have been noted among dentists and oral surgeons [37]. Another study in the USA to estimate the risk of HBV infections among hospital workers, in employees in five hospitals in different parts of the country; Georgetown, Washington, Denver, Colorado, Los Angeles, found 14% serologic markers of HBV infections [38].

The prevalence of HBV markers in Western Europe among nurses, dentists, midwives and physicians was estimated at 10% for northern countries, 20% for middle countries, and 40% for southern Countries. In Western Europe, it was estimated that 16,500 new HBV infections in HCWs occur each year, with 990 becoming chronic infections and 200 expected to die from liver cirrhosis and 40 from primary HCC [39]. A recent study in India, an intermediate endemic zone showed that the estimated prevalence rate of HBV in the healthy general population is around

4.7%, and a 5% HBsAg positivity in HCWs, but a highest seropositivity of around 40% among laboratory technicians [40].

A study from Taiwan, among HCWs who were exposed to high risk patients, showed 16% HBV seropositivity [41]. In a similar study in North West Turkey between 2002 and 2003, the occupational hazard of exposure to HBV was evaluated among 595 nurses; showed 18.7% exposure to HBV infection and 2.7% HBsAg positivity [42]. In a study done in Brazil, out of 474 dentists associated with the Regional Odontology Council, 10.8% were seropositive for HBsAg [43]. In Korea, a study was performed at Sanggye Paik Hospital in 2003, in which 571 HCWs; 56 physicians, 289 nurses, 113 technicians, and 113 aid nurses, between 21 and 74 years of age, were included. The positivity rate for HBsAg was 2.4% [44]. In another study in Japan, out of 141 dental workers, it was found that no worker was HBsAg positive. This indicated that vaccination of healthcare workers and adoption of universal precautions in developed countries pays its dividends [45].

In Sub-Saharan Africa, a study done in Uganda on the seroprevalence and risk factors for HBV infection among HCWs found a seroprevalence of HBV markers of 8.1% indicating current infection, and 48.1% had evidence of previous exposure to HBV [46]. In Ethiopia, a study done on 110 health care and 110 non health care workers, hepatitis B virus was detected in 8 (7.3%) and 1 (0.9%) of health care and non health care workers, respectively. Significant differences were observed in the detection rate of HBV in HCWs compared to non health care workers [47]. Hepatitis B serological markers; HBsAg, anti-HBc and anti-HBs were determined, and the overall prevalence rate was 9.02% for HBsAg, 46.25% for anti-HBs, 73.6% for anti-HBc and 76.38% for at least one marker positive by another study [48].

The fundamental principles and ethics of health care is that the sick persons must receive care. This premise carries an unstated consequence; an occupational risk to healthcare workers who respond to the needs of contagious patients. HBV presents an occupational risk of infections for all HCWs in the world. Determining the prevalence and associated risk factors is important to generate data that will help establish an intervention mechanism including vaccination package to HCWs, compliance with universal precautions (UPs), access to safer injection technologies and post exposure management, as these in turn will help in improving quality of patient care and services access.

2.3. Laboratory Diagnosis of HBV Infections

Diagnosis is based on clinical, laboratory, and epidemiologic findings. HBV infection cannot be differentiated on the basis of clinical symptoms alone, and definitive diagnosis depends on the results of serologic testing. Serologic markers of HBV infection vary depending on whether the infection is acute or chronic.

HBsAg (hepatitis B surface antigen) is the most commonly used test for diagnosing acute HBV infections or detecting carriers. HBsAg can be detected as early as 1 or 2 weeks and as late as 11 or 12weeks after exposure to HBV when sensitive assays are used. The presence of HBsAg indicates that a person is infectious, regardless of whether the infection is acute or chronic. Anti-HBc (core antibody) develops in all HBV infections, appears shortly after HBsAg in acute disease, and indicates HBV infection at some undefined time in the past. Anti-HBc only occurs after HBV infection and does not develop in persons whose immunity to HBV is from vaccine. Anti-HBc generally persists for life and is not a serologic marker for acute infection. IgM anti-HBc appears in persons with acute disease about the time of illness onset and indicates recent infection with HBV. IgM anti-HBc is generally detectable 4 to 6 months after onset of illness and is the best serologic marker of acute HBV infection. A negative test for IgM-anti-HBc together with a positive test for HBsAg in a single blood sample identifies a chronic HBV infection. HBeAg is a useful marker associated strongly with the number of infective HBV particles in the serum and a higher risk of infectivity. Anti-HBs (surface antibody) is a protective, neutralizing antibody. The presence of anti-HBs following acute HBV infection generally indicates recovery and immunity against re-infection. Anti-HBs can also be acquired as an immune response to hepatitis B vaccine or passively transferred by administration of hepatitis B immune globulin (HBIG). When using radioimmunoassay (RIA), a minimum of 10 sample ratio units should be used to designate immunity. The level of anti-HBs may also be expressed in milli-international units/mL (mIU/mL). 10 mIU per mL is considered to indicate a protective level of immunity [3], [49].

2.4.Relevance of the Study

Hepatitis B virus infection is a serious global health problem, with 2 billion people infected worldwide, and 350 million suffering from chronic HBV infection. About 35 million healthcare workers face occupational exposure to bloodborne viruses each year in which about 2 million to hepatitis B virus infections. The infection may be transmitted through sexual intercourse, parenteral contact or from an infected mother to the baby at birth and, if contracted early in life, may lead to chronic liver disease, including cirrhosis and hepatocellular carcinoma [3-5].

The fundamental principles and ethics of health care is that the sick persons must receive care. This premise carries an unstated consequence; an occupational risk to healthcare workers who respond to the needs of contagious patients. HBV presents an occupational risk of infections for all HCWs in the world [19, 46]. The risks for occupational infection have been a source of concern among healthcare because of their frequent and often substantial exposures to patient blood and body fluids. In developed countries, these occupational exposures are routinely reported to their Centers of Diseases Control, but in Ethiopia, such surveillance system is yet very rudimentary, and calls for a need to receive a great attention.

Determining the prevalence and associated risk factors is important to generate data that will help establish an intervention mechanism including vaccination package to HCWs, compliance with universal precautions, access to safer injection technologies and post-exposure management, as these in turn would help in improving safety of HCWs, quality of patient care and access to services. It is also of paramount importance to facilities in establishing surveillance system for registering, reporting and managing of occupational exposures. Besides providing immunity against HBV infection, vaccines indirectly also protect against hepatocarcinoma. Considering the risk of liver cirrhosis, hepatocellular carcinoma and transmission of HBV to patients, there is a need to focus efforts on mitigating transmission through improving the work place environment and making use of the available vaccine to all health care workers who are susceptible to infection. And this shall follow after accurate determination of the magnitude of current rate of infection, successful piloting and screening.

Chapter 3: Objective of the Study

3.1. General Objective

- To determine the magnitude and associated risk factors of Hepatitis B viral infections in healthcare workers.

3.2. Specific Objectives

- To estimate the magnitude of Hepatitis B viral infections in healthcare workers.
- To identify associated risk factors related to Hepatitis B Viral infections in healthcare workers.
- To assess vaccine status against Hepatitis B virus in healthcare workers.

3.3. Research Hypotheses

- The following hypothesis was investigated in this study: The emphasis given concerning recommended precautions for infection prevention, safety and prevention of occupational exposure, the magnitude of Hepatitis B viral infections in Healthcare Workers will follow from that of the general Population.

Chapter 4: Methodology

4.1. Study Area and Study Period

The study was conducted from November 2013 – May 2014 in the capital city of Ethiopia, Addis Ababa. Ethiopia is located in the Eastern part of Africa. The land area is estimated to be about 1.1 million square kilometers. The country is among the three most populous countries in Africa with a total population of 79,221,000. Of whom 65,996,000 are rural and 39,691,000 are males. Addis Ababa has a total population of 3,147,000 [50]. This study was specifically conducted in St Paul Hospital Millennium Medical College, one of Ethiopia's largest specialized federal referral and teaching hospital. At the time of the study, the hospital had about 145 doctors (including specialists), 141 paramedical officers (clinical officers, medical laboratory technologists, pharmacists, radiographers), 419 nurses (including midwives) and 781 general service providers (including cleaner, patient porters) and administrative staffs (Oral communication with HR).

St Paul Hospital Millennium Medical College (SPHMMC) is the second largest public hospital in Ethiopia with the mission to provide preventive, promotive, curative, and rehabilitative health care service in the country. The Hospital was built in 1969 and since then it has been a source of medical care for underserved populations. The hospital currently has more than 300 beds and provides service with an annual average of 200,000 patients through its emergency, outpatient and inpatient services. The hospital has over 1486 clinical and nonclinical staffs and 13 departments including diagnostic laboratory and most recently launching its new Hemodialysis and renal replacement units.

4.2. Study Design

A prospective hospital based cross-sectional study was conducted using a semi-structured questionnaire for sociodemographic and associated risk factor assessment. Venous blood samples were collected and sera were tested for hepatitis B viral infections using immunoassay tests. Data was analyzed using SPSS version 20.0. Chi-square test was used for bivariate analysis of the association between categorical variables with main outcomes. Multivariate logistic regression model has been computed to assess exposure to risk factors by job category, age, sex, practice area, vaccination status and service year as dependent confounding risk factors, and P-value < 0.05 was considered statistically significant.

4.3. Study Population

4.3.1. Source Population

The source population includes all healthcare providers working in St Paul Hospital Millennium Medical College in Addis Ababa, Ethiopia.

4.3.2. Study Population

The study population was all healthcare workers who come into contact with patients, or are potentially exposed to blood and body fluids from patients while attending to or handling samples from patients and handling clinical wastes. These healthcare workers include doctors, nurses, medical laboratory professionals, radiographers and general service providers. Only those who were present at the time of sample collection were recruited.

4.4. Eligibility; Inclusion and Exclusion Criteria

4.4.1. Inclusion Criteria: In this study Doctors, nurses, midwifery nurses, laboratory technicians, cleaners, patient porter and other general service providers, particularly in the departments of surgery, gynecology, medical laboratory, medical and emergency wards were included in this study. Healthcare providers working for at least six months and above have been included in the study.

4.4.2. Exclusion Criteria: Health workers who were not available during the period of sample collection, those who do not have frequent exposure for blood and other body fluids due to the nature of their working environment and who were not voluntary to participate were excluded from the study. Healthcare providers working less than six months, visitors and personnel working only in the administrative positions were also excluded from the study.

4.5. Study Variables:

- **Dependent Variables:** Seroepidemiology of Hepatitis B viral infections in healthcare workers.
- **Independent Variables:** Sociodemographic variables (Sex, Age, marital status), vaccination status for hepatitis B virus, years of services, job category, having training on safety and infection prevention, history of operation, history of jaundiced and diagnosed liver disease, history of tattooing, history of needle sticks and sharp injuries, history of blood or body fluid splash and blood transfusions.

