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PREVALENCE AND ASSOCIATED RISK FACTORS OF HEPATITIS B VIRUS SURFACE ANTIGEN (HBsAg) AMONG INDIVIDUALS VISITING WOLDIA HEALTH CENTER, NORTHEAST ETHIOPIA

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By

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Declaration

I, the undersigned, declare that this study entitled “Prevalence and associated risk factors of Hepatitis B Virus Surface Antigen (HBsAg) among individuals visiting Woldia Health Center, northeast Ethiopia” contains my personal work. In compliance with internationally accepted practices, I duly acknowledged and referred all materials used in this study. I understand that non-obedience to the principles of academic honesty and integrity, misrepresentation or fabrication of any data will constitute sufficient ground for disciplinary action by the University.

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

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BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF BIOLOGY

APPROVAL SHEET

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Acronyms

AIDS	Acquired Immune Deficiency Syndrome
Anti-HBs	Antibody for Hepatitis B surface antigen
AOR	Adjusted Odds Ratio
CI	Confidence Interval
COR	Crude Odds Ratio
DNA	Deoxyribonucleic Acid
HBcAg	Hepatitis B core Antigen
HBeAg	Hepatitis B endogenous Antigen
HBIG	Hepatitis B Immunoglobulin
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HIV	Human Immunodeficiency Virus
IFN	Interferon
IL	Interleukin
LHBs	Large HBV surface protein
MHBs	Middle HBV surface protein
MHC	Major Histocompatibility Complex
mRNA	messenger Ribonucleic Acid
NKT	Natural Killer T-Cell
ORFs	Overlapping Reading Frames
PCR	Polymerase Chain Reaction
Pol	Polymerase

PreS1	PreS1 domain
PreS2	PreS2 domain
RNA	Ribonucleic Acid
S	S domain
SHBs	Small HBV surface protein
SPSS	Statistical Package for Social Sciences
TNF	Tumor Necrosis Factor
WHO	World Health Organization

Abstract

Hepatitis B is the most common and severe infection of the liver in the world including Ethiopia. This study was undertaken to assess the prevalence and possible association between risk factors and sero-positivity of Hepatitis B Virus surface antigen (HBsAg) among individuals visiting Woldia Health Center, northeast, Ethiopia. Hospital based cross-sectional study was conducted from November 2018 to February 2019. Blood samples were randomly collected from 415 individuals. Data on socio-demographic characteristics and potential risk factors were collected using structured questionnaire. HBsAg rapid kits were used to detect the presence of HBsAg using antibodies (if any) against HBV from serum samples of the studied subjects. Chi-square test was used for assessing the association between socio-demographic variables and HBV status. Logistic regression analysis was done to determine the strength of association between risk factors and HBV infection. Prevalence of hepatitis B was 3.37%. Amongst the potential risk factors analyzed in univariate logistic regression, sex (COR= 3.759, CI: 1.274-11.087, *P*= 0.016), smoking (COR= 6.517, CI: 1.286-33.035, *P*= 0.024), previous experience of liver disease (COR= 11.879, CI: 2.822-50.010, *P*= 0.001), previous experience of TB or cancer (COR=8.187, CI: 1.568-42.740, *P*= 0.013), having multiple sexual partners (COR= 6.614, CI: 2.032-21.525, *P*= 0.002), and abortion (COR= 10.082, CI: 1.797-56.574, *P*= 0.009) were significantly associated with hepatitis B infection. Nevertheless, only the variable, previous experience of liver disease, remained as a statistical significant explanatory risk factor in multivariate logistic regression (AOR= 30.69, *P*= 0.013) analysis. This study found an intermediate endemicity (3.37 %) of HBV infection and it was very small, but this needs to be confirmed by other studies at community level. Thus, screening of individuals for HBV infections and administration of health information about the risk factors, the mode of transmissions and prevention is recommended.

Keywords: *Ethiopia, Hepatitis B virus surface antigen, Woldia*

1. Introduction

1.1. Background of the study

Hepatitis is a worldwide disease which is characterized by inflammation of the liver. It is commonly caused by viruses. Additionally, using drugs or other medications, alcohol and toxic substances may cause hepatitis. Hepatitis, which is caused by immunological attacks, is called autoimmune hepatitis. Autoimmune hepatitis occurs when the body thinks the liver is a forging substance or an antigen and it makes antibodies against its own liver tissue. The progress of hepatitis can be either self-limiting or can increase to scaring or liver cancer (cirrhosis) (Kheiri and Makvandi, 2015; Marcin, 2017).

There are five main types of viral infection. These are hepatitis A, B, C, D, and E viruses (WHO, 2018). There is also recently discovered viral hepatitis which is called hepatitis G virus. It is closely related to hepatitis C virus. The virus and its effects are not clearly known and it's under investigation (Davis, 2018). The virus that is responsible for each type of hepatitis is different. Hepatitis A and E cause acute diseases, but hepatitis E is mostly severe in pregnant women. The rest hepatitis which are hepatitis B, C, and D are mostly ongoing and chronic diseases (Marcin, 2017). Particularly, hepatitis B and C cause chronic disease in many people and are the most common causes of liver cancer (WHO, 2018).

Hepatitis A and E are mostly transmitted by eating food or drinking water which is contaminated by feces from person infected with hepatitis A and E. They also spread by having anal-oral sex (Welch, 2018; WHO, 2018). Hepatitis E can also transmitted by consuming undercooked pork or wild game (Welch, 2018). Hepatitis B and C are commonly transmitted by having unprotected contact with an infected person's blood, semen, or other body fluid. Hepatitis D is a co-infection with hepatitis B, the mode of transmission is the same as hepatitis B (Welch, 2018; WHO, 2018).

Hepatitis B virus infection is the most common and severe infection of the liver which is caused by a DNA hepatitis B virus. It attacks the liver and cause constant liver injuries, liver cirrhosis, liver cancer and it may lead to failure of the liver and death (Dahlström and Viberg, 2013; Endeshaw Abateneh *et al.*, 2018). Hepatitis B is also called serum hepatitis (MedlinePlus, 2009) and is highly spreadable. It is transmitted through the contact of blood, semen, vaginal fluids, and mucous membranes; by having unprotected sex, unsafe blood transfusions, using unsterilized needles, from mother to child during pregnancy and at birth, and using illegal

drugs. Hepatitis B virus can be prevented by vaccine and this behavior gives a unique character for it (Dahlström and Viberg, 2013).

Viral hepatitis caused 1.34 million deaths in 2015, a number comparable to deaths caused by tuberculosis and higher than those caused by HIV. However, the number of deaths due to viral hepatitis is increasing over time. Most viral hepatitis deaths in 2015 were due to chronic liver disease (720 000 deaths due to cirrhosis) and primary liver cancer (470 000 deaths due to hepatocellular carcinoma). The prevalence of HBV infection is high and it causes the most morbidity and mortality rate in the world. Globally, in 2015, an estimated 257 million people were living with chronic HBV infection. The epidemic caused by HBV affects mostly the WHO African Region and the Western Pacific Region (WHO, 2017).

Hepatitis B virus prevalence is highest in sub-Saharan Africa and east Asia, where between 5–10% of the adult population is chronically infected. Mother-to-child transmission of hepatitis B virus is a major mode of transmission in high prevalence settings. High rates of chronic infections are also found in the Amazon region of South America and the southern parts of eastern and central Europe. In the Middle East and the Indian subcontinent, an estimated 2–5% of the general population is chronically infected (WHO, 2016). HBV is endemic to sub-Saharan Africa. The rate of HBV infection varies between 5% and 19% in different African countries (Chotun, 2012). Ethiopia is one of the endemic sub-Saharan African countries for HBV (Letebrhan Weldemhret *et al.*, 2016).

1.2. Statement of the problem

In Ethiopia there is no enough data on prevalence of HBV rather in selected group of people with risk factors such as pregnant women (Zerihun Belay, 2018), military personnel (Tigist Birku *et al.*, 2015), HBV with diabetes (Daniel Mekonnen *et al.*, 2014) and HIV patients (Bayeh Abera *et al.*, 2017). For instance, the overall pooled prevalence of hepatitis B virus was 7.4%. The pooled prevalence among subgroups showed 5.2% in human immunodefeciency virus infected individuals, 8.0% in community based studies, 8.4% in blood donors, 11.0% in immigrants and 6.9% in other groups (Yeshambel Belyhun *et al.*, 2016).

The prevalence of HBV in Amhara regional states; for instance in Gojjam zones were 3.1% (Bayeh Abera *et al.*, 2017). The Sero-prevalence of HBV infection was 4.2 in military personnel (Tigist Birku *et al.*, 2015).

The prevalence of HBV in patients with diabetes mellitus in Woldia was 3.7% in a year of November, 2010 to January, 2011 (Daniel Mekonnen *et al.*, 2014). Previous studies on prevalence of HBsAg in Woldia showed its association with other infection like diabetes and HIV, and they barely associated it with risk factors. Woldia has a high flow of transportation, both passenger and business. It also serves as a rest point for high number of heavy trucks. This makes the area exposed to a higher rate of STDs. Therefore, the aim of this study was to assess the prevalence and associated risk factors of HBsAg in Woldia Health Center.

1.3. Objectives of the study

1.3.1. General objective

The general objective of this study was to assess the prevalence and associated risk factors of Hepatitis B Virus surface antigen (HBsAg) among individuals visiting in Woldia Health Center, northeast Ethiopia.

1.3.2. Specific objectives

- ✓ To determine the prevalence of HBsAg among individuals visiting Woldia Health Center
- ✓ To investigate possible association between risk factors and prevalence of HBsAg

1.4. Significance of the study

Most people do not know their hepatitis status because of the lack of simple and effective hepatitis testing strategies and tools; as a result, less than 5% of people with chronic hepatitis infection know their status. For this reason, diagnosis often occurs late and appropriate tests to assess liver disease and guide treatment decisions, including when to start treatment, are seldom available (WHO, 2016).

Medicines and diagnosis are unaffordable for most people. The high prices of new medicines are a major barrier to access in most countries. Treatments for chronic hepatitis B virus infection are lifelong for most people. The challenge will be to ensure that such medicines are affordable and that those people in need of treatment have access to those medicines without experiencing financial hardship (WHO, 2016). Therefore, screening for hepatitis B is necessary to identify individuals who have HBV infection so they can receive appropriate medical treatment. The finding obtained from this study could be used by professionals in health sector for effective prevention and control of the disease. In addition, the result of this study would serve as a baseline for further studies on the same subject.

2. Literature review

2.1. General description of HBV

Hepatitis B virus belongs to the *hepadnaviridae* family. Its members are divided in to two genera; *Orthohepadna* viruses infecting mammals, and *Avihepadna* viruses affecting birds. *Orthohepadna* viruses infect humans and other primates whereas *Avihepadna* viruses have been found in ducks, herons and strokes. HBsAg was discovered by Blumberg in the serum of an Australian aborigine in 1965 and was called the Australian antigen. He was awarded the Nobel Prize in 1976 (Alestig, 2011).