4.6. Sample Size Determination

The healthcare facility was first selected based on convenience from teaching, and staff size from other hospitals in Addis Ababa. Based on different professional categories, healthcare workers (doctors, nurses including midwifery nurses, laboratorians, pharmacists, porters and cleaners) working in their respective practice areas during the study period were approached conveniently to participate in the study and a total of 313 subjects were involved.

4.7. Sample Collection Procedures

A structured questionnaire was used to capture individual sociodemographic characteristics (age, sex, marital status), and associated risk factors (history of exposure to patient blood and body fluids, department of practice, history of operation, history of blood transfusion, duration in service, use of protective wear, vaccination status to Hepatitis B virus, trainings on safety and infection prevention). About 5ml of blood were collected, from all study subjects, and serum was separated from all samples and stored at -20^{oc} waiting for analysis.

4.8. Sample Processing and Laboratory Diagnosis

The serologic markers; hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (anti-HBs) and hepatitis B core antibody (anti-HBc) were analyzed using Chemiluminescent Microparticle immunoassay (CMIA) at International Clinical Laboratories, a Joint Commission International Accredited laboratory in Addis Ababa, Ethiopia. The Presence of HBsAg in blood signifies acute or chronic persistent hepatitis B viral infections. Anti-HBs are produced in response to HBsAg and confer immunity to re-infection and their presence indicates immunity to hepatitis B viral infections following an infection or successful immunization with hepatitis B vaccine. Anti-HBc is directed against the core antigen following a natural infection and normally persists for life. Its presence may indicate a current or past resolved infection. Sample collection and questionnaire administration were done by laboratorians. The left over samples and all material used in sample collection has been disposed according to the Hospital's and the Laboratory's waste disposal and management system.

4.8.1. ARCHITECT HBsAg: The ARCHITECT HBsAg assay is a chemiluminescent microparticle immunoassay (CMIA) which uses microparticles coated with monoclonal anti-HBs for the qualitative determination of hepatitis B surface antigen (HBsAg) in human serum and

plasma. HBsAg assays are routinely used to aid in the diagnosis of suspected hepatitis B viral (HBV) infection and to monitor the status of infected individuals, whether the patient's infection has resolved or the patient has become a chronic carrier of the virus. The ultimate goal of HBV therapy is the maximum reduction or loss of HBsAg with but not necessarily including seroconversion to anti-HBs. Prolonged suppression of HBV DNA has been shown to decrease the risk of the development of cirrhosis and hepatocellular carcinoma. Quantitation of HBV has a growing clinical utility in the monitoring of therapy in the case of chronic Hepatitis B. Therapy in these cases may include treatment by pegylated interferon or with nucleo (s) tide analogues. Studies have suggested the use of HBsAg as a biomarker for the prognosis and response to therapy in cases of chronic Hepatitis B. It has been shown that HBsAg titers can correlate with Serum HBV DNA and intrahepatic cccDNA levels, with some variation in the different disease phases. Quantitation of serum HBsAg may also be utilized to distinguish between different phases of chronic Hepatitis B infection and serum HBsAg may act as a marker for the identification of inactive carriers.

The ARCHITECT HBsAg assay is a two-step immunoassay, using chemiluminescent microparticle immunoassay (CMIA) technology, with flexible assay protocols referred to as Chemiflex, for the quantitative determination of HBsAg in human serum and plasma. In the first step, sample and anti-HBs coated paramagnetic microparticles are combined. HBsAg present in the sample binds to the anti-HBs coated microparticles. After washing, acridinium-labeled anti-HBs conjugate is added in the second step. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT *i** System optics. The concentration of hepatitis B surface antigen in the specimen is determined using a previously generated ARCHITECT HBsAg calibration curve. If the concentration of the specimen is greater than or equal to 0.05 IU per ml, the specimen is considered reactive for HBsAg (Manufacturer insert paper).

4.8.2. ARCHITECT Anti-HBc II: The ARCHITECT Anti-HBc II assay is a chemiluminescent Microparticle immunoassay (CMIA) for qualitative detection of antibody to hepatitis B core antigen (anti-HBc) in human serum and plasma. The ARCHITECT Anti-HBc II assay is

intended to be used as a screen for blood and plasma to prevent transmission of hepatitis B virus (HBV) to recipients of blood and blood components and as an aid in the diagnosis of HBV infection. The ARCHITECT Ant-HBc II assay utilizes Microparticles coated with recombinant hepatitis B virus core antigen (rHBcAg) for the detection of anti-HBc. Anti-HBc determinations can be used as an indicator of current or past HBV infection. Anti-HBc is found in serum shortly after the appearance of hepatitis B surface antigen (HBsAg) in acute HBV infections. It will persist after the disappearance of HBsAg and before the appearance of detectable antibody to HBsAg (anti-HBs). In the absence of information about any other HBV markers, it must be considered that an individual with detectable levels of anti-HBc may be actively infected with HBV or that the infection may have resolved, leaving the person immune. Anti-HBc may be the only serological marker of HBV infection and potentially infectious blood. The presence of anti-HBc does not differentiate between acute or chronic hepatitis B infections.

The ARCHITECT Anti-HBc II 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 diluents, specimen diluents, and rHBcAg coated paramagnetic micro particles 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 chemiluminescent 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 (Manufacturer insert paper).

4.8.3. ARCHITECT Anti-HBs: The ARCHITECT Anti-HBs assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of antibody to Hepatitis B surface antigen (anti-HBs) in human serum and plasma. The ARCHITECT Anti-HBs assay determines the concentration of antibody to Hepatitis B surface antigen (anti-HBs) present in

human serum and plasma. Anti-HBs assays are often used to monitor the success of Hepatitis B vaccination. The presence of anti-HBs has been shown to be important in protection against Hepatitis B virus (HBV) infection. Numerous studies have demonstrated the effectiveness of the Hepatitis B vaccine to stimulate the immune system to produce anti-HBs and to prevent HBV infection. Assays for anti-HBs are also used to monitor the convalescence and recovery of Hepatitis B infected individuals. The presence of anti-HBs after acute HBV infection and loss of Hepatitis B virus surface antigen (HBsAg) can be a useful indicator of disease resolution. Detection of anti-HBs in an asymptomatic individual may indicate previous exposure to HBV. Samples with anti-HBs concentration less than 10.0 mIU per mL are considered nonreactive by the ARCHITECT Anti-HBs assay.

The ARCHITECT Anti-HBs assay is a two-step immunoassay, using chemiluminescent microparticle immunoassay (CMIA) technology, for the quantitative determination of anti-HBs in human serum and plasma. In the first step, sample and recombinant HBsAg (rHBsAg) coated paramagnetic microparticles are combined. Anti-HBs present in the sample binds to the rHBsAg coated microparticles. After washing, acridinium-labeled rHBsAg conjugate is added in the second step. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti-HBs in the sample and the RLUs detected by the ARCHITECT *i** System optics. The concentration of anti-HBs in the specimen is determined using a previously generated ARCHITECT Anti-HBs calibration curve. If the concentration of the specimen is greater than or equal to 10.0 mIU per mL the specimen is considered reactive for anti-HBs (Manufacturer insert paper).

4.9. Data quality Management

The questionnaire were pretested in HCWs who were not included in the study to identify the clarity of their sensitiveness as well as gap on risk factors of hepatitis B viral infections, and based on the result of the pre-test correction was made properly. All the data were checked for completeness, accuracy and consistency by the principal investigator immediately during sample collection and administration of questionnaire. Sample collectors were trained; in addition, there was a daily follow up by the principal investigator. Standard operating procedure (SOPs) were followed properly during both sample collection and laboratory analysis, and calibrators, positive and negative controls for hepatitis B virus were used.

4.10. Data Processing and Analysis

Quantitative data were coded and entered into computer and analyzed by SPSS 20.0 V soft-wares after cleaning. In the analysis process, frequency distributions of variables were worked out in order to describe in relation with the study population. The association between dependent and independent variables was measured by means of odds ratio for which 95% confidence interval has been calculated. Variable that showed a statistically significant association ($p<0.05$) were analyzed at multivariate level.

4.11. Operational Definitions

The following definitions were used in this study. A citation follows definitions that were taken from other works. Definitions which are not cited were developed for the purpose of this study.

Acute Hepatitis: A patient is said to have acute infection of hepatitis B virus if both HBsAg and anti-HBc antibody IgM is detected in patients' blood. Usually a self-limiting disease marked by inflammation in the liver in association with a transient hepatitis B viral infections.

Chronic HBV Infection: An individual is considered chronically infected if HBsAg is present for more than six months. Three markers are used to determine the stage of chronic infection: HBsAg, HBeAg, and anti-HBc total, with persistent hepatitis B viral infection accompanied by ongoing liver injury and resulting risk of cirrhosis and HCC.

HealthCare Workers (HCWs): persons who work in a healthcare setting in direct contact with patients, blood and body fluids, including clinical and health sciences students, cleaners and patient supporting workers-porters.

Hepatitis: An inflammation of the liver that tends to cause a severe acute infection and may progress to chronic infection and permanent liver damage.

Needle stick: Injury, penetration of the skin by needle or other sharp object resulting in a cut or other entry.

Standard Precautions: Mandatory precautions which apply to blood, all body fluids except sweat, non-intact skin, and mucous membranes and are to be used in the care of all hospitalized patients, regardless of diagnosis or infection status.

Universal Precautions: Common terminology for the desired protective behaviors for prevention of transmission of infectious diseases, based on the modification of isolation

precautions to include universal application to all patients, regardless of diagnosis or infection status.

Risk Factor Definition: The studied risk consists in a percutaneous exposure of a HCW with at least one sharp object contaminated with HBV, during the past year in their work environment. It was assumed that the contamination of the sharp object occurred through previous contact with blood or body fluids of another person.

4.12. Ethical Considerations

Ethical approval was prescribed from the ethical review committee of the Department of Clinical Laboratory Sciences, School of Allied Health Sciences, College of Health Science, Addis Ababa University. And the study was conducted in St Paul Hospital Millennium Medical College after permission was obtained from the office of the academic vice provost following IRB approval. Names and any other sensitive personal information of individual study subjects were not recorded during sample collection. Moreover the sample collectors were professionals working in the laboratory department of the hospital and were mentored daily by senior medical laboratory technologists and the principal investigator. Samples were collected after getting consent from the study healthcare workers.

4.13. Result Dissemination

The findings of this study will be distributed to the participating health institutions, federal ministry of health, funding organization and different stakeholders through the appropriate channel. And the finding will also be published in local or international journal.