2.2. Epidemiology of HBV

Based on the prevalence of HBV marker, the world is divided into three areas; high (> 8%), intermediate (2-8%) and low (< 2%) HBV endemicity. East and Southeast Asia, the pacific, sub-Saharan Africa, and parts of southern Europe are highly endemic areas in the world. HBV infection is comparatively rare in North America, and western and northern Europe, with the prevalence rate of around 0.1% (Chen and Chang, 2010).

The prevalence of HBsAg in Europe widely varies, ranging from 0.3 to 12% even within a single country. Asia and pacific region countries has the highest prevalence level of HBV infection in the world. Next to Asia, Africa also has the highest prevalence rate which is the second largest number of individuals with chronic HBV infection, approximately 58 million. Overall, Africa is considered as high endemic area with 7-26% prevalence of HBsAg (Chen and Chang, 2010).

2.3. Morphology of HBV

HBV is also known as the Dane particle. It is a small, circular, partially double-stranded DNA molecule of approximately 3.2 kb nucleotides (Chen and Chang, 2010). HBV is an enveloped virus, measuring 42-47nm in diameter, with an icosahedral nucleocapsid that encloses a partially double-stranded relaxed-circular (rc) DNA genome covalently bound to the viral polymerase. The envelope comprises a small amount of lipid of cellular origin and three hepatitis B surface proteins (HBs); large, medium, and small hepatitis surface protein, which form disulfide-linked homo and heterodimers (Kann, 2002).

The serum of infected individuals contains, two types of subviral particles; small spherical particles with diameter of 20-27nm nucleocapsid made of 180 copies of protein which

containing the polymerase and HBV DNA. Outer envelope containing lipid and three forms of HBsAg. The filamentous particles also with diameter of about 20nm but it has variable length (Kann, 2002; WHO, 2002).

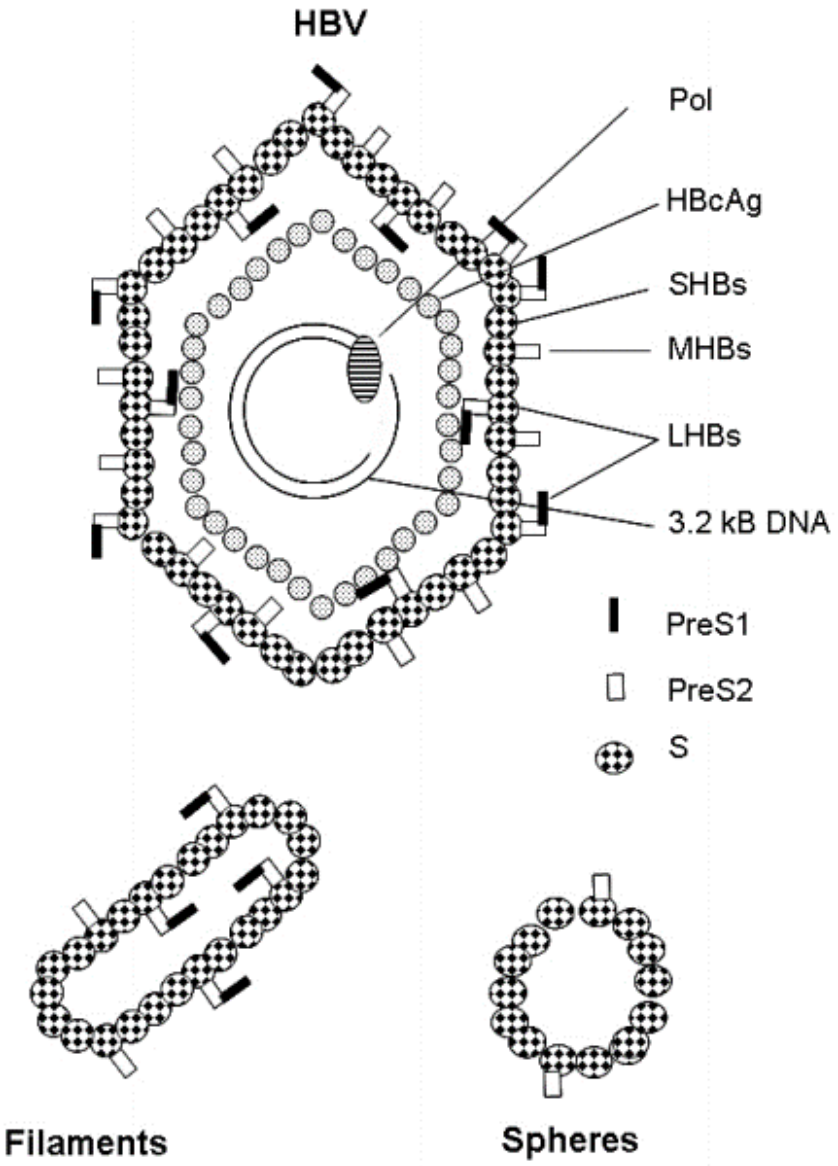

Figure 1: Schematic structure of the HBV particle and subviral particles. Source: (Schädler and Hildt, 2009)

The nucleocapsid is formed by multiple copies of core protein. Of the total 183-185 amino acids which is depending upon genotype, the N-terminal 149-151 amino acids are responsible for self-assembly of nucleocapsid. The nucleocapsid contains pores that allow the diffusion of nucleotides during the synthesis of the DNA genome. The C-terminal amino acids of the core

protein play a role in the packaging of the pregenome-polymerase complex with in the nucleocapsid (Bruss, 2007).

2.4. Replication of HBV

The replication of HBV is unique. Although a DNA virus, it encodes a reverse transcriptase and replicates through an RNA intermediate. The mechanism of entry in to the hepatocyte is largely unknown, but probably HBV attaches to hepatocytes via pre-S1 part of HBsAg. This receptor ligand binding may then, at least partly, account for the high specificity of liver cells, although HBV infection has also been found in lymphocytes, pancreas and the kidney. On penetration into the cell, the capsid is delivered to the nucleus where the partial DNA strand is synthesized to completion (Alestig, 2011).

The HBV genome utilizes four overlapping reading frames (ORFs) to efficiently store the coding instructions for its proteins and regulatory sequences. They are termed P (polymerase), S (surface), and C (core) and X (HBx protein) (Tiollais *et al.*, 1985; Kann, 2002). In the nucleus, the DNA is transcribed into four mRNAs of different sizes (3.5kb, 2.4kb, 2.1kb, and 0.9kb), which are transported into the cytoplasm. The large 3.5kb mRNA that are longer than the genome encodes HBc and HBe antigens and the HBc mRNA also codes for the polymerase and serves as template for replication of the whole genome. HBcAg and HBeAg share 90% of their protein sequence but are translated differently, and HBeAg is secreted into serum and does not self-assemble like a capsid antigen. The 2.4 kb fragment encodes the large S protein, and the smaller M and L proteins are transcribed from the 2.1 kb fragment. The small 0.9 kb fragments encode the X protein, which probably is involved in transcription regulation and acts as a protein kinase. A new virion particle is assembled as the core proteins encapsulate the large 3.5 kb mRNA and negative-sense DNA is synthesized by a reverse transcription, RNA degradation and a positive-sense DNA synthesizes. Finally, the core is enveloped and the viron leaves the cell by exocytosis (Alestig, 2011).

Replication and transcription are controlled by two enhancers (Enh I, Enh II) and four promoters (preC/C, preS1, S, X) initiating transcription of each ORF. Transcription factors binding to Enh I and Enh II are more abundant in liver cells than in other cells, resulting in a higher viral replication and specificity for these cells (Chen and Chang, 2010).

2.5. Pathogenesis of HBV

The HBV replication cycle is not directly cytotoxic to cells. This fact accords well with the observation that many HBV carriers are asymptomatic and have minimal liver injury, despite extensive and ongoing intrahepatic replication of the virus. It is now thought that host immune responses to viral antigens displayed on infected hepatocytes are the principal determinants of hepatocellular injury. This notion is consistent with the clinical observation that patients with immune defects who are infected with HBV often have mild acute liver injury but high rates of chronic carriage (Stevens *et al.*, 1975).

The immune responses to HBV and their role in the pathogenesis of hepatitis B are incompletely understood. In acute, self-limited hepatitis B virus infection, strong T cell give responses to many HBV antigens are readily demonstrable in the peripheral blood. These responses involve both major histocompatibility complex (MHC) class II restricted, CD4+ helper T cells and MHC class I–restricted, CD8+ cytotoxic T lymphocytes. The antiviral cytotoxic T-lymphocyte response is directed against multiple epitopes within the HBV core, polymerase, and envelope proteins; strong helper T-cell responses to C and P proteins have also been demonstrated in acute infection. By contrast, in chronic carriers of HBV, such virus-specific T-cell responses are greatly attenuated, at least as assayed in cells from the peripheral blood. However, antibody responses are vigorous and sustained in both situations (although free antibodies against HBsAg (anti-HBs antibodies) are not detectable in carriers because of the excess of circulating HBsAg). This pattern strongly suggests that T-cell responses, especially the responses of cytotoxic T lymphocytes, play a central role in viral clearance (Chisari and Ferrari, 1995; Chisari, 1996).

The mechanisms by which cytotoxic T lymphocytes kill liver cells causing viral clearance have been incisively investigated in transgenic mice that express viral antigens or contain replication-competent viral genomes in the liver. Because these mice harbor HBV genes in their germ-line DNA, they are largely tolerant to HBV proteins, and accordingly, clinically significant liver injury does not develop. However, if antiviral cytotoxic T lymphocytes of syngeneic animals are transferred into such mice, acute liver injury with many of the features of clinical hepatitis B develops. It is striking that, in this model, the number of hepatocytes killed by direct engagement between cytotoxic T lymphocytes and their targets is very small and clearly insufficient to account for most of the liver damage. This suggests that much of the injury is due to secondary antigen-nonspecific inflammatory responses that are set in motion

by the response of the cytotoxic T lymphocytes. Presumably, much of the damage occurring in this context is due to cytotoxic by-products of the inflammatory response, such as tumor necrosis factor (TNF), free radicals, and proteases. Other immune-cell populations, notably natural killer T cells, probably also contribute to liver injury (Guidotti *et al.*, 1996).

Recent experiments suggest that some of the inflammatory by-products, notably interferon- γ (IFN-γ) and TNF-α, can have antiviral effects that do not involve killing the target cells. When cytotoxic T lymphocytes are transferred to mice that bear replicating HBV, viral DNA and RNA throughout the liver rapidly disappear, even from viable, uninjured hepatocytes, an effect that can be blocked by the administration of antibodies to TNF-α and IFN-γ. Such non-cytocidal antiviral effects may be important for viral clearance in natural infection. In fact, cytokine release triggered by unrelated hepatic infections in HBV-transgenic mice can have the same effect. This phenomenon may explain the suppression and occasional clearance of chronic HBV infection in patients with superimposed acute hepatitis caused by unrelated viruses (Cavanaugh *et al.*, 1998; Baron *et al.*, 2002; Ganem and Prince, 2004).