Chapter 5: Results

5.1. Socio-demographic characteristics of the study subjects

A total of 313 healthcare workers were investigated for Hepatitis B viral infections from November 2013 to May 2014 in St Paul Hospital Millennium Medical College Addis Ababa, Ethiopia. Females account for 67.4 % (211/313) and males account the remaining 32.6 % (102/313) of the study subjects, resulting in female to male ratio of 2:1. The mean and median age of healthcare worker was 30.99 ± 8.766 and 28.0 ± 8.766 years respectively. The age range of the studied subjects was 40.5 years (21-60 years). The majority of healthcare worker 32.6 % (102/ 313) were between the ages of 21 - 25years while health care workers with age of 51 years and above accounts the least group 4.1 % (13/ 313). Of the 313 healthcare workers, 53.7 % (168/ 313) were single. The sex, age, and marital status distributions of all the study subjects are shown in Table 5.1.

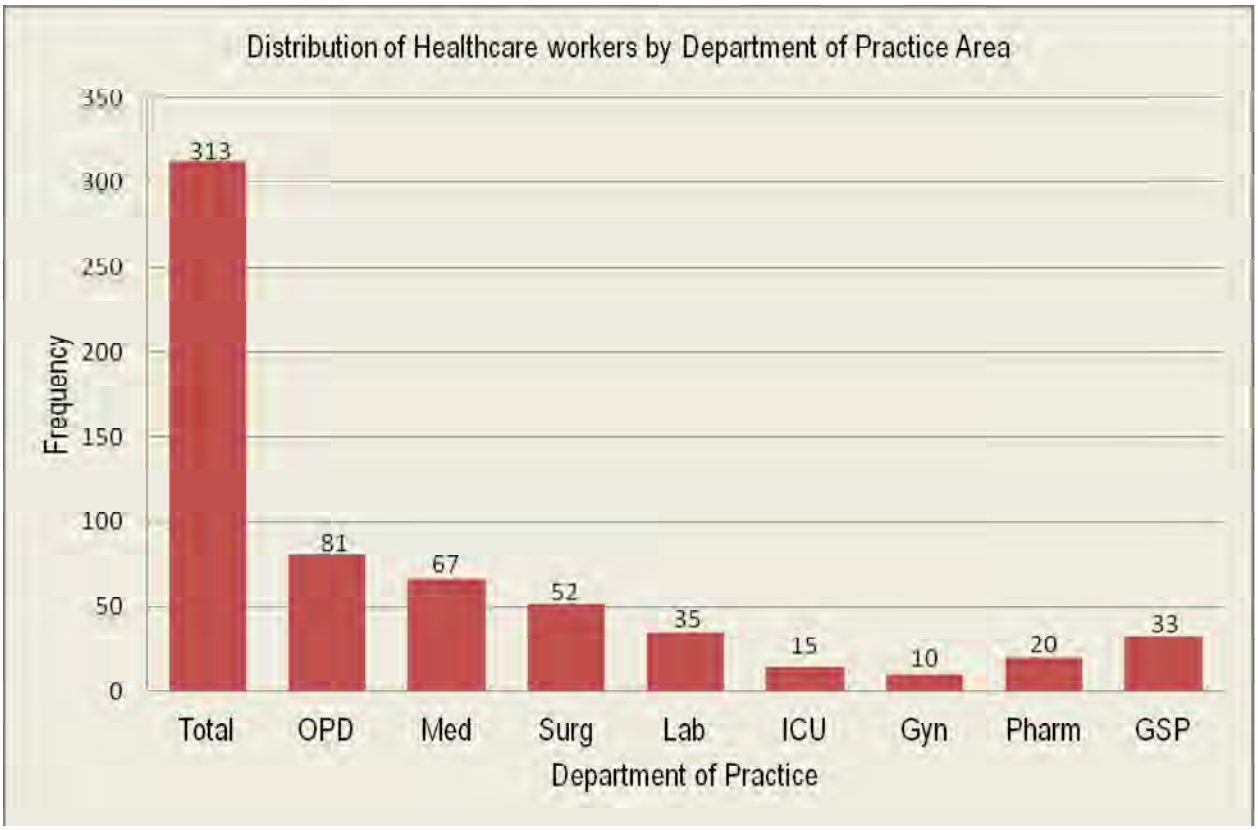
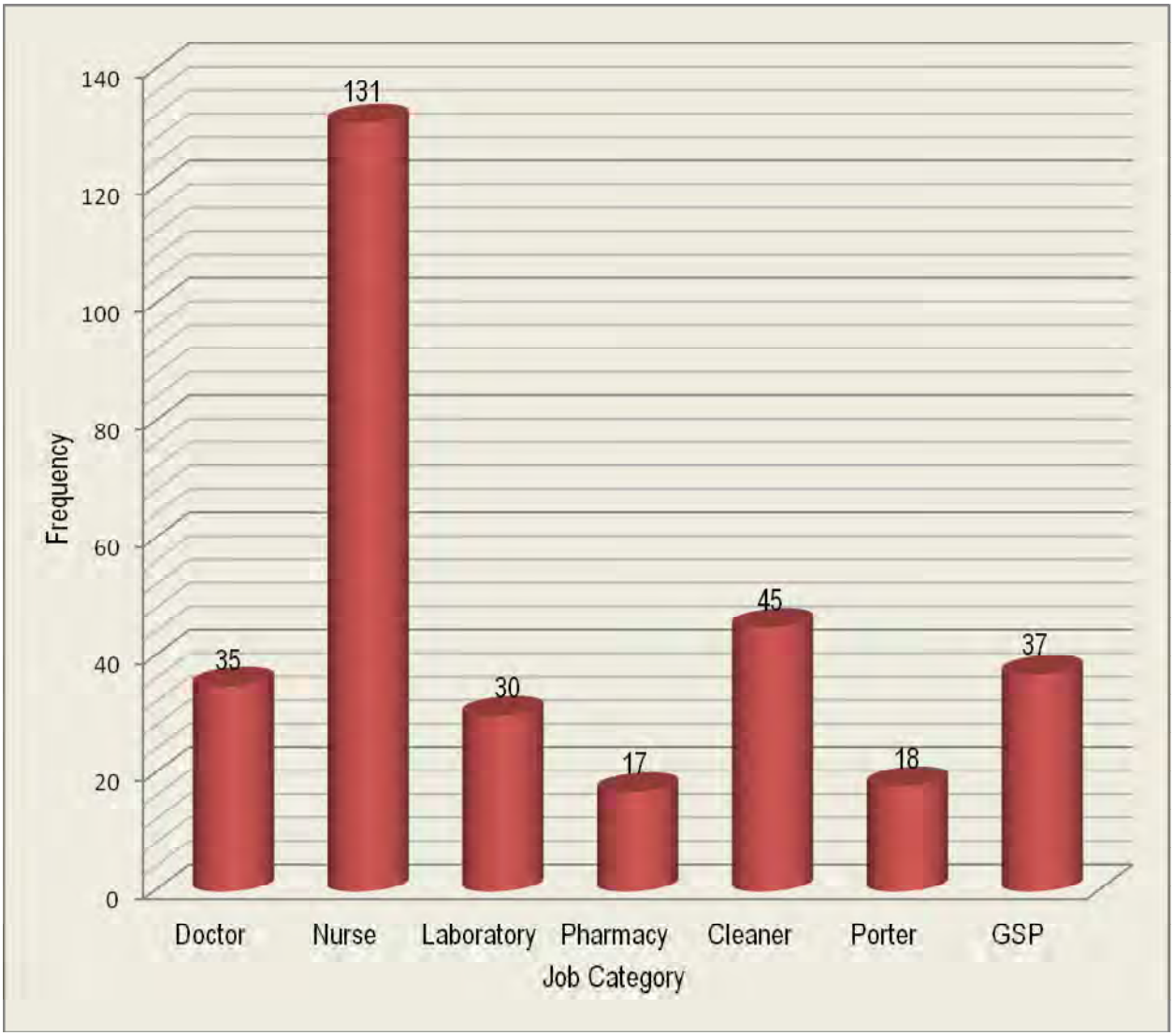


Figure-5.1. Frequency distribution healthcare workers by department of practice at St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 – May 2014.

Table-5.1. Socio-demographic characteristics of healthcare workers at St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 - May 2014.

Variables	Number	Percent
Sex Distribution		
Female	211	67.4
Male	102	32.6
Age Group		
21 – 25	102	32.6
26 – 30	95	30.3
31 – 35	47	15.0
36 – 40	28	8.9
41 – 45	9	2.9
46 – 50	19	6.1
>=51	13	4.2
Marital Status		
Single	168	53.7
Married	141	45.0
Divorced	3	1.0
Widowed	1	0.3

The professional distributions of the 313 HCW's are summarized in figure 5.2. Nurses and cleaners accounted the highest proportion, 41.9 % (131/ 313) and 14.4 % (45/ 313) respectively. Pharmacists and porters had the lowest proportion 5.4 % (17/313) and 5.8 % (18/3130) respectively.



**GSP: General Service Provider which includes: Drivers, Data Recorders, Accountants, Cashiers, Guards, Mourn Workers, Laundry Workers and Food Preparation Workers.*

Figure-5.2. Frequency distribution by job category of healthcare workers at St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 - May 2014.

The duration of service varied between less than one year (6 – 12 months) and greater than 35 years. Majority of the HCWs 88(28.2%) had duration of service from 2 – 5 years followed by 74(23.7%) had duration of service from 5 – 10 years. The remaining 54(13.3%) had duration of service from 1 – 2 year(s) and greater than 10 years. 42(13.5%), had duration of service less than one year (6 – 12 months), (Figure 5.3).

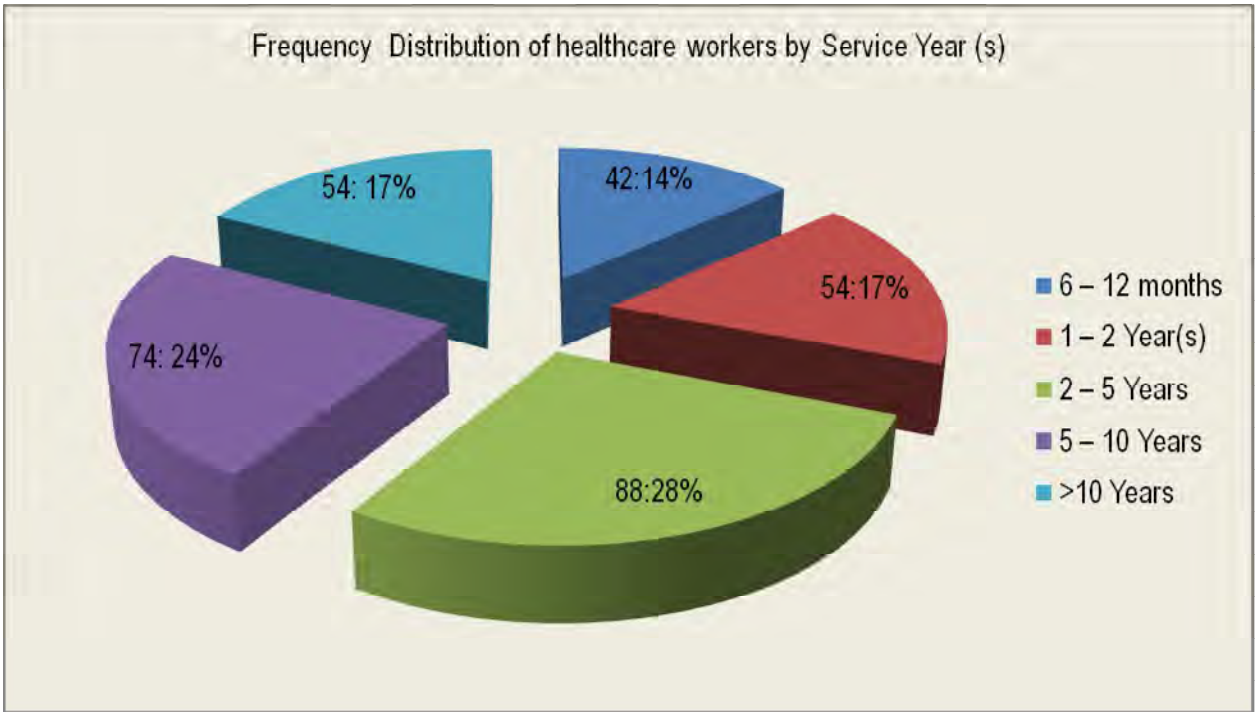


Figure-5.3. Frequency distribution by service year(s) of healthcare workers at St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 - May 2014.

5.2. Potential for Exposure to Risk Factors among Healthcare Workers

From all the 313 healthcare workers, 179 (57.2 %) had history of occupational exposure to blood and blood products with ungloved practices , 139 (44.4%) had history of occupational exposure to body fluids and 119 (38%) had history of blood or body fluids splash to exposed face and 95(30.4%) were exposed within the last six months. Among all the 313 healthcare workers 156 (49.8%) used glove consistently, meaning use of glove each time they carried out a procedure involving biologically infectious materials as a means of prevention from risk. In this study the nurses, porters and the laboratorian categories were the least likely to consistently use gloves accounting 48.9 %, 44.4 % and 40 % respectively, while 71.4 % of doctors reported consistent use of gloves. Of the total of 313 studied subjects 106 (33.9 %), 111(35.5%), 165(52.7%), 47(15%), 15(4.8%), 14(4.5%), 21(6.7%) had history of exposure to needle sticks injuries, sharp injuries, taken care of known hepatitis patients, operations, blood transfusion, jaundiced or diagnosed liver disease and tattoo respectively. Frequency of occupational exposure to risk factors in healthcare workers is summarized in Table 5.2.

Table-5.2. Frequency and history of Exposure to Risk Factors among the 313 HCWs in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 - May 2014.

Type (Exposure)	Total N (%)	Prevalence of Exposure by Professional Category						
		Doct N=35	Nurse N=131	Lab N=30	Phar N=17	Cleaner N=45	Porter N=18	GSP N=37
Needle Stick Injury	106(33.9)	12	69	5	2	10	2	6
Sharp Injury	111(35.5)	11	47	5	6	20	5	17
Blood and BP*	179(57.2)	27	110	11	2	15	9	5
Body Fluid	139(44.4)	26	84	7	0	13	6	3
Liver Disease	14(4.5)	2	7	0	1	1	0	3
Operation	47(15.0)	8	16	3	3	5	2	10
Tattooing	21(6.7)	0	8	2	2	3	4	2
Blood Trans	15(4.8)	1	8	1	0	0	1	4
Caring Hepatitis Pt*	165(52.7)	31	104	7	1	10	5	7

Blood and BP = blood and blood products, Caring Hepatitis Pt* = Caring of Hepatitis patients, Doct = Doctors, Lab=Laboratory, Phar = Pharmacy, GSP = General Service Provider.*

Of the 313 HCW's, 102(32.6 %), and 64(20.4 %) had history of very frequent exposure to blood and blood products, and at least three times frequencies of exposure to body fluids respectively. From all the studied subjects, 12.8 % (40/313) of healthcare workers had at least one time exposure to needle stick injuries, and 11.8 % (37 /313) had history of repeated exposure to sharp injuries. Although continuing medical education and in-service training is becoming mandatory for all healthcare workers, training in safety precautions and infection prevention control were

reported by 25.2 % (79/313) of the studied subjects. Of all the study subjects, 95(30.4%) had at least one health professional family member (defined to father / mother, sister / brother or husband / wife), being the majority 55 (17.6) were accounted by either sister or brother of the study subjects.

Exposure to potentially infectious body fluids at the work place was captured using a set of variables. Among all professional categories, nurses suffered from exposure to blood and blood products 83. 9 % (110 / 313) and needle stick injuries 52.7 % (69 / 313), while doctors mainly suffered from exposure to body fluids 74.2 % (26 / 313). The least exposure observations were accounted in pharmacists, 2(11.2%), 2(11.2%), 0(0%) from needle sick injuries, blood and blood products and exposure to body fluids respectively.

5.3. The Magnitude of Hepatitis B Infections among Health care workers

The diagnosis of Hepatitis B viral infections was determined by detecting either anti-HBc antibody or HBsAg marker. The presence of either of these markers was taken as a measure of infections. The presence of anti-HBs marker with and without anti-HBc indicates immunity through natural infection and or following complete scheduled immunization respectively. From all studied healthcare workers, the overall prevalence rate was 2.6 % (8 / 313) for HBsAg ,25.6 % (80 / 313) for anti-HBc,26.2 % (82 / 313) for anti-HBs, and 28.8 % (90 / 313) for at least one marker positive, (Table 5.3).

Table-5.3. Hepatitis B viral markers among healthcare workers in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 – May 2014 (n= 313).

Viral Markers	Negative	Positive	Prevalence
HBsAg*	305	8	2.6%
Anti-HBs*	231	82	26.2%
Anti-HBc*	233	80	25.6%
At least one Marker	223	90	28.8%

**HBsAg = Hepatitis B surface antigen, *Anti-HBs = Antibody to Hepatitis B surface antigen, *Anti-HBc = Antibody to Hepatitis B core antigen.*

There is no indeterminate results, meaning positive anti-HBc and negative for both HBsAg and anti-HBs markers found in this study.

The prevalence rate of hepatitis B viral infections was higher in males (3.9%) than females (1.9 %) with HBsAg marker and the same higher prevalence in males (30.4%) than females (23.2%) was identified with anti-HBc marker. However, the difference is not statistically significant ($p > 0.05$). The prevalence of hepatitis B viral infections was highest in the age group of 41 – 45 years (11.1%) followed by 31 – 35 (4.26 %) with HBsAg, (Table 5.4).

Table-5.4. Prevalence of hepatitis B virus by age of healthcare workers in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 – May 2014.

Age Group (in Years)	Marker Prevalence			
	HBsAg (+)		Anti-HBc (+)	
	Number	Percent (%)	Number	Percent (%)
21 – 25	2(102)	1.96	21(102)	20.6
26 – 30	3(95)	3.16	25(95)	26.3
31 – 35	2(47)	4.26	14(47)	29.8
36 – 40	0(28)	0	8(28)	28.6
41 - 45	1(9)	11.1	2(9)	22.2
46 – 50	0(19)	0	6(19)	31.6
>50	0(13)	0	4(13)	30.8
Total	8(313)	2.6	80(313)	25.6

The magnitude of hepatitis B viral infections in healthcare workers was highest among the general service providers (8.1%), followed by patient porters (5.6 %), cleaners (4.4%), laboratorians (3.3%), and nurses (0.8%) by HBsAg marker. While the magnitude of life time exposure to hepatitis B viral infections as evidenced by anti-HBc marker were highest among cleaners (35.6%), followed by patient porters (33.3%), and general service providers (27.0%), (Table 5.5).

Table-5.5. The Magnitude of Hepatitis B viral markers with job categories of healthcare workers in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 – May 2014.

Job Category	HBsAg (+) %N	Anti-HBS (+) %N	Anti-HBc (+) %N
Doctors	0.0 (0/35)	28.6(10/35)	25.7(9/35)
Nurses	0.8(1/131)	26.7(35/131)	23.7(31/131)
Laboratory	3.3(1/30)	26.7(8/30)	20.0(6/30)
Pharmacy	0.0(0/17)	11.8(2/17)	11.8(2/17)
Cleaners	4.4(2/45)	31.1(14/45)	35.6(16/45)
Porters	5.6(1/18)	27.8(5/18)	33.3(6/18)
GSP	8.1(3/37)	21.6(8/37)	27.0(10/37)
Total	2.6(8/313)	26.2(82/313)	25.6(80/313)

HBsAg=Hepatitis B surface antigen, Anti-HBs=Antibody to Hepatitis B surface antigen, Anti-HBc= Antibody to Hepatitis B core antigen, GSP = General Service Provider: includes Data Recorders, Ambulance Drivers, Laundry and Mourn Workers.

As to the correlation of hepatitis B viral infection between department of practice, healthcare workers practicing in gynecology had higher prevalence and life time exposure to hepatitis B viral infections 40.0% (4/10) followed by general service providers 30.3%(10/33), surgery 28.8%(15/52), internal medicine 26.9 %(18/67), out-patient department 25.9% (21/81), ICU 20.0% (3/15) and laboratory 17.1% (6/35), but the difference is not statistically significant ($p > 0.05$), (Figure 5.4).