2.6. Natural immune responses to HBV

2.6.1. Innate immunity

Important effectors of innate immunity defensive lines are type I Interferons (IFN-α and IFN-β) and proinflammatory cytokines such as interleukins. Toll-like receptors (TLRs), a pathogen recognition receptor, mediate the production of type I IFNs, which in turn stimulates antigen-presenting cells (APCs) such as dendritic cells and Kupffer cells that could lead to the production of interleukin-8 (IL-8), IL-12, IL-18 and other cytokines (Boehme and Compton, 2004).

During the first weeks of HBV infection, no changes in the expression of intrahepatic genes are observed (Wieland *et al.*, 2004). Therefore, it seems that HBV cannot induce strong innate immune responses such as induction of type I interferon and production of proinflammatory cytokines early in the course of infection (Wieland *et al.*, 2004). However, *in vitro* studies indicated that innate immunity of hepatocytes may sense and limit the HBV infection (Lucifora *et al.*, 2010).

2.6.2. Cellular immunity

A fundamental role of T-cell responses in HBV clearance was shown in a chimpanzee model showing that *in vivo* depletion of CD4+ or CD8+ T cells prevents HBV clearance. The strength

of specific T cell responses determines the outcome of HBV infection. Previous studies suggested that strong, polyclonal, and multi-specific CD8+ and CD4+ T cell responses are correlated with acute self-limited HBV infection whereas weak and limited focused T cell responses are observed more often in chronic HBV infection (Bertoletti and Ferrari, 2012). HBeAg induces a T-helper 2 (Th2) immune response whereas HBcAg stimulates a Th1 response. Additionally, polymerase and X antigens can also induce CD4+ T cell responses (Chang and Lewin, 2007).

The Th2 response to HBeAg outperforms the Th1 response to the HBcAg. Therefore, the HBcAg-specific T cells have been shown to be depleted *in vivo* (Huang *et al.*, 2006). Interestingly, it seems that different doses of virus generate different responses. A Th1-mediated response is produced by low doses of the virus while a Th2-mediated response is produced by high doses of the virus (Huang *et al.*, 2006).

CD8+ T cells recognize HBV epitopes, especially HBcAg epitopes that are presented on the surface of infected liver cells through HLA class I molecules. Upon recognition of infected cells, cytotoxic T lymphocytes begin a direct cell killing process along with secretion of IFN-γ and TNF-α (tumor necrosis factor). These two cytokines induce non-cytolytic down regulation of HBV replication through multiple mechanisms. Even though it is produced by HBV specific CD8+ T cells, IFN-γ produced by macrophages, NKT cells, and HBV-non-specific T cells in response to other pathogens such as the choriomeningitis virus can also down regulate HBV replication (Kakimi *et al.*, 2000).

2.6.3. Humoral immunity

Acute HBV infection recovery results in a lifelong protective immunity. HBsAg-specific antibodies, as well as HBV-specific CD4+ and CD8+ T cells, are responsible for this protection. HBV DNA disappearance from blood and liver is followed by maximal CD4+ and CD8+ T cell responses in the liver and blood, maximum alanine aminotransferase (ALT) levels, and the presence of HBeAg and HBsAg specific antibodies in the blood (Wieland *et al.*, 2004). In addition to the role of humoral immunity in HBV protection, HBV-specific antibodies could help understand different stages of the disease. HBcAg specific IgM is the first antibody detected in the course of infection, whereas antibodies against HBeAg and HBsAg appear late and imply a better prognosis. The appearance of HBsAg-specific antibodies is correlated with protective immunity. Both HBsAg-specific antibodies and HBcAg-specific IgG persist lifelong after clinical recovery (Fattovich *et al.*, 2008).

Even after clinical recovery of acute hepatitis B infection and mediation of lifelong protective immunity, minimal amounts of HBV persist in the blood and are controlled by adaptive immune responses. Accordingly, clinically recovered patients with protective HBsAg and HBeAg-specific antibodies may experience reactivation of HBV after immunosuppression due to cancer chemotherapy (Kawatani *et al.*, 2001). Moreover, organ transplantation from HBsAg specific antibody positive donors may transmit HBV to immunosuppressed transplant recipients (Chazouilleres *et al.*, 1994). Some studies suggest that booster vaccinations are required for maintenance of vaccine-induced HBV specific humoral and cellular responses. However, this is still a controversy because others consider the antigen-specific B and T cells' immunological memory to be sufficient for efficient responses against later infections, even when vaccine-induced antibodies is reduced to undetectable levels (Rahman *et al.*, 2000).

2.7. Transmission and risk factors of HBV

HBV is spread through contact with infected body fluids and the only natural host is human. Blood is the most important vehicle for transmission, but other body fluids have also been implicated, including semen and saliva. Currently, three modes of HBV transmission have been recognized: perinatal, sexual and parenteral/percutaneous transmission. There is no reliable evidence that airborne infections occur and feces are not a source of infection. HBV is not transmitted by contaminated food or water, insects or other vectors (Hou *et al.*, 2005).

Perinatal Transmission

Transmission of HBV from carrier mothers to their babies can occur during the perinatal period, and appears to be the most important factor in determining the prevalence of the infection in high endemicity areas, particularly in China and Southeast Asia. Before HBV vaccine was integrated into the routine immunization program, the proportion of babies that become HBV carriers is about 10-30% for mothers who are HBsAg-positive but HBeAg-negative. However, the incidence of perinatal infection is even greater, around 70-90%, when the mother is both HBsAg-positive and HBeAg-positive (Stevens *et al.*, 1979; Xu *et al.*, 1985). There are three possible routes of transmission of HBV from infected mothers to infants: transplacental transmission of HBV in utero; natal transmission during delivery; or postnatal transmission during care or through breast milk. Since transplacental transmission occurs antenatally, hepatitis B vaccine and HBIG cannot block this route. Epidemiological studies on HBV intrauterine infection in China showed that intrauterine infection occurs in 3.7-9.9% pregnancy women with positive HBsAg and in 9.8-17.39% with positive HBsAg/HBeAg (Xu

et al., 1998, 2002)and it was suggested that a mother with positive HBeAg (OR = 17. 07) and a history of threatened premature labor (OR = 5. 44) are the main risk factors for intrauterine infection. The studies on transplacental transmission of HBV suggested two possible mechanisms (1) hemagenous route: a certain of factors, such as threaten abortion, can make the placental microvascular broken, thus the high-titer HBV maternal blood leak into fetus' circulation (Lin *et al.*, 1987; Ohto *et al.*, 1987); (2) cellular transfer: the placental tissue is infected by high titer of HBV in maternal blood from mother's side to fetus' step by step, and finally, HBV reach fetus' circulation through the villous capillary endothelial cells (Xu *et al.*, 1998, 2001, 2002; Yan *et al.*, 1999). For neonates and children younger than 1 year who acquire HBV infection perinatally, the risk of the infection becoming chronic is 90% (Hyams, 1995), presumably because neonates have an immature immune system. One of the possible reasons for the high rate of chronicity is that transplacental passage of HBeAg may induce immunological tolerance to HBV in fetus. (Hou *et al.*, 2005)

Sexual Transmission

Sexual transmission of hepatitis B is a major source of infection in all areas of the world, especially in the low endemic areas, such as North America. Hepatitis B is considered to be a sexually transmitted disease (STD). For a long time, homosexual men have been considered to be at the highest risk of infection due to sexual contact (70% of homosexual men were infected after 5 years of sexual activity) (Alter, 2003). However, heterosexual transmission accounts for an increasing proportion of HBV infections. In heterosexuals, factors associated with increased risk of HBV infection include duration of sexual activity, number of sexual partners, history of sexual transmitted disease, and positive serology for syphilis. Sexual partners of injection drug users, prostitutes, and clients of prostitutes are at particularly high risk for infection (Alter and Mast, 1994).

Parenteral/percutaneous Transmission

The parenteral transmission includes injection drug use, transfusions and dialysis, acupuncture, working in a health-care setting, tattooing and household contact. In the United States and Western Europe, injection drug use remains a very important mode of HBV transmission (23% of all patients) (Margolis *et al.*, 1991). Risk of acquiring infection increases with duration of injection drug use. Although the risk for transfusion-associate HBV infection has been greatly reduced since the screening of blood for HBV markers and the exclusion of donors who engage in high-risk activities, the transmission is still possible when the blood donors are

asymptomatic carrier with HBsAg negative (Luo *et al.*, 1993). Obvious sources of infection include HBV-contaminated blood and blood products, with contaminated surgical instruments and utensils being other possible hazards. Parenteral/percutaneous transmission can occur during surgery, after needle-stick injuries, intravenous drug use, and following procedures such as ear piercing, tattooing, acupuncture, circumcision and scarification. The nosocomial spread of HBV infection in the hospital, particularly in dialysis units, as well as in dental units, has been well described (Margolis *et al.*, 1991), even when infection control practices are followed. As with other modes of transmission, high vial titers have been related to an increased risk of transmission. People at high-risk of infection include those requiring frequent transfusions or hemodialysis, physicians, dentists, nurses and other healthcare workers, laboratory technicians, intravenous drug users, police, firemen, laundry workers and others who are likely to come into contact with potentially infected blood and blood products. The risk of chronicity is low (less than 5%) for transmission through sexual contact, intravenous drug use, acupuncture, and transfusion (Hyams, 1995). Individuals at risk for these transmission modes usually acquire HBV infection during adolescence or adulthood without immune tolerance. Instead, the disease progresses directly to the immune clearance phase and are of short duration, which probably accounts for high spontaneous recovery.(Hou *et al.*, 2005)

2.8. Diagnosis of HBV

Serological markers are used to diagnose and monitor Hepatitis B virus infection. The first antigen present is the Hepatitis B surface antigen (HBsAg) and is the only antigen present during the first 3-5 weeks in newly infected individuals. HBsAg is also useful because it is detectable before clinical symptoms appear. People who chronically carry HBsAg can be considered infectious. The body normally produces antibodies to HBsAg as part of the normal immune response. Individuals who have anti-HBs antibody have had a past infection and are not susceptible to reinfection. Anti-HBs also develop in those who have been successfully vaccinated against Hepatitis B (Rivier and Helen, 2015).

The hepatitis B core antigen (HBcAg) is serologically significant because it is present in the nuclei of hepatocytes only during an acute infection. In an acute infection, the antibody to HBc, anti-HBc, develops before anti-Hbs. Anti-HBc appears at the onset of symptoms and can persist for life. The presence of anti-HBc indicates previous or ongoing infection with HBV in an undefined timeframe. However, the IgM antibody to hepatitis B core antigen positively indicates recent infection with HBV (< 6 months). Its presence indicates an acute infection.