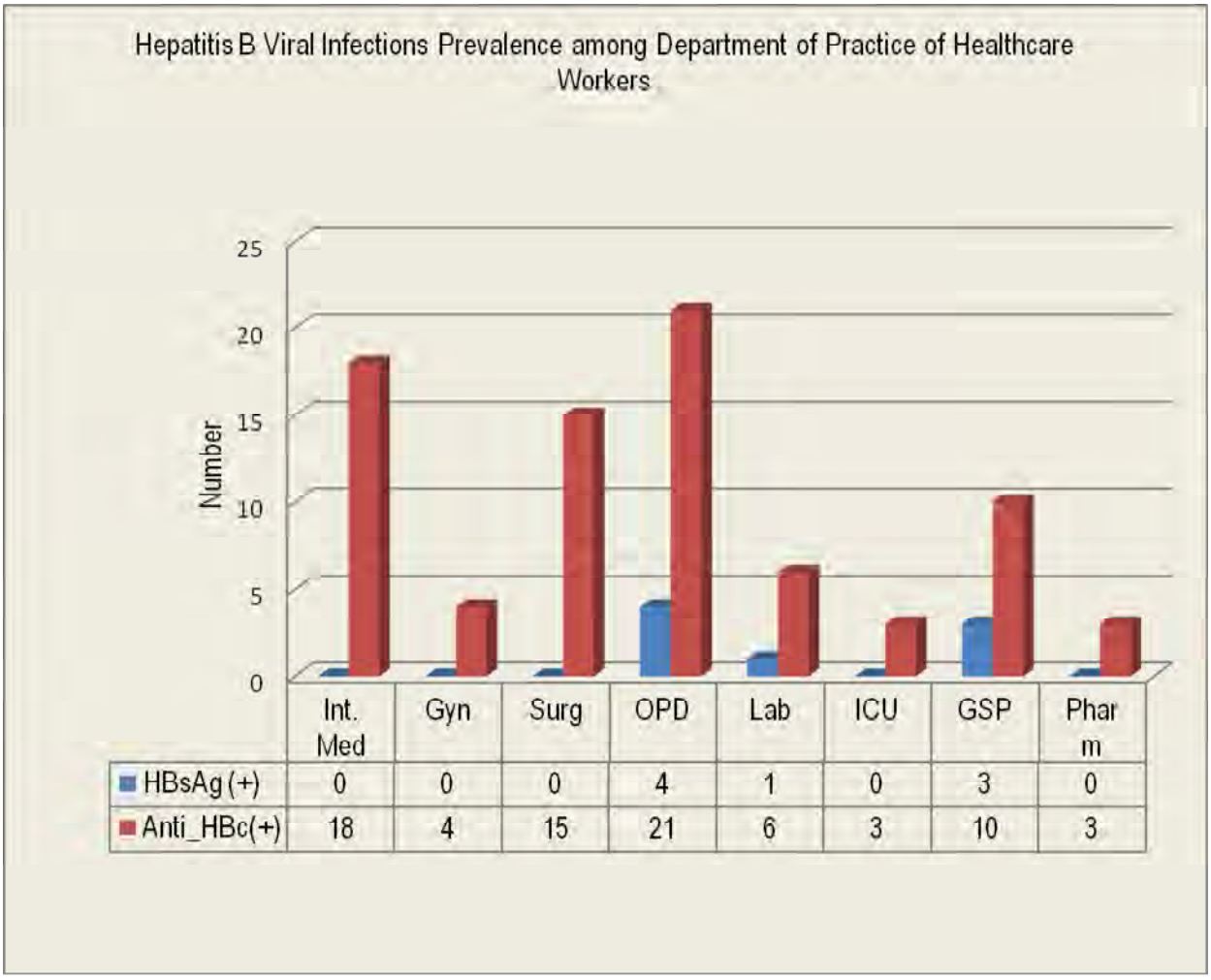


Figure-5.4. The Magnitude of Hepatitis B viral markers among department of practice of healthcare workers in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 – May 2014.

With regard to the sociodemographic data, although married healthcare workers had higher prevalence and life time exposure to hepatitis B viral infections (32.6%) than the unmarried counter parts (19.6 %), neither sex (P = 0.241), nor age (P = 0.538) and marital status (P = 0.065) were found responsible for any statistically significant differences in between hepatitis B viral negative and hepatitis B viral positive healthcare workers. However, a strong correlation was found with, exposure to blood and blood products (P = 0.012), jaundiced and diagnosed liver disease (P = 0.032), exposure to body fluids (P = 0.016), exposure within the last six months (P = 0.048), and history of vaccination (P = 0.002).

Immunity against hepatitis B viral infections (defined as anti-HBs titre >10 mIU per mL was detected in 26.2 % (82/313) of healthcare workers, (Figure 5.5).

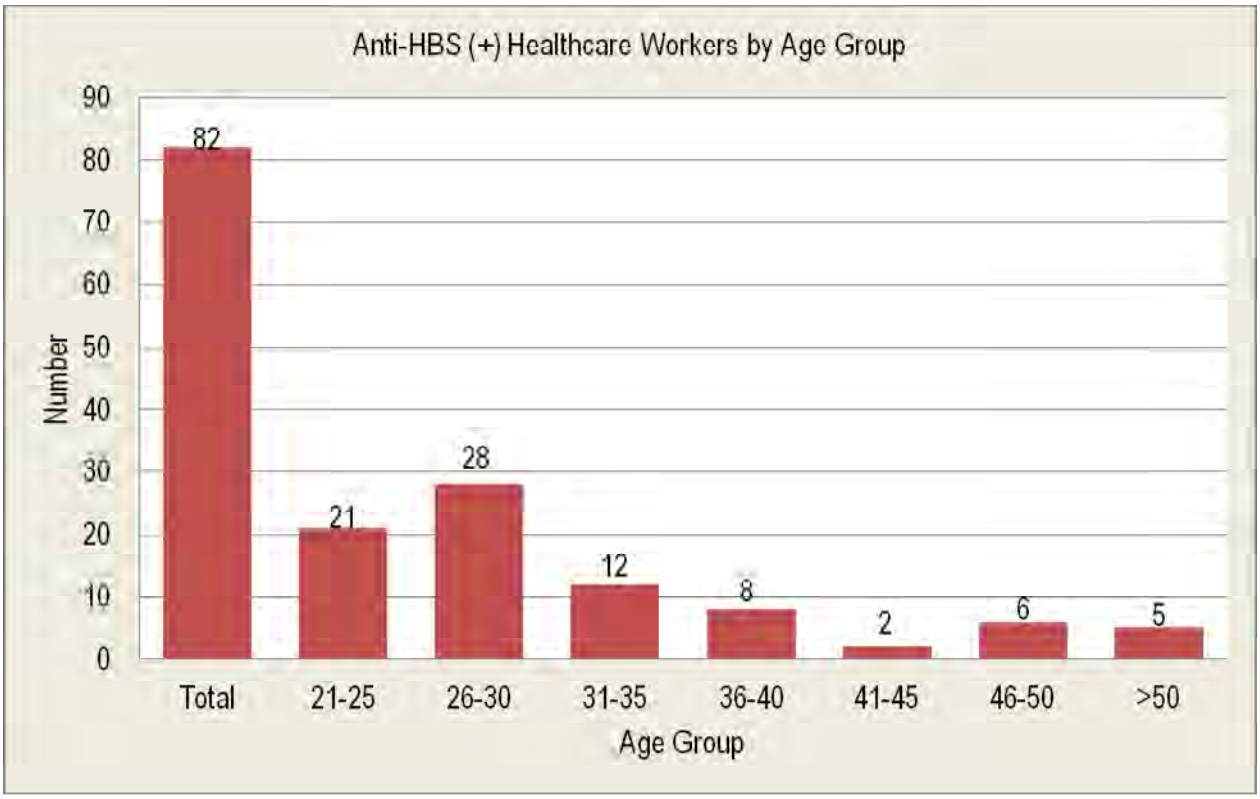


Figure-5.5. Anti-HBs viral markers among specific age groups of the 313 healthcare workers in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 – May 2014.

Table-5.6. Hepatitis B viral markers with glove use among healthcare workers in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 – May 2014 (n= 313).

Glove User	HBsAg (+) (%)N	Anti-HBc (+) (%)N
Consistent Users	1.92% (3 / 156)	26.3% (41/156)
Intermittent Users	1.75% (2 / 114)	24.6% (28/114)
Non-User, considered N/A*	6.97 % (3 / 43)	25.6% (11/43)

N/A = Considered non-appropriate by the study subject themselves during data collection.*

5.4. Hepatitis B viral markers in relation to Vaccination status of health care workers

Out of the 313 studied subjects only 1.6 % (5 /313) and 4.8 % (15 / 313) healthcare workers had completed the recommended three doses of scheduled vaccination and incomplete vaccination status respectively. Even though, information about the source of the vaccination (whether it is commercial or non-commercial, government or private provider) was not captured in this study, from those study subjects with a history of complete vaccination and incomplete vaccination, 20 % (1 / 5) and 60% (9/15) had no serological evidence of immunization (anti-HBs < 10 mIU per mL).

Table-5.7. Hepatitis B Viral markers in relation to vaccination status of the study subjects in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, November 2013 – May 2014 (n= 313)

Status	Number (%)	HBsAg (+)	Anti-HBs (+)	Anti-HBC (+)
Vacc Complete	5(1.6%)	0(%)	4(80%)	0(0%)
Vacc Incomplete	15(4.8%)	0(%)	6(40%)	0(0%)
Non-Vaccinated	293(93.6%)	8(2.7%)	72(24.6%)	80(27.3%)
Total	313(100%)	8(2.6%)	82(26.2%)	80(25.6%)

About 73.8 % (231 /313) did not have any of the markers and thus classified as susceptible to infection. The sera of about 27.3 % (80 / 313) and 24.6 % (72 /313) were positive for anti-HBc and anti-HBs markers respectively, an indication that they were immune to hepatitis B viral infections following a natural course of infections, while only 3.19 % (10 / 313) were tested positive for anti-HBs and ascertain immunity following vaccination.

5.5. Risk Factors for Hepatitis B Viral Infection

In the univariate analysis HBsAg positive and anti-HBc positive study subjects were included. Among the sociodemographic characteristics, the crude odds ratio of being hepatitis B infected indicates a greater risk in male than females (COR=1.357, 95% CI: 0.676 - 2.276). Exposure to blood and blood products was also subjected for univariate analysis and there was statistical significant association with hepatitis B infection (COR = 9.351, 95% CI: 1.164 – 75.095).

Table-5.8. Risk factors: results from logistic regression model among all healthcare workers in St Paul Hospital Millennium Medical College; Addis Ababa, Ethiopia, November 2013 – May 2014.

Risk Factor	Odds Ratio (95%CI)	P – Value
Age	1	0.538
Sex:		
▪ Male	1.357(0.676 – 2.276)	0.111
▪ Female	1.444(0.850 – 2.451)	0.241
Blood and Blood Product contact	9.351(1.164 – 75.095)	0.012
Body Fluids exposure	5.592(0.696 – 44.913)	0.065
Needle Stick Injuries	1.536(0.315 – 7.481)	0.454
Sharp Injuries	0.916(0.223 – 3.761)	0.483
Jaundiced and Diagnosed Liver Disease	3.096(1.051 – 9.120)	0.032
History of Operation	0.970(0.950 – 0.991)	0.268
Blood Transfusion	0.973(0.955 – 0.992)	0.672
Job Category	1	0.171
Department of Practice	1	0.063
Splash of Blood and Body Fluids	1.200(0.715 – 2.016)	2.88
Tattoo	1.500(0.583 – 3.860)	0.271

Following univariate analysis, significant risk factors for hepatitis B viral infection among studied healthcare workers were found to be history of exposure to blood and blood products (COR = 9.351, 95% CI: 1.164 – 75.095), history of jaundiced and diagnosed liver disease (COR = 3.096, 95%CI:1.051 – 9.120), and vaccination status (χ^2 = 11.145, P = 0.002).

The variables found to be associated with Hepatitis B viral infection in the univariate analysis were entered into the logistic regression model controlling confounders and for evaluating the effects of risk variables on hepatitis B viral infections among studied group. Following the multivariate analysis history of jaundiced and diagnosed liver disease had remained statistically significant association with hepatitis B viral infections among all the studied healthcare workers (OR = 4.092; 95%CI: 1.060 – 9.513); P = 0.039. Vaccination status was also showed a significantly strong association (χ^2 = 11.145, P = 0.002).

Chapter 6: Discussion

6. Discussion

In this study the magnitude of hepatitis B virus infection is prevalent among healthcare workers in St Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia. The overall prevalence rate was 2.6 % for HBsAg, 25.6% for anti-HBc, 26.2% for anti-HBs, and 28.8% for at least one marker positive. However this finding is much lower than findings of similar studies previously conducted among healthcare workers in Ethiopia, (9.02 % for HBsAg, 73.6 % for anti-HBc, 46.25 % for anti-HBs, and 76.4 % for at least one marker positive (48), 7.3 % of HBsAg seroprevalence of hepatitis B virus infections among health care workers at Bulle Hora Woreda, Oromia [47] and 9.7% HBsAg seroprevalence of hepatitis B viral infections among healthcare workers at Tikur Anbessa University Hospital and Ras Desta Damtew Memorial Hospital in Addis Ababa [51]. The possible justification for this marked variation could be; limited number of published articles, differences in demographic characteristics of the study subjects, differences in diagnostic technologies used, differences in diagnostic markers used to measure infection, and probably changes over the times in trend of hepatitis B viral infections following the many awareness interventions.

The current prevalence of 2.6% HBsAg and 25.6 % anti-HBc markers of hepatitis B viral infections in our study were even much more lower than other findings from a community based studies among the general population in Ethiopia, 10.8 % for HBsAg and 73.3% for at least one marker positive [8]. Comparison of our result with other countries on healthcare workers also compared and showed a prevalence of 9.9 % in Yemen, a highly endemic country [52], in Senegal 17.8% [53] and in Uganda 8.1% [46]. However, our findings are in consistent with previous studies conducted on community based seroepidemiological studies in Ethiopia among different settings and groups, 5 % of HBsAg and 37 % any marker prevalence in pregnant women in Addis Ababa [54], 3.7 % HBsAg prevalence in pregnant women in Jimma [36], 5.3 % of HBsAg prevalence in pregnant women attending Debre-Tabor Hospital [35], 5.7 % of HBsAg prevalence among visitors of Shashemene General Hospital, Oromia [34], 7 % of HBsAg prevalence, with higher prevalence in males than females [55]. Comparison of our result with other studies from other countries on community based seroepidemiological studies showed variable results with seroprevalence of 1.0 % in people born in Canada, 6.9% in the Inuit population in Canada, 7.4%

in immigrants from highly endemic counties in Canada [27], and 4.6% seroprevalence from Pakistan [31].

A comparable an intermediate endemicity level of current hepatitis B viral infections among healthcare workers were also reported from different parts of the world, 4.9 % of HBsAg seroprevalence from Sudan, Khartoum [9], 5 % of HBsAg seropositivity from India [40], 2.18% of HBsAg seroprevalence with significant difference between vaccinated and non-vaccinated healthcare workers from Pakistan [56], 2.7 % of seroprevalence from Turkey [42], 2.4% of HBsAg seroprevalence from Korea [44], 2.8% of HBsAg prevalence from Syria [57]. However, our finding is higher than other seroprevalence studies conducted on the general population in different countries; 1.2 % in Mexico [58], 1% in Morocco [59], 0.5 % in Pakistan [60], and 0.0% in Japan [45]. The reasons for the relatively lower rate of seroprevalence in our study compared with other studies can be attributed to the difference in demographic characteristics of the study population, the difference in hepatitis B virus geographical epidemiology in these countries, awareness of the routes of hepatitis B virus transmission, efforts made to implement universal precautions by healthcare workers and different degree of benefits due to the initiation of national and global immunization programmes all over the world might explain these discrepancies accordingly.

The World Health Organization estimates that about 3 million healthcare workers face occupational exposure to blood borne viruses each year in which about 2 million to hepatitis B viral infections, 900,000 to hepatitis C virus, and 300,000 to human immunodeficiency virus, of which 90 % of the infections that result from these exposures are in low income countries [61].

Recognizing this threat, the United States Centers for Disease Control and Prevention proposed a series of procedures including standard precautions, advise health care workers to practice regular personal hygiene, use protective barriers such as gloves and gown as required and proper disposal of sharps and other clinical wastes for preventing occupational exposures and for handling potentially infectious materials [62].

Compliance to safety, infections prevention and universal precaution were assessed by consistent use of glove as a tool kit to capture the data and only 49.8% used glove consistently; with higher 71.4% consistent use of glove reported from doctors and the least 40.0% reported from medical

laboratory professionals. This suggests the low rate of compliance found in our study, and indicates yet there is a problem in compliance to universal precaution. In our study, needle stick and sharp injuries accounted for 33.9 %, and 35.5 % respectively, involving all the studied healthcare workers and their respective job categories. With regard to needle stick injuries, a similar study revealed in Ethiopia a one year prevalence of needle stick and sharps injury of 17.5% and 13.5% respectively [63], where as a comparable report of needle stick injury in Hawassa, Ethiopia indicated 35.8% [64]. This shows needle stick and sharp injuries are quite common occupational exposures in healthcare settings in this country.

On the other hand, exposure to blood and body fluids in the studied healthcare workers reported were 57.2% and 44.4% ungloved respectively. This finding is in consistent with other reports from Ethiopia, which were done previously in different regions; 30.5% of needle stick injuries, 25.7% of sharps injuries and 28.8% of exposures to blood and body fluids were reported from eastern Ethiopia [63], while 35.8% of prevalence for needle sticks injuries reported in Hawassa [64], and lower than the previous study from Addis Ababa, 82.8% of exposure to blood and body fluids [51]. Our study recognized lower than half of the study subjects encountered needle stick and sharp injuries this is in agreement with previous report from Addis Ababa, 31.1% of sharp injuries, but a lower exposure rate for needle stick injuries 59.0% [51].

The possible reasons for high prevalence of these needle stick and sharp injuries include lack of specific programme measures to address occupational challenges, lack of safer sharp devices, lack of information and failing to adherence to standard precautions. National Institute of Occupational Safety and Health, United States identifies needle stick injuries; over use of injections and unnecessary sharps, lack of disposable supplies, safer needle devices, sharps disposal containers, lack of access to and failure to use sharps container immediately after use, poorly trained staff, needle recapping, passing instruments from hand to hand as seen in operating room, and lack of awareness and trainings on safety and infection preventions are possible predisposing factors for viral infections [65]. This is in agreement with findings from this study in which more than 74.8 % of healthcare workers were not trained on infection prevention and safety precautions.

The logistic regression model indicated exposure to blood and blood products, jaundiced and diagnosed liver diseases, and vaccination are strongly associated with each other, indicating the clustering nature of exposure incidents on groups of healthcare workers probably based on negligence and substandard compliances with infection prevention and safety precautions. With regard to the frequencies of exposure, 32.6 % and 20.4 % of healthcare workers had reported frequent and repeated exposure to blood and body fluids respectively, while at least one time exposure of needle stick injuries accounted 12.8 % and 11.8 % of frequent exposure reported for that of sharp injuries. Although, this finding is much lower than reports in Syria, 76.6 % of sustained at least one needle stick injuries [57], the investigation suggests that exposure to blood and body fluid; needle stick and sharp injuries are still considerable burden for healthcare workers. Although all healthcare workers in contact with patients are at risk of exposure to blood and body fluids, needle stick and sharp injuries, nurses experienced the highest prevalence of injuries 83.9 % from blood and blood products, and 52,7 % from needle stick injuries. Doctors followed with 74.2 % from body fluids. According to other studies, nurses experience the majority of needle stick injuries in the world [66]. Nurses are more likely to handle sharp devices and have more contact with patients and this is in agreement with our study.

Hepatitis B vaccination among healthcare workers in this study was very low at 1.6% (fully vaccinated) and 4.8% incompletely vaccinated, with at least two scheduled doses. This is a very much lowered prevalence of vaccination among similar studies, according to the world health organization estimates; vaccination coverage varies from 18 % in Africa to 77 % in Australia and New Zealand [66]. A study in Kenya showed 12.8 % of HCWs were vaccinated [67]. There are many potential reasons for low HBV vaccine coverage, the most common being unavailability of the vaccine at the health facility. While the vaccine is available at the market at a cost, healthcare workers had relied on provision from their institutions. Other potential reasons identified in our study and supported by others include lack of knowledge about severity and vaccine efficacy, and low risk perception of hepatitis B viral infections [66]. Although, there is a moderately good awareness among healthcare workers that in most of countries in the world health organization African region have implemented hepatitis B vaccine in their routine national EPI since 2005[68]. However, availability of a safe and efficacious vaccine and adoption of appropriate immunization strategies are the most effective means to prevent HBV infection and its consequences.

On the other hand, in our study 20.0% (1/5) of healthcare workers with history of complete scheduled vaccination, and 60.0% (9/15) of healthcare workers with history of incomplete (at least two doses) scheduled vaccination, had no serological evidence of immunity (anti-HBs < 10 mIU/ml). Similar study in India from 153 healthcare workers who received three doses of scheduled vaccination only 67% (32/48) of males and 72% (76/105) of females had serological evidence of immunity (anti-HBs < 10 mIU/ml) with gender difference [69]. Another study in Iran from 129 HBV vaccinated healthcare workers, 68.2% (103/129) had serological evidence of immunity (anti-HBs > 10 mIU/ml) and 17.2 % (26/129) had no serological evidence of immunity (anti-HBs < 10 mIU/ml) with no association between gender and anti-HBs titer, but vaccination and adequate completion of its courses were associated with higher anti-HBs titer [70]. While, in the booster dose of vaccine it was 94.3% and 100% with the first and second booster dose of vaccination, respectively [71]. This indicates a need to measure the concentration of anti-HBs titer in all vaccinees after the third dose of scheduled vaccination and consider reassessment of vaccination in HCWs according to their anti-HBs levels 10 years after vaccination [72].