The hepatitis B e antigen or HBeAg is the secreted product of the nucleocapsid gene of HBV that is found in serum during acute and chronic hepatitis B. Its presence indicates that the virus is replicating and the infected person has high levels of HBV and is highly infectious. The presence of HBeAg and HBsAg generally indicates a poor prognosis and is indicative of a chronic infection with chronic liver disease. Alternatively, the presence of anti-HBe means recovery and a very low infectivity. Conversion from e antigen to e antibody is a predictor of long-term clearance of HBV in patients undergoing antiviral therapy and indicates lower levels of HBV (Rivier and Helen, 2015).

HBV antigens and antibodies are an excellent way to determine the status of the individual. Another way to measure disease progression is by the use of polymerase chain reaction (PCR) technique to isolate specific HBV DNA. PCR is highly specific and can be used to monitor effectiveness of antiviral therapy. Other ways to measure disease progression are liver function tests, blood electrolytes, CT-scan of the liver to look for extent of damage, and liver biopsy (Rivier and Helen, 2015).

2.9. Prevention and control

Three main strategies are available for the prevention of HBV infection: behavior modification to prevent disease transmission, passive immunoprophylaxis, and active immunization (Kedir Yimer, 2008).

I) Behavior Modification

Improved screening measures of blood products have reduced the risk of transfusion-associated hepatitis. Behavior modification is thought to be more beneficial in developed countries than in developing countries, where neonates and children in early childhood are at the greatest risk of acquiring infection. In these groups, immunoprophylaxis, both passive and active, will be more effective (Alter, 2003).

II) Passive Immunoprophylaxis

Hepatitis B Immune Globulin (HBIG) is a sterile solution of ready-made antibodies against hepatitis B. HBIG is prepared from human blood from selected donors who already have a high level of antibodies to hepatitis B and used in passive immunoprophylaxis. Passive immunoprophylaxis is used in five situations (1) after needle stick exposure (2) newborns of mothers infected with hepatitis B; (3) after sexual exposure, and (4) after liver transplantation 5) after contamination of the eye (Banatvala and Van Damme, 2003).

III) Active Immunization

Immunization against HBV has made this infection a vaccine preventable infectious disease. The first vaccines to be licensed (1981) were plasma derived, but these have largely been replaced by recombinant derived ones, which were introduced in 1986. In 1991, the WHO recommended that HB vaccine should be introduced into the Expanded Programme of Immunization (EPI). The objectives of vaccination against hepatitis B are primarily to prevent infection, thereby reducing the incidence of persistent HBV infection and chronic liver disease, and in addition eliminating the pool of chronic carriers, thus limiting transmission of infection to susceptible contacts (Bock *et al.*, 1995).

3. Materials and Methods

3.1. Description of the study area

Woldia is located 360 kilometers away from Bahir Dar and 521 kilometers from Addis Ababa. It is found in northeast Ethiopia. The global positioning system coordinates of Woldia in terms of latitude and longitude indicate that 11° 49’ 59.99” N and 39° 40’ 59.99” E, respectively. The elevation is 2,112 meter above sea level. The average annual temperature is 18.5°C while the average annual rainfall is 994mm. Woldia is a business center which connects roads from Addis Ababa, Bahir Dar and Mekelle. Due to this, it has a high flow of transportation, both passenger and business. It also serves as a rest point for a high number of heavy trucks. For this reason, the majority of the population leads their lives doing small business activities. Woldia has an estimated total population of 46,139 people. Out of this 23, 000 are males and 23,139 are females. In Woldia there is one General Hospital and two Health Centers (as Endeshaw Abateneh *et al.*, 2018). Woldia Health Center has a total of 75 employees (26 males and 49 females) including 5 health officers, 6 clinical nurses, 3 laboratory technicians and 4 midwives.

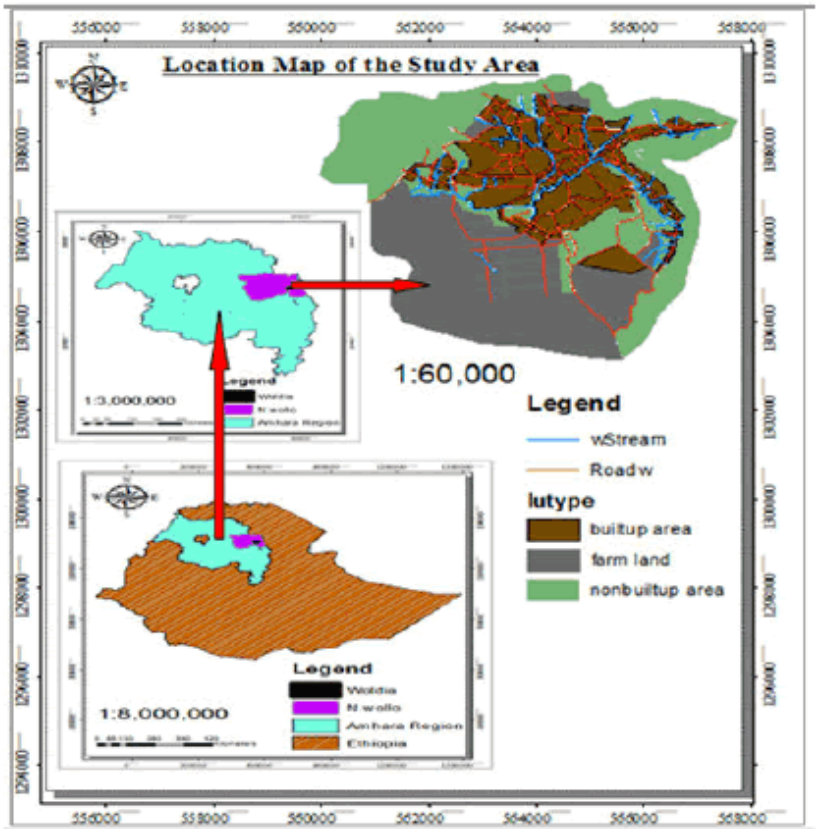

Figure 2: Map of the study area (www.omocsonline.org)

3.2. Study design and period

Hospital based cross-sectional study was conducted from November 2018 to February 2019 so as to assess the prevalence and associated risk factors of HBV among individuals visiting Woldia Health Center, Woldia, Northeast Ethiopia.

3.3. Source and study population

All individuals who were visiting Woldia Health Center served as source population whereas those who visited the center during the period of sample collection served as study population.

3.4. Sampling technique and sample size

Study participants were selected by using simple random sampling technique. The sample size was estimated by using statistical formula (Naing *et al.*, 2006). As prevalence of these infectious diseases was not known in the study area, the sample size of the proposed study was calculated using 50 % prevalence. Based on this, sample size was calculated to be 384, but in order to minimize the error 10% of the sample size was added and the total sample size was 422.

$$n = \frac{Z^2}{d^2} P(1 - P)$$

Where n= sample size

d = margin of error (5 %)

P = prevalence (50 %)

z = critical value at 5 % level (1.96)

3.5. Data collection method

A pre-tested structured questionnaire was delivered to voluntary individuals to obtain socio-demographic information including sex, age, occupation, educational status, and other information on risk factors for transmission of HBV including a history of previous blood transfusion, place of previous delivery, any surgical procedure, tattooing, and hospital admission. The questionnaires were first prepared in English then translated into Amharic.

After completion of the questionnaire, about 5ml of blood sample was aseptically collected by venipuncture from each participant into sterile test tube because the blood was withdrawn for various types of blood tests. The blood samples were left to form clots; after which they were centrifuged for 10 minutes at 2000 revolutions per minute (rpm) to separate serum from clot.

Sample testing was done using rapid strip test which detects HBsAg through visual interpretation of color development on the internal strip. Anti-HBsAg antibodies are immobilized on the test region of the membrane. During testing, the specimen reacts with anti-HBsAg antibodies conjugated to colored particles and precoated onto the sample pad of the test. The mixture then migrates through the membrane by capillary action, and interacts with reagents on the membrane (Assure Tech (Hangzhou) co., Ltd., Zhejiang, China). The separated serum was allowed to equilibrate to room temperature (15-30^{0}C). To perform the laboratory test, the sealed pouch was removed, and the strip was placed on a clean, level surface. Three drops of serum were transferred to the sample pad of the strip with the micro-pipette. Finally, waited until the colored band(s) was appeared. The result was read after 15 minutes.

Interpretation of the result was made as follows: individuals were considered positive when two colored bands appeared on the membrane of the test strip; when one band appeared in the control region (C) and the another band appeared in the test region (T).Whereas the individuals were considered negative when only one colored band appeared in the control region (C) and no apparent colored band appeared in the test region (T). The test result was considered invalid when control bands failed to appear on the test strip. In this case the procedure was repeated with a new test strip.

3.6. Data analysis

The association between risk factors and HBsAg was determined by using Chi-square test and then logistic regression analysis was employed to measure the strength of the association between dependent and independent variables. Variables with p-value less than 0.05 were considered as significant. Crude odds ratio (COR) with 95% confidence interval was determined and then for the identification of the most explanatory variables in multivariate logistic regression, adjusted odds ratio (AOR) was calculated using variables which had p-value less than 0.25 in univariate logistic regression analysis (Bursac *et al.*, 2008). The data were processed by using SPSS version 20.

3.7. Study variables

3.7.1. Dependent variable

Serum status of HBsAg was dependent variable which was studied.

3.7.2. Independent variables

Socio-demographic characteristics like, age, educational status, occupational status, and marital status, history of blood transfusion, history of abortion, surgical procedure, dental surgery, tattooing, unsafe injection, history of house hold contact, and multiple sexual exposures were independent variables.

3.8. Inclusion and exclusion criteria

All individuals who were visiting Woldia Health Center and who were willing to participate in the study were included. However, people who were HBV positive, those who were vaccinated against the virus, those who were on antiviral treatment, who were seriously ill and those who were not willing to participate in the study were not included.

3.9. Validity and reliability of the study

To increase the degree of validity and reliability of the study, all the required information was collected after pre-testing the questionnaire. By taking 5 % of the sample size, the questionnaire was tested to check whether it was understandable or not. The quality control for HBsAg test kits was monitored by using positive and negative control samples before testing the patients' serum, and in every opening of new kits.

3.10. Ethical Considerations

Ethical clearance and permission was obtained from Ethical Clearance Committee of College of Science, Bahir Dar University, and then letter of support was written to Woldia Health Center.

The study participants were informed about the purpose of the study and the importance of their participation in the study. And also they were informed about their right to participate in the study, or not participate; stop at any time in between data collection or decline to answer some of the questions if they felt uncomfortable without losing the benefit that they would get from the institution. Their participation was purely on voluntary basis. They were asked to sign on the Amharic written consent and patient information sheet for this particular study. Those study subjects who were positive for HBsAg were informed about their status and they were followed up by physicians working at the health center.