The possible justification for lack of adequate anti-HBs antibody formation may be due to the persistent exposure of HCWs to HBV infection, low level viremia, and infection with mutant forms from patients on certain antiviral treatments. The persistence of anti-HBs depends on the peak antibody level achieved after three doses [73]. Unresponsiveness to hepatitis B vaccine has been attributed to a number of environmental and genetic factors, the most important ones being the haplotype of HLA antigens and immunological tolerance [74]. A variety of HLA class I and II antigens have been reported to be associated with unresponsiveness to the vaccine in different ethnic populations [75].

7. Limitations of the Study

We recognized certain limitations in our study. Due to insufficient immunoassay logistics provided, there is no data available from this study regarding IgM anti-HBc and HBeAg markers. This limited us to classify whether infections are acute or chronic and whether the degree of infectivity correlates with a high level of hepatitis B viral replication or not. Since the data regarding exposure to risk factors were collected by a self administered questionnaire, there is a possibility of recall bias among the healthcare workers of their risk factors. The choice of

serologic markers, temporal influences, and representativeness of the study population may also have limitation in comparability of HBV seroprevalence results.

8. Conclusion and Recommendations

The prevalence of current hepatitis B virus infection and life time exposure to hepatitis B virus infection among health care workers was high. Exposure to potentially infectious body fluids was also high and yet only a small proportion of healthcare workers are vaccinated against hepatitis B virus infection. Most of the HBV infected healthcare workers in the present study had undergraduate level of education. This fact is enough to put a rigorous emphasis on proper continuous medical education and training on infection prevention and safety precautions which is most likely to serve as an effective tool in controlling exposure to injuries. Besides the doctors, nurses and medical laboratory professionals; cleaners, porters and general service providers were at a comparably high or more risk of HBV infection as they interact with patients and clinical wastes.

Based on our results, the following recommendations are forwarded accordingly:

- Efficient intervention strategies to protect healthcare workers from occupational exposures to needle stick and sharp injuries, and blood and body fluids should be identified, implemented and monitored.
- Assurance of compliance with universal precautions, access to safer injection technologies, post-exposure management and continuing medical education on infection prevention and safety precautions are also valid recommendations.
- Facilities should also establish surveillance system for registering, reporting and managing of occupational exposures.
- High risk adult groups of subpopulations, healthcare workers need to be vaccinated.
- Although immunizing with universal vaccination is the only way to control HBV infection, recent advances in the specific treatment have enabled suppression of the chronic viral infection. Strong and effective suppression of chronic hepatitis B in human carriers (HBsAg-Positives) will help to prevent the spread of hepatitis B viral infections, and this can be made possible with the use of either α-interferon (IFN- α; regular or pegylated) or nucleoside analogs.

9. References

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10. Annexes

Annex- I. Questionnaire for the Investigation of the Prevalence and Associated Risk Factors of HBV Infections in Health Care Workers at Black Lion University, Zewditu and St. Paul Specialized Hospitals in Addis Ababa, Ethiopia

Participant Code no: ☐☐☐☐☐

I. Identification

1. Age (in yrs): _____
2. Sex: _____
3. Marital Status: _____
4. Occupation: _____
5. Department of Practice: _____
6. Total Years of Service:_____

II. Risk assessment

7. History of Occupational exposure to blood and blood products ungloved?	☐ Yes
	☐ No
8. If yes, how many times exposed ungloved?	☐ Once
	☐ Twice
	☐ Three times
	☐ Several Times
9. History of occupational exposure to body fluid (peritoneal, pericardial, pleural, synovial, CSF, Amniotic fluid)?	☐ Yes
	☐ No
10. This exposure in the last 6 months?	☐ Yes
	☐ No
11. If yes, how many times exposed ungloved? Unprotected?	☐ Once
	☐ Twice
	☐ Three times
	☐ Several Times
12. Use of Gloves during exposure prone procedures?	☐ Consistently
	☐ Intermittently
13. Splash of blood or body fluid to exposed face?	☐ Yes
	☐ No

14. History of needle sticks injury?	□ Yes
	□ No
15. If yes, how many times you have been exposed?	□ Once
	□ Twice
	□ Three times
	□ Several Times
16. History of sharp injury?	□ Yes
	□ No
17. If yes, how many times you have been exposed?	□ Once
	□ Twice
	□ Three times
	□ Several Times
18. Have you ever taken care of Hepatitis Patient?	□ Yes
	□ No
19. Do you have History of Operation?	□ Yes
	□ No
20. Do you have History of blood transfusion?	□ Yes
	□ No
21. History of Jaundice or Diagnosed liver disease?	□ Yes
	□ No
22. Do you have History of tattooing?	□ Yes
	□ No
23. Previous history of hepatitis B vaccination?	□ Fully Vaccinated
	□ Incompletely Vacc.
	□ Non-Vaccinated
24. Do you have any family member who is health worker?	□ Yes
	□ No
25. If yes, please tell us who is he in relation to you?	□ My Mother
	□ My Father
	□ Sister/Brother
	□ Wife/Husband
26. Have you taken trainings on IP and safety precautions?	□ Yes
	□ No

III. Laboratory Results:

1. HBs Ag Positive ☐ Negative ☐
2. Anti-HBs Positive ☐ Negative ☐
3. Anti HBc Positive ☐ Negative ☐

IV.Comments_____
_____
_____

Annex-II: Information Sheet of the study subject

Purpose: We are studying the prevalence and associated risk factors of HBV infections in healthcare workers who spend much of their time in contact with blood and other body fluids which contain infectious organism. Hepatitis virus is the etiological agent of chronic hepatopathies and that patients with acute infection may not develop symptoms in most cases and serve as a source of infection. Early detection of the affected prevents complication and transmission to others. The objective of this study is to determine the magnitude of HBV in healthcare workers. They will assess the implications of the study for the safety of healthcare workers in Ethiopia.

Participation: We are asking you and others to voluntarily participate in this study. What is expected from everyone is to respond some question which take about fifty minutes and give 5 ml of venous blood. The blood samples are collected using sterile materials and disposables.

Risks: While you are participating, you are likely to have some risks. The risks associated with this study could be some discomforts and in a rare occasion a hematoma may be developed when we draw 5ml of venous blood from you. However, these things are not produce serious pain and if in case any problems arise during and following sample collection, we shall offer you necessary medical interventions in this regard.

Benefits: If you are positive for HBV during investigation, opportunities for treatment will be arranged and you will be followed. If you are negative for HBV, Vaccination for HBV will be given to prevent future HBV infection by dealing with the concerned body.

Confidentiality: Any information that we will collect about you during this study will be kept confidential and your identity will be put away after re-coding your file and kept in a secured place. Only the principal investigators will be able to link your identity with the code number, if this becomes necessary to assist you in any way.

Sharing the result: At the end of the study we will present the result to responsible bodies, the report will not bear any information relevant to your personality. We assure you the confidentiality of such information. Thus, we also need your permission to use the test results for writing a report and to use for publication.

Right to refuse: Since participation in this study is entirely voluntarily, you can refuse to participate in this study at any time. Your refusal will not affect your job.

Any question regarding this study can be addressed to:

Principal Investigator (PI): Gizachew Taddesse Akalu

Contact Address: Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences. Phone (Cell Phone): +251-911 31 99 58, P.o.Box: 18951, E-mail: gtakalu@yahoo.com.

Annex-III: Consent Form

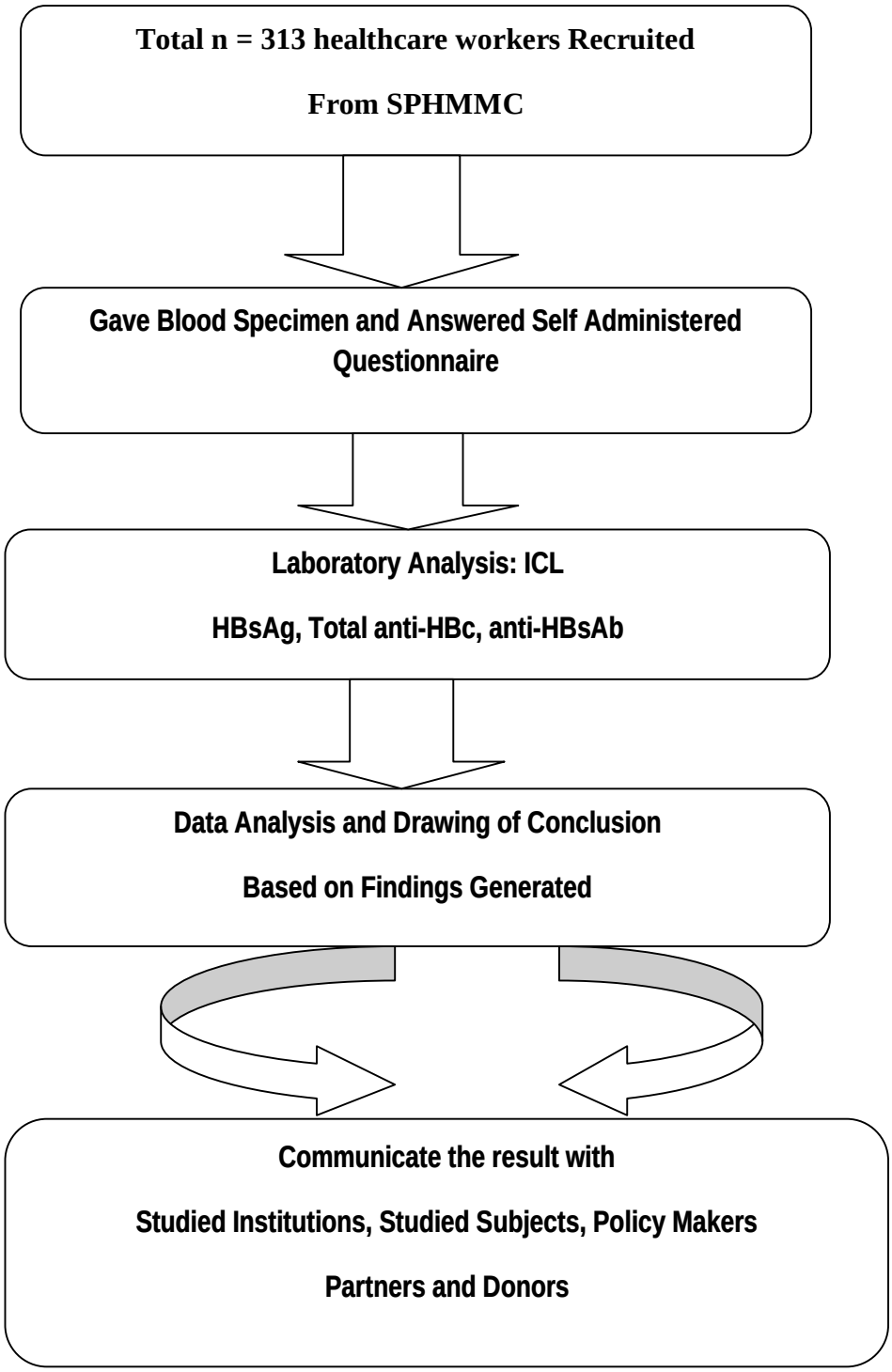
I have been requested to participate in a research project that aims to determine the prevalence and associated risk factors of Hepatitis B virus infections in healthcare workers. I have been informed that all information I will be giving will be kept confidential was provided the opportunity to ask questions and given adequate time to rethink the issue. The aim and objectives of the study are sufficiently clear to me. I have not been pressurized to participate in any way. I understand that participation in this study is completely voluntary and that I may withdraw from it at any time and without supplying reasons. I am fully aware that the results of this Study will be used for scientific purposes and may be published. I agree to this, provided my privacy is guaranteed. And I confirm my agreement by putting my signature below. I hereby give my consent for giving of blood specimens.

Full Name: _____

Signature: _____

Date: _____

Annex-IV: Conceptual Frame Work



Annex-V: Laboratory Test Procedures

Assay Procedure ARCHITECT Anti-HBs

1. Before loading the ARCHITECT Anti-HBs Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment.
2. Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
3. 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.
4. Order calibration, if necessary. Order tests. For information on ordering patient specimens, calibrators, and controls, and general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
5. Load the ARCHITECT Anti-HBs Reagent Kit on the ARCHITECT *i* System. Verify that all necessary reagents are present. Ensure that septums are present on all reagent bottles.
6. The minimum sample cup volume is calculated by the system and is printed on the Order list report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation verify adequate sample cup volume is present prior to running the test.
7. Priority: 125 µL for the first Anti-HBs test plus 75 µL for each additional Anti-HBs test from the same sample cup. ≤ 3 hours on board: 150 µL for the first Anti-HBs test plus 75 µL for each additional Anti-HBs test from the same sample cup. > 3 hours on board: additional sample volume is required. Refer to the ARCHITECT System Operations Manual, Section 5, for information on sample evaporation and volumes. If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
8. ARCHITECT Anti-HBs Calibrators and Controls should be mixed by gentle inversion prior to use. To obtain the recommended volume requirements for the ARCHITECT Anti-HBs Calibrators and Controls, hold the bottles **vertically**, and dispense 7 drops of each Calibrator (for 2 replicates), or 5 drops of each Control (for 1 replicate) into each respective sample cup.
9. Load samples. For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
10. Press RUN. The ARCHITECT *i* System performs the following functions:

    Moves the sample to the aspiration point, loads a reaction vessel (RV) into the process path, aspirates and transfers sample into the RV, advances the RV one position and transfers microparticles into the RV, mixes, incubates, and washes the reaction mixture, adds conjugate to the

RV, mixes, incubates, and washes the reaction mixture, adds Pre-Trigger and Trigger Solutions, measures chemiluminescent emission to determine the quantity of Anti-HBs in the sample, aspirates contents of RV to liquid waste and unloads RV to solid waste, and Calculates the result.

Calibration

1. To perform an ARCHITECT Anti-HBs calibration, test Calibrators 1 and 2 in duplicate. A single sample of all levels of Anti-HBs controls must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the Control package insert. Calibrators should be priority loaded.
2. Calibrator Range: 0 - 1000 mIU/mL
3. Once ARCHITECT Anti-HBs calibration is accepted and stored, all subsequent samples may be tested without further calibration unless: A reagent kit with a new lot number is used, and Controls are out of range.

Quality Control Procedures

1. It is recommended that the ARCHITECT Anti-HBs Positive Control 1, Anti-HBs Positive Control 2, and Negative Control be run in order to verify the calibration.

2. The recommended control requirement for the ARCHITECT Anti-HBs assay is a single sample of each control tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory specific procedures. Ensure that assay Control values are within the concentration ranges specified in the Control package insert.

Interpretation of Results

1. Specimens with concentration values < 10.00 mIU/mL are considered nonreactive by the criteria of ARCHITECT Anti-HBs.
2. Specimens with concentration values ≥10.00 mIU/mL are considered reactive by the criteria of ARCHITECT Anti-HBs.

Assay Procedure ARCHITECT Anti-HBc II

1. Before loading the ARCHITECT Anti-HBc Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment.
2. Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
3. Once the microparticles have been resuspended, place a septum on the bottle.
4. Load the ARCHITECT Anti-HBc Reagent Kit on the ARCHITECT *i* System. Verify that all necessary reagents are present. Ensure that septums are present on all reagent bottles.
5. Order calibration, if necessary. Order tests. For information on ordering patient specimens, calibrators, and controls, and general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
6. The minimum sample cup volume is calculated by the system and is printed on the Order list report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation verify adequate sample cup volume is present prior to running the test.
7. Priority: 75 µL for the first ARCHITECT Anti-HBc II test plus 75 µL for each additional Anti-HBs test from the same sample cup. ≤ 3 hours on board: 150 µL for the first Anti-HBc II test plus 25 µL for each additional Anti-HBc II test from the same sample cup. > 3 hours on board: replace with a fresh sample (patient specimen, calibrator and control). If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
8. ARCHITECT Anti-HBc II Calibrators and Controls should be mixed by gentle inversion prior to use. To obtain the recommended volume requirements for the ARCHITECT Anti-HBc II Calibrators and Controls, hold the bottles **vertically**, and dispense 5 drops of each Calibrator or 4 drops of each Control into each respective sample cup.
9. Load samples. For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
10. Press RUN. The ARCHITECT *i* System performs the following functions:

    Moves the sample to the aspiration point, loads a reaction vessel (RV) into the process path, aspirates and transfers sample into the RV, advances the RV one position and transfers microparticles into the RV, mixes, incubates, and washes the reaction mixture, adds conjugate to the RV, mixes, incubates, and washes the reaction mixture, adds Pre-Trigger and Trigger Solutions, measures chemiluminescent emission to determine the quantity of Anti-HBs in the sample, aspirates contents of RV to liquid waste and unloads RV to solid waste, and Calculates the result.

Calibration

1. To perform an ARCHITECT Anti-HBc II calibration, test Calibrators 1 and 2 in duplicate. A single sample of each Anti-HBc II control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the Control package insert. Calibrators should be priority loaded.
2. Once ARCHITECT Anti-HBs calibration is accepted and stored, all subsequent samples may be tested without further calibration unless: A reagent kit with a new lot number is used, and Controls are out of range.

Quality Control Procedures

1. The recommended control requirement for the ARCHITECT Anti-HBc II assay is a single sample of each control tested once every 24 hours each day of use. If the laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures.

2. The ARCHITECT Anti-HBc II control value must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the association test results are invalid and samples must be retested. Recalibration may be indicated.

Interpretation of Results

1. Specimens with concentration values < 1.00 S/CO are considered nonreactive by the criteria of ARCHITECT Anti-HBc II.
2. Specimens with concentration values ≥1.00 S/CO are considered reactive by the criteria of ARCHITECT Anti-HBc II.

Assay Procedure ARCHITECT HBsAg

1. Before loading the ARCHITECT HBsAg Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment.
2. Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
3. 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.

4. Order calibration, if necessary. Order tests. For information on ordering patient specimens, calibrators, and controls, and general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
5. Load the ARCHITECT HBsAg Reagent Kit on the ARCHITECT *i* System. Verify that all necessary reagents are present. Ensure that septums are present on all reagent bottles.
6. The minimum sample cup volume required to perform a single HBsAg test on the ARCHITECT *i* system is 150µl for the first HBsAg plus 75 µl for each additional HBsAg test from the same sample cup. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation verify adequate sample cup volume is present prior to running the test. The minimum sample cup volume is calculated by the system and is displayed on the patient, calibrator and control order screen and on the Order list report.
7. Priority for three or fewer replicates: 75µL for the first HBsAg test plus 50 µL for each additional Anti-HBs test from the same sample cup.
8. To minimize the effects of evaporation all samples, calibrators and controls must be tested within 3hours of being placed on the ARCHITECT *i* system. If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
9. ARCHITECT HBsAg Calibrators and Controls should be mixed by gentle inversion prior to use. To obtain the recommended volume requirements for the ARCHITECT HBsAg Calibrators and Controls, hold the bottles **vertically**, and dispense 10 drops of each Calibrator (for 2 replicates), or 6 drops of each Control (for 1 replicate) into each respective sample cup.
10. Load samples. For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
11. Press RUN.

Calibration

1. To perform an ARCHITECT HBsAg calibration, test Calibrators 1 and 2 in duplicate. A single sample of all levels of HBsAg controls must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the Control package insert. Calibrators should be priority loaded.

2. Calibrator Range: 0 – 250 IU/mL

3. Once ARCHITECT HBsAg calibration is accepted and stored, all subsequent samples may be tested without further calibration unless: A reagent kit with a new lot number is used, and Controls are out of range.

Quality Control Procedures

3. It is recommended that the ARCHITECT HBsAg Positive Control 1, HBsAg Positive Control 2, and Negative Control be run in order to verify the calibration.

4. The recommended control requirement for the ARCHITECT HBsAg assay is a single sample of each control tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory specific procedures. Ensure that assay Control values are within the concentration ranges specified in the Control package insert.

Interpretation of Results

1. Specimens with concentration values < 0.05 IU/mL are considered nonreactive by the criteria of ARCHITECT HBsAg.

2. Specimens with concentration values ≥ 0.05 IU/mL are considered reactive by the criteria of ARCHITECT HBsAg.

Annex- VI. Declaration

I the undersigned, declare that this MSc thesis is my own original work and it has not been presented for a degree or some other purpose in any universities, colleges or institutions and that all sources of material used for the thesis have been dully acknowledged.

Name: **Gizachew Taddesse Akalu**

Signature: _____

Place: **Addis Ababa, Ethiopia**

Date of Submission: **26 June 2014**

This thesis has been submitted for examination with my approval as a University advisor.

Name: **Kassu Desta (MSc, PhD Fellow)**

Signature: _____

Place: **Addis Ababa, Ethiopia**

Date of submission: **26 June 2014**