4. Results

4.1. Socio-demographic characteristics and history of exposure of study participants

4.1.1. Socio-demographic characteristics of study participants

A total of 422 individuals were participated in the study. Due to incomplete data, seven of the participants were excluded, leaving only 415 individuals for result analysis. The minimum and maximum age of the study participants were 4 and 80 years, respectively. The mean (±SD) age of the study participants was 26.87 ± 10.53. Females take the majority of the study participants, 302 (72.8 %), while the male were 113 (27.2%). Most of the study participants, 259 (62.4%), were married. One hundred sixty (38.6%) study participants attended 7 – 10 grade. One hundred seven (25.8%) and 105 (25.3%) engaged in private job and housewife, respectively. Out of the total study participants, only 12 (2.9%) and 133 (32.0%) were smoker and alcoholic, respectively. Only 58 (14.0%) participants had a history of hepatitis diagnosis before this study (Table 1).

Table 1: Socio-demographic characteristics of individuals visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Frequency	Percentage
Age (year)		
≤ 10.00	13	3.1
11.00 - 20.00	79	19.0
21.00 - 30.00	226	54.5
31.00 - 40.00	56	13.5
41.00 - 50.00	23	5.5
51.00 - 60.00	15	3.6
61.00 - 70.00	2	0.5
71.00+	1	0.2
Sex		
Female	302	72.8
Male	113	27.2
Marital status		
Married	259	62.4
Single	131	31.6
Divorced	16	3.9
Widowed	9	2.2

Educational status		
Illiterate	46	11.1
Read and write	6	1.4
1-6 Grade	84	20.2
7-10 Grade	160	38.6
11-12 Grade	18	4.3
>12 Grade	101	24.3
Occupation		
Jobless	21	5.1
Government employee	88	21.2
Farmer	27	6.5
House wife	105	25.3
Private	107	25.8
Student	67	16.1
Smoking		
No	403	97.1
Yes	12	2.9
Alcohol drinking		
No	282	68.0
Yes	133	32.0
Hepatitis diagnosis history		
No	357	86.0
Yes	58	14.0

4.1.2. History of exposure of the study participants

Among the 415 study participants, 78 (18.8%) were hospitalized before, 39 (9.4%) had surgical operation and 10 (2.4%) received blood transfusions. Twelve (2.9%) participants had previous experience of liver disease while 10 (2.4%) had previous experience of TB and cancer disease. Of study participants, 120 (28.9%) had multiple sexual partners. Most participants, 304 (73.3%), were ear pierced whereas 137 (33%) had tattoo. One hundred ten (26.5%) participants shared sharp materials with others (Table 2).

Table 2: History of exposure of individuals to HBV infection visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Frequency	Percentage
Previous hospital admission		
No	337	81.2
Yes	78	18.8
Surgical Operation		
No	376	90.6
Yes	39	9.4
Blood transfusion		
No	405	97.6
Yes	10	2.4
Intravenous drug use		
No	414	99.8
Yes	1	0.2
Tattoo		
No	278	67.0
Yes	137	33.0
Hemodialysis		
No	370	89.2
Yes	45	10.8
Previous experience of liver disease		
No	403	97.1
Yes	12	2.9
Previous experience of TB and cancer		
No	405	97.6
Yes	10	2.4
Receiving corticosteroids or immunosuppressive drugs		
No	371	89.4
Yes	44	10.6
Multiple sexual partners		
No	295	71.1
Yes	120	28.9
Tooth extraction		
No	351	84.6
Yes	64	15.4
Ear piercing		
No	111	26.7
Yes	304	73.3
Sharing sharp materials		
No	305	73.5
Yes	110	26.5

4.1.3. History of exposure to HBV among female participants

Additional factors associated with giving birth and abortion were considered for female participants only. From the 302 female study participants, 53 (17.5%) had abortion. One hundred forty-six (48.3 %) female participants had children and of whom 108 (74.0%) delivered at hospital (Table 3).

Table 3: Potential risk factors of HBV infection associated with female study participants visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Frequency	Percentage
History of abortion		
No	249	82.5
Yes	53	17.5
Do you have children?		
No	156	51.7
Yes	146	48.3
Place of delivery		
Home	38	26.0
Hospital	108	74.0
Number of children		
One child	63	43.2
Two children	42	28.8
Three children	28	19.2
More than three children	13	8.9

4.2. Prevalence of HBsAg and its association with socio-demographic characteristics and exposure history of study participants

4.2.1. Prevalence of HBsAg and its association with socio-demographic characteristics

In this study, 14 (3.37%) of the 415 participants were positive for HBsAg. Of the total HBsAg positive individuals, eight (57.14%) were males. Six (42.88%) of the positive participants were in the age group of 21-30 years while seven (50%) of the positive participants were married. Seven (50%) attended 7-10th grade education and six (42.88%) had private jobs. Eleven (78.57%) of the participants with HBsAg positive results were not diagnosed for hepatitis before this study (Table 4)

The association between the HBsAg test result and the socio-demographic characteristics of study participants were tested by using pearson chi-square (χ^2) test of association. The result of the test showed that age, marital status, educational status, occupation, habit of alcohol drinking, and hepatitis diagnosis were not statistically significant. While sex (χ^2 (1) = 6.54, *P* = 0.011) and smoking (χ^2 (1) = 6.69, *P* = 0.010) were statistically significant (Table 4).

Table 4: The relationship between socio-demographic characteristics and HBV infection among individuals visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Total (415)	Hepatitis B test result		P-Value
		Negative (401)	Positive (14)	
Age (year)				
<= 10.00	13	13 (100%)	0 (0%)	
11.00 - 20.00	79	78 (98.73%)	1 (1.27%)	
21.00 - 30.00	226	220 (97.35%)	6 (2.65%)	
31.00 - 40.00	56	51 (91.07%)	5 (8.93%)	0.345
41.00 - 50.00	23	22 (95.65%)	1 (4.35%)	
51.00 - 60.00	15	14 (93.33%)	1 (6.67%)	
61.00 - 70.00	2	2 (100%)	0 (0%)	
71.00+	1	1 (100%)	0 (0%)	
Sex				
Female	302	296 (98.01%)	6 (1.99%)	0.011*
Male	113	105 (92.92%)	8 (7.08%)	
Marital status				
Married	259	252 (97.3%)	7 (2.7%)	
Single	131	126 (96.18%)	5 (3.82%)	0.184
Divorced	16	14 (87.5%)	2 (12.5%)	
Widowed	9	9 (100%)	0 (0%)	
Educational status				
Illiterate	46	44 (95.65%)	2 (4.35%)	
Read and write	6	6 (100%)	0 (0%)	
1-6 Grade	84	82 (97.62%)	2 (2.38%)	0.855
7-10 Grade	160	153 (95.63%)	7 (4.38%)	
11-12 Grade	18	17 (94.44%)	1 (5.56%)	
>12 Grade	101	99 (98.02%)	2 (1.98%)	
Occupation				
Jobless	21	21 (100%)	0 (0%)	
Government employee	88	84 (95.45%)	4 (4.55%)	
Farmer	27	26 (96.3%)	1 (3.7%)	0.395
House wife	105	102 (97.14%)	3 (2.86%)	
Private	107	101 (94.39%)	6 (5.61%)	
Student	67	67 (100%)	0 (0%)	

Smoking				
No	403	391 (97.02%)	12 (2.98%)	0.010*
Yes	12	10 (83.33%)	2 (16.67%)	
Alcohol drinking				
No	282	275 (97.52%)	7 (2.48%)	0.143
Yes	133	126 (94.74%)	7 (5.26%)	
Hepatitis diagnosis				
No	357	346 (96.92%)	11 (3.08%)	0.413
Yes	58	55 (94.83%)	3 (5.17%)	

** Statistically significant at P<0.05*

4.2.2. Pevalence of HBsAg and its association with risk factors

Of the total HBsAg positive individuals, six (42.86%) had tattoos on their body, seven (50%) had ear piercings, ten (71.43%) had multiple sexual partners (Table 5). Three (21.43%) of the positive participants had previous experience with liver disease, two (14.29%) had experience of TB or cancer (Table 5).

The result from Chi-square test analysis showed that some of the risk factors were statistically significant and the others were not. Previous experience of liver disease (χ^2 (1) = 17.3, *P* <0.001), previous experience of TB and cancer (χ^2 (1) = 8.69, *P* = 0.003), having multiple sexual partners ($\chi 2$ (1) = 12.74, *P* < 0.001), and ear piercing (χ^2 (1) = 3.99, *P* = 0.046) showed statistical significant association with HBsAg. While the other risk factors such as sharing sharp materials, tooth extraction, tattoo, hemodialysis and the rest were not associated with HBV infection (*P*> 0.05) (Table 5).

Table 5: The relationship between risk factors and HBV infection among individuals visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Total (415)	**Hepatitis B test result**		**P-Value**
		Negative (401)	**Positive (14)**	
Previous hospital admission				
No	337	327 (97.03%)	10 (2.97%)	0.341
Yes	78	74 (94.87%)	4 (5.13%)	
Surgical Operation				
No	376	363 (96.54%)	13 (3.46%)	0.769
Yes	39	38 (97.44%)	1 (2.56%)	
Blood transfusion				
No	405	391 (96.54%)	14 (3.46%)	0.550
Yes	10	10 (100%)	0 (0%)	

Intravenous drug use				
No	414	400 (96.62%)	14 (3.38%)	0.852
Yes	1	1 (100%)	0 (0%)	
Tattoo				
No	278	270 (97.12%)	8 (2.88%)	0.426
Yes	137	131 (95.62%)	6 (4.38%)	
Hemodialysis				
No	370	358 (96.76%)	12 (3.24%)	0.673
Yes	45	43 (95.56%)	2 (4.44%)	
Previous experience of liver disease				
No	403	392 (97.27%)	11 (2.73%)	<0.001*
Yes	12	9 (75%)	3 (25%)	
Previous experience of TB and cancer				
No	405	393 (97.04%)	12 (2.96%)	0.003*
Yes	10	8 (80%)	2 (20%)	
Receiving corticosteroids or immunosuppressive drugs				
No	371	360 (97.04%)	11 (2.96%)	0.181
Yes	44	41 (93.18%)	3 (6.82%)	
Multiple sexual partners				
No	295	291 (98.64%)	4 (1.36%)	<0.001*
Yes	120	110 (91.67%)	10 (8.33%)	
Tooth extraction				
No	351	337 (96.01%)	14 (3.99%)	0.104
Yes	64	64 (100%)	0 (0%)	
Ear piercing				
No	111	104 (93.69%)	7 (6.31%)	0.046*
Yes	304	297 (97.7%)	7 (2.3%)	
Sharing sharp materials				
No	305	293 (96.07%)	12 (3.93%)	0.292
Yes	110	108 (98.18%)	2 (1.82%)	

** Statistically significant at P<0.05*

4.2.3. Prevalence of HBsAg and its risk factors associated with female participants

From the 302 females who took part in this study, six (1.99%) were positive for HBsAg. Out of these six, four (66.67%) of them had experienced abortion. All six (100%) positive female participants had children. From these, four (66.67%) delivered their children at hospitals while two (33.33%) delivered at home (Table 6).

The chi-square test result showed that, the variables abortion (χ^2 (1) = 10.21, P = 0.001) and having children (χ^2 (1) = 6.54, P = 0.011) showed statistical association with HBV infection

whereas place of delivery and number of children were not statistically associated with HBV infection (Table 6).

Table 6: The relationship between risk factors and HBV infection among females visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Total	Hepatitis B test result		P-Value
		Negative	Positive	
History of abortion				
No	249	247 (99.2%)	2 (0.8%)	0.001*
Yes	53	49 (92.45%)	4 (7.55%)	
Total	302	296	6	
Do you have children?				
No	156	156 (100%)	0 (0%)	0.011*
Yes	146	140 (95.89%)	6 (4.11%)	
Total	302	296	6	
Place of delivery				
Home	38	36 (94.74%)	2 (5.26%)	0.677
Hospital	108	104 (96.3%)	4 (3.7%)	
Total	146	140	6	
Number of children				
One child	63	59 (93.65%)	4 (6.35%)	0.433
Two children	42	41 (97.62%)	1 (2.38%)	
Three children	28	28 (100%)	0 (0%)	
More than three children	13	12 (92.31%)	1 (7.69%)	
Total	146	140	6	

** Statistically significant at P<0.05*

4.3. Univariate analysis of risk factors in relation to the prevalence of HBsAg

4.3.1. Univariate analysis of socio-demographic characteristics

Univariate logistic regression analysis was used to determine the strength of association between the characters and HBsAg test result by calculating crude odds ratio. The result showed that high strength for some characteristics; the odds for males to get HBsAg were about four times higher than females (COR = 3.759, 95% CI: 1.274-11.087), and the odds for smokers to get HBsAg were about seven times higher than non-smokers (COR = 6.517, 95% CI: 1.286-33.035) and these associations were statistically significant with *P*-value of 0.016 and *P*= 0.024, respectively. The chance for alcoholics to get HBsAg was 2.183 times higher than non-alcoholics (COR = 2.183, 95% CI: 0.750-6.354), but the association was not statistically significant (*P*> 0.05) (Table 7).

Table 7: Univariate analysis of socio-demographic characteristics in relation to HBV infection among individuals visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Total (415)	Hepatitis B test result		COR	95% CI	P-Value
		Negative (401)	Positive (14)			
Age (year)						
<= 10.00	13	13 (100%)	0 (0%)			
11.00 - 20.00	79	78 (98.73%)	1 (1.27%)			
21.00 - 30.00	226	220 (97.35%)	6 (2.65%)			
31.00 - 40.00	56	51 (91.07%)	5 (8.93%)	-	-	-
41.00 - 50.00	23	22 (95.65%)	1 (4.35%)			
51.00 - 60.00	15	14 (93.33%)	1 (6.67%)			
61.00 - 70.00	2	2 (100%)	0 (0%)			
71.00+	1	1 (100%)	0 (0%)			
Sex						
Female	302	296 (98.01%)	6 (1.99%)	1.0		0.016*
Male	113	105 (92.92%)	8 (7.08%)	3.759	1.274 - 11.087	
Marital status						
Married	259	252 (97.3%)	7 (2.7%)	1.0		
Single	131	126 (96.18%)	5 (3.82%)	1.429	0.445 - 4.591	0.292
Divorced	16	14 (87.5%)	2 (12.5%)	5.143	0.977 - 27.077	
Widowed	9	9 (100%)	0 (0%)	-	-	
Educational status						
Illiterate	46	44 (95.65%)	2 (4.35%)	2.250	0.307 - 16.491	
Read and write	6	6 (100%)	0 (0%)	-	-	
1-6 Grade	84	82 (97.62%)	2 (2.38%)	1.207	0.166 - 8.759	0.897
7-10 Grade	160	153 (95.63%)	7 (4.38%)	2.265	0.461 - 11.124	
11-12 Grade	18	17 (94.44%)	1 (5.56%)	2.912	0.250 - 33.911	
>12 Grade	101	99 (98.02%)	2 (1.98%)	1.0		
Occupation						
Jobless	21	21 (100%)	0 (0%)			
Government employee	88	84 (95.45%)	4 (4.55%)			
Farmer	27	26 (96.3%)	1 (3.7%)	-	-	-
House wife	105	102 (97.14%)	3 (2.86%)			
Private	107	101 (94.39%)	6 (5.61%)			
Student	67	67 (100%)	0 (0%)			
Smoking						
No	403	391 (97.02%)	12 (2.98%)	1.0		0.024*
Yes	12	10 (83.33%)	2 (16.67%)	6.517	1.286 - 33.035	

Alcohol drinking						
No	282	275 (97.52%)	7 (2.48%)	1.0		0.152
Yes	133	126 (94.74%)	7 (5.26%)	2.183	0.750 - 6.354	
Hepatitis diagnosis						
No	357	346 (96.92%)	11 (3.08%)	1.0		0.419
Yes	58	55 (94.83%)	3 (5.17%)	0.583	0.158 - 2.156	

COR – Crude odds ratio

CI – Confidence interval

** Statistically significant at P<0.05*

4.3.2. Univariate analysis of medical history and personal practices potentially associated with HBV infection

Similar with socio-demographic characteristics, the strength of association was also done for medical history and personal practices. The odds of getting HBV infection for individuals who had previous experience of liver disease were about 12 times higher than those individuals who had no previous experience (COR= 11.879, 95% CI: 2.822-50.010) and the association was statistically significant (*P*= 0.001). Individuals with previous experience of TB and cancer had 8.19 times higher odds to get HBV than those who did not (COR =8.187, 95% CI: 1.568-42.740) and the association was statistically significant (*P*= 0.013). The odds of being infected with HBV infection among individuals who had multiple sexual partners were about seven times higher than those individuals who had no multiple sexual partners (COR= 6.614, 95% CI: 2.032-21.525), and the association was statistically significant (*P*= 0.002) (Table 8).

Table 8: Univariate analysis of medical history and personal practices in relation to HBV infection among individuals visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Total (415)	**Hepatitis B test result**		**COR**	**95% CI**	**P-Value**
		Negative (401)	**Positive (14)**			
Previous hospital admission						
No	337	327 (97.03%)	10 (2.97%)	1.0		0.347
Yes	78	74 (94.87%)	4 (5.13%)	1.768	0.540 - 5.791	
Surgical Operation						
No	376	363 (96.54%)	13 (3.46%)	1.0		0.770
Yes	39	38 (97.44%)	1 (2.56%)	0.735	0.094 - 5.772	
Blood transfusion						
No	405	391 (96.54%)	14 (3.46%)	-	-	-
Yes	10	10 (100%)	0 (0%)			

Intravenous drug use						
No	414	400 (96.62%)	14 (3.38%)	-	-	-
Yes	1	1 (100%)	0 (0%)			
Tattoo						
No	278	270 (97.12%)	8 (2.88%)	1.0		0.429
Yes	137	131 (95.62%)	6 (4.38%)	1.546	0.526 - 4.547	
Hemodialysis						
No	370	358 (96.76%)	12 (3.24%)	1.0		0.675
Yes	45	43 (95.56%)	2 (4.44%)	1.388	0.300 - 6.408	
Previous experience of liver disease						
No	403	392 (97.27%)	11 (2.73%)	1.0		0.001*
Yes	12	9 (75%)	3 (25%)	11.879	2.822 - 50.010	
Previous experience of TB and cancer						
No	405	393 (97.04%)	12 (2.96%)	1.0		0.013*
Yes	10	8 (80%)	2 (20%)	8.187	1.568 - 42.740	
Receiving corticosteroids or immunosuppressive drugs						
No	371	360 (97.04%)	11 (2.96%)	1.0		0.194
Yes	44	41 (93.18%)	3 (6.82%)	2.395	0.642 - 8.936	
Multiple sexual partners						
No	295	291 (98.64%)	4 (1.36%)	1.0		0.002*
Yes	120	110 (91.67%)	10 (8.33%)	6.614	2.032 - 21.525	
Tooth extraction						
No	351	337 (96.01%)	14 (3.99%)	-	-	-
Yes	64	64 (100%)	0 (0%)			
Ear piercing						
No	111	104 (93.69%)	7 (6.31%)	1.0		0.055
Yes	304	297 (97.7%)	7 (2.3%)	0.350	0.120 - 1.022	
Sharing sharp materials						
No	305	293 (96.07%)	12 (3.93%)	1.0	0.100 - 2.053	0.304
Yes	110	108 (98.18%)	2 (1.82%)	0.452		

COR – Crude odds ratio
CI – Confidence interval
** Statistically significant at P<0.05*

4.3.3. Univariate analysis of risk factors associated with female participants

The analysis showed that females who had abortion were about 10 times at risk to get HBV than females who had no abortion ((COR) = 10.082, 95% CI: 1.797-56.574), and the association was statistically significant (*P*= 0.009) (Table 9).

Table 9: Univariate analysis of risk factors in relation to HBV infection among females visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Total	Hepatitis B test result		COR	95% CI	P- Value
		Negative	Positive			
History of abortion						
No	249	247 (99.2%)	2 (0.8%)	1.0		0.009*
Yes	53	49 (92.45%)	4 (7.55%)	10.082	1.797 - 56.574	
Total	302	296	6			
Do you have children?						
No	156	156 (100%)	0 (0%)			
Yes	146	140 (95.89%)	6 (4.11%)	-	-	-
Total	302	296	6			
Place of delivery						
Home	38	36 (94.74%)	2 (5.26%)	0.692	0.122 - 3.941	0.679
Hospital	108	104 (96.3%)	4 (3.7%)	1.0		
Total	146	140	6			
Number of children						
One child	63	59 (93.65%)	4 (6.35%)	1.0		
Two children	42	41 (97.62%)	1 (2.38%)	.360	0.039 - 3.336	
Three children	28	28 (100%)	0 (0%)	-	-	0.815
More than three children	13	12 (92.31%)	1 (7.69%)	1.229	0.126 - 11.987	
Total	146	140	6			

COR – Crude odds ratio
CI – Confidence interval
** Statistically significant at P<0.05*

4.4. Multivariate logistic regression analysis of selected variables

All socio-demographic characteristics and other medical history and personal practices with P-value less than 0.25 in univariate analysis were selected and entered for multivariate logistic regression analysis to identify the most important explanatory variables of HBV infection. In the univariate analysis sex, smoking, alcohol, previous experience of liver disease, previous experience of TB or cancer, receiving immunosuppressive drug, having multiple sexual partners, ear piercing, and history of abortion showed statistically significant association with

HBsAg seropositivity. The only significant explanatory variables for HBV infection in the present study subject was previous experience of liver disease (AOR= 30.69; 95% CI 2.031-463.708, *P*= 0.013) (Table 10).

Table 10: Multivariate analysis of selected variables in relation to HBV infection among individuals visiting Woldia Health Center, northeast Ethiopia in 2018-2019

	Total	Hepatitis B test result		AOR	95% CI	P-Value
		Negative	Positive			
Smoking						
No	403	391 (97.02%)	12 (2.98%)			
Yes	12	10 (83.33%)	2 (16.67%)	-	-	-
Total	415	401	14			
Alcohol drinking						
No	282	275 (97.52%)	7 (2.48%)	1.0		
Yes	133	126 (94.74%)	7 (5.26%)	8.434	0.675 – 105.334	0.098
Total	415	401	14			
Previous experience of liver disease						
No	403	392 (97.27%)	11 (2.73%)	1.0		
Yes	12	9 (75%)	3 (25%)	30.685	2.031 – 463.708	0.013*
Total	415	401	14			
Previous experience of TB and cancer						
No	405	393 (97.04%)	12 (2.96%)	1.0		
Yes	10	8 (80%)	2 (20%)	42.303	0.880 – 2033.577	0.058
Total	415	401	14			
Receiving corticosteroids or immunosuppressive drugs						
No	371	360 (97.04%)	11 (2.96%)	1.0		
Yes	44	41 (93.18%)	3 (6.82%)	0.362	0.020 – 6.562	0.492
Total	415	401	14			
Multiple sexual partners						
No	295	291 (98.64%)	4 (1.36%)	1.0		
Yes	120	110 (91.67%)	10 (8.33%)	6.917	0.635 – 75.379	0.113
Total	415	401	14			

Ear piercing						
No	111	104 (93.69%)	7 (6.31%)			
Yes	304	297 (97.7%)	7 (2.3%)	-	-	-
Total	415	401	14			
History of abortion						
No	249	247 (99.2%)	2 (0.8%)	1.0		
Yes	53	49 (92.45%)	4 (7.55%)	5.070	0.567 – 45.372	0.147
Total	302	296	6			

AOR – Adjusted odds ratio

CI – Confidence interval

** Statistically significant at P<0.05*

5. Discussion

According to the existence of HBV marker in the body, the world is divided into three areas of high (> 8%), intermediate (2-8%) and low (< 2%) HBV endemicity (Chen and Chang, 2010). Knowing the prevalence and risk factors are important for planning measures for hepatitis B infection. In addition, the study of prevalence is important to provide a basis of action, education and clinical practice.

In this study, the prevalence of HBsAg was 3.37%, which was intermediate endemicity. This finding was almost similar with 3.1% reported from Gojjam, northwest Ethiopia (Bayeh Abera *et al.*, 2017), 4.4% among pregnant women in Felege Hiwot Referral Hospital (c, 2.6% among health care workers and medical waste handlers in primary hospitals of northwest Ethiopia (Endalew Yizengaw *et al.*, 2018), and 3.7% among diabetic individuals attending Woldia General Hospital (Daniel Mekonnen *et al.*, 2014). A slightly high prevalence (5.7%) of HBsAg was reported from Debre Markos among people attending HIV VCT center (Abebe Mazengia *et al.*, 2013). a study from Tigray also showed 5.9% among individuals attending ART clinic at Mekele Hospital (Letebrhan Weldemhret *et al.*, 2016). In addition, in Eritrea the prevalence of HBsAg among pregnant women was 3.2% (Nahom Fessehaye *et al.*, 2018), the prevalence of HBsAg in India was 3.9% (Khatoon and Jahan, 2016), and 4.04% in China (Yang *et al.*, 2017).

This study showed a relatively higher prevalence compared to prevalence rates reported elsewhere in the world. For example, a study conducted in Iran showed HBV prevalence in pregnant women to be 0.59% (Kheiri and Makvandi, 2015), 1.14% among blood donors in Iraq (Nawfal, 2018), and 1.81% in Morocco (Baha *et al.*, 2013), 0.5% among prisoners of Brazilian prison system (Silva *et al.*, 2017), and 1.8% in Rio de Jeneiro, in Southern Brazil (Villar *et al.*, 2014). This might be due to regular screening and vaccination of HBV may provide in their country.

The prevalence of HBsAg in this study was lower compared to prevalence rates reported in Arsi, Asella Referral and Teaching Hospital where the prevalence was 9.7% (Zerihun Belay, 2018), 6.9% at Deder Hospital eastern Ethiopia (Abdi Umare *et al.*, 2016), 10.9% among pregnant women at Assela Referral Hospital (Birhanu Betela *et al.*, 2018), 7.7% in Cameroon (Abongwa *et al.*, 2016), 31.3% among pregnant women at Hargeisa Group Hospital, Somaliland (Al-Mamari, 2019), 13.6% in Nigeria (Musa *et al.*, 2015), 9.1% in Sudan (Badawi

et al., 2018), and 7.9% in south-western Saudi Arabia (Al-Humayed, 2017). This might be due to different sample population, difference in socioeconomic status and traditional practices.

The present study showed that the prevalence of HBV was higher among males than among females (7.08% and 1.99% respectively) which is similar with the study conducted in Debre Markos (6.3% and 4.8% respectively) (Abebe Mazengia *et al.*, 2013), and in Mekele, Tigray (9.4% and 3.6% respectively) (Letebrhan Weldemhret *et al.*, 2016). In this study, sex (being male or female) was significantly associated with HBV infection and males were about four times more likely to expose with HBV infection than females and this finding was in agreement with the study done in Mekele, Tigray Ethiopia in which males were 2.8 times more exposed to HBV infection than females (Letebrhan Weldemhret *et al.*, 2016). The reason behind this might be due to their job nature as males travel more frequently than females and this activity might have exposed them to multiple sexual partners, and thus highly likely of getting the infection. Also, males are most likely to begin early sex than females which could be another reason for exposure of males to HBV infection than females (O'Donnell *et al.*, 2001; Imaledo *et al.*, 2012).

In the current study, of the total positive cases, the most frequent cases were detected among those with age group 21-30 years but the prevalence was higher among the age group 31-40 years which accounts 8.93%. Similarly, in the study done in Cameroon, the prevalence of HBV infection was highest among the age group of 25-34 years (11.8%) (Abongwa *et al.*, 2016). The possible reason might be at this age the individuals might be sexually active and HBV infection is transmitted through sexual intercourse due to this higher prevalence of HBV infection in this age group. In contrast to our study, a study done at Bishoftu Hospital, Oromia Regional State, of the total positive cases, the majority were detected among those with the age group of 25-29, but the prevalence was higher among the old age group, above 41 years old (50%) (Zelalem Desalegn *et al.*, 2016).

The current study showed that smoking and HBV infection had statistically significant association and the odds for smokers to get HBV were about seven times higher than non-smokers but this finding contradicted with the study done in Woldia General Hospital, Woldia, Ethiopia (Daniel Mekonnen *et al.*, 2014), the reason could be the two study subjects were different which is the latter study was only on diabetic patients.

The prevalence of HBV infection in the current study was significantly associated with having multiple sexual partners and it showed about seven times higher likely of getting HBV among

individuals having multiple sexual partners. In agreement with the current study, a study done in Mekele, Tigray Ethiopia showed that having multiple sexual partners was significantly associated with HBV infection (Letebrhan Weldemhret *et al.*, 2016). The study done in northwest Ethiopia among health care workers and medical waste handlers also showed similar association (Endalew Yizengaw *et al.*, 2018). Similar type of studies done in India (Khatoon and Jahan, 2016) and Cameroon (Abongwa *et al.*, 2016) agreed with the current study, in which a statistical significant association was shown between HBV and having multiple sexual partners and HBV infection. This could have ascribed to one of the major transmission routes of HBV infection which is sexual intercourse.

The result was contradicted to the result from study conducted in East Wolega Zone, West Oromia Ethiopia (Regea Dabsu and Eyasu Ejeta, 2018). This difference might be due to the reason that our study included individuals visiting Woldia Health Center, but the study in Wolega focused only on pregnant women, so the chance of having multiple sexual partners is not like in the presentt study. In addition, the study conducted in Woldia General Hospital on diabetes patients showed no significant association between HBV infection and having multiple sexual partners (Daniel Mekonnen *et al.*, 2014) this may be due to the difference in studied subjects and sampling technique where in the latter study only diabetic patients were involved. Furthermore, the variation might also have ascribed to sample size difference. The sample size of the current study was 415 individuals, whereas that of Daniel Mekonnen *et al.* was 216.

In this study, socio-demographic characteristics like, age, marital status, occupation, and educational status were not significantly associated with HBsAg positivity. Similarly, history of exposure of blood transfusion, tooth extraction, tattoo, unsafe injection and history of surgery did not show statistically significant association with HBV infection. Additionally, previous hospital admission, surgical operation, tattoo, hemodialysis, tooth extraction, ear piercing, sharing sharp materials, and place of delivery did not show significant association with HBV infection.

The current study showed that abortion and HBV infection had statistical significant association, which showed that females who had abortion were 10 times at risk of getting HBV infection than females who had no abortion. This finding was consistent with the study done at Deder Hospital, eastern Ethiopia among pregnant women attending antenatal care clinic (Abdi Umare *et al.*, 2016), at Bishoftu General Hospital, Ethiopia (Zelalem Desalegn *et al.*, 2016),

and in Iran (Kheiri and Makvandi, 2015). On the other hand, our finding was not in agreement with the study done at Debre Markos Referral Hospital (Abebe Mazengia *et al.*, 2013) and at Mekelle Hospital, Tigray Ethiopia (Letebrhan Weldemhret *et al.*, 2016). In both studies, abortion and HBV infection were not significantly associated.

In the present study, previous exposure to liver disease was significantly associated with HBV infection. Similarly, the study done among cleaners in Addis Ababa, Ethiopia showed found there is a significant association between previous experience of liver disease and HBV infection (Asmare Mekonnen *et al.*, 2015).

6. Conclusion

An intermediate prevalence (3.37%) of HBsAg was found in this study. In the current study, previous experience of liver disease, having multiple sexual partners, previous experience of TB or cancer, and abortion were significantly associated with HBV infection. Previous experience of liver disease was the most explanatory risk factor for HBV infection. No statistically significant association was observed between the acquisition of HBV infection and socio-demographic characteristics except sex and smoking. Similarly, no significant association was seen between HBV infection and other variables like surgical operation, tattoo, ear piercing.

7. Recommendation

Health information on the risk factors, mode of transmission, treatment, prevention and health impact of HBV should be administered. As having multiple sexual partners is major mode of transmission, further detailed study is required at community level to reduce the transmission of HBV in Woldia town and its surrounding. In the future large-scale studies which employ additional serological markers (to distinguish active Vs. chronic infections) and molecular techniques are required.

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Appendix

Appendix 1 – Informed Consent and Patient Information Sheet (English Version)

My name is Selamawit Getachew and I am M.Sc student in Biomedical Science at BDU. I am doing a research entitled "Prevalence and associated risk factors of Hepatitis B Virus surface antigen (HBsAg) among individuals visiting Woldia Health Center".

The objective of the study is to determine the prevalence of Hepatitis B among individuals visiting Woldia Health Center and to investigate possible association between risk factors and prevalence of Hepatitis B. If you are agree to participate in the study, about 5 ml of blood will be collected from you or you will allow us to use the sample that you will give for your medical examination and you will be interviewed. During collection of blood, you may feel some discomfort, but this does not produce serious pain. All the data obtained will be kept strictly confidential by using only code numbers and locking the data, only study personnel will have access to the files. Anonymous testing will be undertaken, that is the sample will be coded and positive results will not be identified by names. There will be no cost on you as a result of taking part in this study and you are not asked to pay for the laboratory examination. Your contributions will help for professionals in health sector for effective prevention and control of the disease. Your participation is purely voluntary, and you can withdraw any time after you get involved in the study. You can also jump (decline) to answer some of the questions if you feel uncomfortable. Participation and not participation has no influence on the service you seek to get.

Participant's response: I am free to decline to be in this study, or to withdraw from it at any point and also to jump a question that feels me discomfort. She promised that no costs are needed for participating in the study as well as for the laboratory examination. My decision as to whether or not to participate in this study will have no influence on my present or future medical service.

My signature below indicates that I agree to participate in this study.

Subject's signature_____ Date of signature_____

Signature of Person Obtaining Consent_____ Date of signature_____

Appendix 2 – Informed Consent and Patient Information Sheet (Amharic Version)

በጥናቱ ለሚሳተፉ ግለሰቦች የፈቃድ መጠየቂያ እና መቀበያ ፎርም/ሺት/

ሰላማዊት ጌታቸው እባላለሁ:: የባ/ዳር ዩኒቨርሲቲ የባዮሜዲካል ሳይንስ የማስተርስ ድግሪ ተማሪ ነኝ ፣በአሁኑ ሰአት የጉበት/የወፍ/ በሽታ የሚያመጣውን ሄፓታይተስ የተባለውን ቫይረስ ስርጭት ለማወቅ ጥናት እያካሄድኩ ነው ።

የጥናቱ አላማ ሄፓታይተስ የሚያመጣውን የጉበት/የወፍ/ በሽታ ስርጭት እንዲሁም በስርጭቱ እና ተጓዳኝ ምክንያቶች ያለውን አንድነት መኖሩን እና አለመኖሩን ለማየት ነው። እርስዎ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ 5 ሚ.ሊ.. ደም ይሰጣሉ ወይም ለሌላ ምርመራ የሰጡትን ደም እንድንጠቀም ይፈቅዱልናል ፣ ለቃለ መጠይቅም ይተባበሩናል። ደም በሚሰጡበት ሰዓት የተወሰነ የህመም ስሜት ይኖረዋል ነገር ግን ይህ ምንም አይነት የከፋ ጉዳት አያደርስም። የሚሰጡት መረጃ ሁሉም ሚስጥራዊነቱ የተጠበቀ ነው፣ በስም አይገለጽም።በጥናቱ በመሳተፈዎ ምንም አይነት ክፍያ አይጠየቁም ወይም የሚያገኙት ገንዘብ አይኖርም፣ ነገር ግን በጥናቱ በመሳተፍዎ የጤና ባለሙያዎች በሽታውን መቆጣጠር እና መከላከል ላይ ተግተው እንዲሰሩ ያደርጋቸዋል። በጥናቱ የሚሳተፉት ፈቃ□ኛ ከሆኑ ብቻ ነው።ስለዚህ መሳተፍ፣ አለመሳተፍ፣ ከጀመሩ በኋላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ካለ ይለፈኝ የማለት ሙሉ መብት አለዎ። በጥናቱ መሳተፍ ወይም አለመሳተፍ በሚያገኙት አገልግሎት ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት አይኖረውም።

የተሳታፊው ፈቃድ መግለጫ፡ መሳተፍ፣ አለመሳተፍ፣ ከጀመሩ በኋላ ማቋረጥ ወይም መመለስ የማልፈልገው ጥያቄ ካለ ይለፈኝ ማለት እንደምችል፤ በጥናቱ መሳተፍ ወይም አለመሳተፍ በማገኘው አገልግሎት ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት እንደሌለው እና በመሳተፌ ምንም አይነት ክፍያ እንደማይሰጠኝ እና እኔም እንደማልከፍል ተገልጾልኛል። የጥናቱ አላማ ግልጽ ስለሆነልኝ ለመሳተፍ ተስማምቻለሁ ። ፊርማየንም እንደሚከተለው አስቀምጫለሁ።

የጥናቱ ተሳታፊ ፊርማ _____ ቀን_____

ፈቃደኝነትን የጠየቀው ሰው ፊርማ _____ ቀን_____

Appendix 3 – Questionnaire (English Version)

Questionnaire to be filled by each participant

Interviewer: ---------- Date of interview: ----------

I. Identification

1. Code number----------

II. Socio demographic variables

1. Age_____
   - <5
   - 5-18
   - 19-45
   - 46-60
   - >60
2. Sex:
   - Male
   - Female
3. Marital status:
   - Single
   - Married
   - Divorced
   - Widowed
4. Educational status:
   - Illiterate
   - Reading and writing
   - 1-6 Grade
   - 7-10grade
   - 11-12 grade
   - >12+
5. Occupation:
   - Have no job
   - Private employee
   - Government
   - Farmer
   - House wife
   - Sex worker/prostitute/
   - Student
6. Active smoking:
   - Yes
   - No
7. Alcohol consumptions:
   - Yes
   - No
8. Have you tasted before?
   - Yes
   - No

III. History of exposure for potentially associated with the risk of hepatitis

1. Hospital Admission:
   - Yes
   - No
2. Surgical Operation:
   - Yes
   - No
3. Blood Transfusions:
   - Yes
   - No
4. Intravenous Drug Use:
   - Yes
   - No
5. Tattooing:
   - Yes
   - No

6. Hemodialysis (Chronic Renal Failure, Catheterization):

- Yes
- No

7. Previous Experiences of Jaundice (Liver Disease):

- Yes
- No

8. Leukemia, lymphoma,TB and cancer:

- Yes
- No

9. Receiving Corticosteroids or Immunosuppressive Drugs:

- Yes
- No

10. History of Multiple Sexual Exposures:

- Yes
- No

11. Tooth Extraction:

- Yes
- No

12. Have you pierced your ear

- Yes
- No

13. Do you share sharp things with others

- Yes
- No

IV. For Females Only):

1. Abortion
- Yes
- No

1. Do you have children
    - Yes
    - No
2. Where did you gave birth?
    - At hospital
    - At home
3. How many children do you have?
    - One
    - Two
    - Three
    - More than three

Appendix 4 – Questionnaire (Amharic Version)

በጥናቱ ተሳታፊ በሆኑ ግለሰቦች የሚሞላ መጠይቅ ፎርም

የጠያቂው ስም_____ቀን_____መለያ ቁጥር_____

I. የግለሰቡ አካሊዊ ማህበራዊ እና ኢኮኖሚያዊ ዝርዝር

ሁኔታ

1. *ዕድሜ_____*
   - <5
   - 6-18
   - 19-45
   - 46-60
   - >60
2. **ጸታ**:
   - **ሴት**
   - **ወንድ**
3. **የጋብቻ ሁኔታ**:
   - **ያላገባ**
   - **ያገባ**
   - **የተፋቱ**
   - **የትዳር ጓደኛዎ የሞተብዎ**
4. **የትምህርት ሁኔታ**:
   - **ያልተማረ**
   - **ማንበብ እና መጻፍ ብቻ**
   - **ከ1-6 ክፍል**
   - **ከ7-10 ክፍል**
   - **ከ 11-12ኛ ክፍል**
   - **ከ12 ክፍል በላይ**
5. **ስራ**:
   - **ስራ አጥ**
   - **የመንግስት ሰራተኛ**
   - **ገበሬ**
   - **የቤት እመቤት**
   - **ሴትኛ አዳሪ**
   - **የግል ስራ**
   - **ተማሪ**
6. **ሲጋራ ያጨሳለ**:
   - **አዎ አጨሳለሁ**
   - **አላጨስም**
7. **መጠጥ ይጠጣለ**:
   - **አዎ**
   - **አልጠጣም**
8. **የጉበት ምርመራ አድርገዉ ያዉቃሉ**
   - **አዎ**
   - **አላውቅም**

III. በቫይረስ የሚመጣ የጉበት በሽታ የተጋሊጭነት ዝርዝር

ሁኔታ

1. **ሆስፒታል ተኝተው ያውቃሉ**:
   - **አዎ**
   - **ተኝቼ አላውቅም**
2. **ቀዶ ጥገና ተሰርተዉ ያውቃሉ**:
   - **አዎ**
   - **አላውቅም**
3. **ደም ተልግሰው ያውቃሉ**:
   - **አዎ**
   - **አያውቅም**
4. **ዕጽነት ያለው በደም ስር የሚወሰድ መድሃኒት ይወስዳሉ?**

- አዎ
- አልወስድም

5. ለጌጥ ብለው በሰውነትዎ ሊይ ያደረጉት ንቅሳት አለ:

- አዎ
- የለም

6. የቆየ የኩላሊት ህመም አለብዎ:

- አዎ
- የለብኝም

7. የጉበት ህመም ታመው ያውቃለ:

- አዎ
- አሞኝ አያውቅም

8. ካንሰር ወይም ቲቢ አለብዎት

- አዎ
- የለብኝም

9. ሰውነት በሺታን የመከላከል አቅም ዕንዲቀንስ የሚያደርግ መድሃኒት ይወስዳሉ:

- አዎ
- አልወስድም

10. ከአንድ ሰው በላይ የግብረ ስጋ ጓደኛ ኑሮዎት ያውቃል:

- አዎ
- ኑሮኝ አያውቅም

11. ጥርስ አስነቅለው ያውቃሉ:

- አዎ
- አስነቅየ አላውቅም

12. ጆሮዎን ተበስተው ያውቃሉ

- አዎ
- አላውቅም

13. ስለታማ ነገሮችን በጋራ ተጠቅመው ያውቃሉ

- አዎ
- አላውቅም

IV. ለሴቶች ብቻ

1. ጽንስ አቋርጠዉ ያዉቃሉ
   - አዎ
   - አላውቅም
2. ልጆች አለዎት
   - አዎ
   - የለኝም
3. ልጅ የት ነው የወለዱት
   - ሆስፒታል
   - ቤት
4. ስንት ልጆች አለዎት
   - አንድ
   - ሁለት
   - ሶስት
   - ከሶስት በላይ