# ADDIS ABABA UNIVERSITY

# SCHOOL OF GRADUATE STUDIES

# Hepatitis B virus infection among HIV infected individuals with and without antiretroviral therapy in North Shoa Zone, Amhara region, Ethiopia

BY

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June, 2011

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**A thesis Submitted to the Department of Microbiology, Immunology and Parasitology, Addis Ababa University in Partial Fulfilment of the Requirements for the Degree of Masters in Medical Microbiology**

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## Acknowledgements

My sincere and deepest gratitude goes to my advisor and instructor Dr. Solomon G/Selassie (Associate professor, Microbiology) for his unreserved assistance in giving me timely comments and relevant guidance from the beginning of the research proposal to the write-up of the final thesis paper and his understanding of my problems and possible solutions he forwarded.

I am grateful to the DebreBerhan Hospital for granting approval of this study and communicating to the different departments of the Hospital

My hearty respect goes to the owner and general manager of International Clinical Laboratories, Ato Tamrat Beqele who sponsored the full cost of the confirmatory tests.

I am also very grateful and would like to extend my heartfelt thanks and appreciation to the study participants, the data collectors and the staff at the institutions involved for their full participation, responsible data collection and support.

Last but not least, I would like to acknowledge AAU, School of Graduate studies for sponsoring this study financially and the Department of Medical Microbiology, Immunology and Parasitology for the supports rendered to me in accomplishing this thesis.

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## Abbreviations and Acronyms

3TC- Lamivudin

ALT-Alanine TransAminase

ART-Anti Retroviral Therapy

AST- Aspartate TransAminase

ART- Antiretroviral therapy

CD- Cluster of differentiation molecules

CD4 count- CD4+ T-cell (T-lymphocyte bearing CD4 receptor)

D4t- Stavudine

ELISA -Enzyme-linked immunosorbent assay

FTC- Emtricitabine

HAART- Highly Active Antiretroviral Therapy

HBcAg-Hepatitis B core antigen

HBeAg- Hepatitis e Antigen

HbsAg- Hepatitis B surface Antigen

HBV- Hepatitis B Virus

HCC- Hepatocellular carcinoma

HIV- Human immunodeficiency virus

GSHV- Ground squirrel hepatitis virus.

NNRTI - Non-nucleoside reverse transcriptase inhibitor

NRTI - Nucleoside reverse transcriptase inhibitor

PCR- Polymerase chain reaction

TB- Tuberculosis

TDF- Tenofovir

USAID-United States Agency for International Development

WHO- World Health Organization

WHV- Woodchuck hepatitis virus

WMHV- Woolly monkey hepatitis B virus

# Abstract

**Background**- The introduction of highly active antiretroviral treatment (HAART) has greatly decreased morbidity and mortality in HIV-infected individuals. In this regard, HBV co infection with HIV is becoming a major challenge. Because of shared routes of transmission, 90% of people living with HIV have serological markers of HBV infection and 5-15% of them chronically infected with Hepatitis B Virus. Conditions associated with hepatitis B infection are currently among the leading causes of hospital admission and recent studies have shown increasing rates of liver disease and related death among those with HIV.
The impact of co-infection is especially apparent in regions with widespread use of highly active antiretroviral therapy (HAART) where HBV co infection increase hepatotoxicity of HAART and delay immune recovery

**Objective-** To determine the prevalence of hepatitis B virus (HBV) infection among HIV/infected individuals attending care and treatment services in North Shoa zone

**Methods –** A Cohort study was conducted in North Shoa zone from November 2010 to May 2011.HIV infected individuals who were grouped into antiretroviral treatment initiated and Pre- treatment follow up were included in the study. Socio-demographic and clinical data were collected from patient interview, intake form, follow up form and medical record review using structured questionnaire. HBV sero-status was determined by testing presence of Hepatitis B surface antigen using 100ul of serum or plasma detected by SD BIOLINE HBsAg rapid kit confirmed with AxSYM HBsAg(V2) confirmatory test from blood collected for patients follow up. Levels of Alanine transaminase and Aspartate transaminase enzymes and $CD4^{+}$ count were recorded from laboratory registry and patient follow up forms. Usage of HAART was included to assess if treatment change the natural history of HBV infection. Comparison groups were HBV positive antiretroviral receiving patients and HBV positive antiretroviral Naïve ones.

**Results**- The cumulative prevalence of HBsAg in HIV infected individuals was 3.9%.The prevalence was higher in ART initiated than Pre-ART groups, 5.3% and 2.6%, respectively. Despite the difference is not significant. Sex was independently associated with HBsAg prevalence (P=0.03). Males were in increased risk of developing a positive HBsAg test result. (RR=2.32 95%CI: 1.09, 4.96) HBV/HIV co infection was a strong predictor of sharp drop in CD4 cell recovery before starting ART (RR=3.98 95%CI: 1.02,15.48) There was no significant difference observed in the rate of immune recovery and incidence of hepatotoxicity between hepatitis B virus Co-infected and non infected individuals after initiation of ART.
Hepatitis B Co-infected individuals isolated were found to be in the chronic Hepatitis B stage with low or moderate Alanine and Aspartate transaminases levels. (41.5IU/L)

**Conclusion-** The prevalence of hepatitis B infection is higher in ART initiated individuals than Pre-ART. Neither HBsAg sero positivity nor a particular ART regimen affect immune recovery in ART initiated individuals. Since chronically infected individuals are the first candidates of HIV/HBV treatment all markers of HBV especially HBV DNA should be determined to initiate treatment. Since HBsAg positivity is higher in individuals taking combination therapies that has dual effect for HBV and HIV, further study is necessary to identify the cause. Recommendation was forwarded according to the findings.

Key words: HBV, HIV, HAART, Co-infection, HBsAg serostatus, Northshoa

# 1. Introduction

## *1.1. Background*

A person who is infected with both HIV and hepatitis B viruses is said to have a **HIV/HBV Co-infection.(Hepatitis B foundation, 2009)**

Infection with HIV and hepatitis B virus (HBV) are often found in the same individual because of shared routes of transmission. (Sexual intercourse and blood transfusion are the most important routes) (Omland etal, 2008) Many of the countries with a high HBV disease burden are also affected by a high HIV burden, leading to frequent HIV/HBV co-infection. (Hoffmann and Thio, 2007)

Hepatitis B virus (HBV) is the leading cause of chronic liver disease and liver-related death worldwide, with the majority of these cases occurring in areas of Africa and Asia where HBV prevalence is high. (Population prevalence is greater than 8%). Around the world, 90% of HIV-infected persons have biological signs of prior HBV infection (defined by the presence of serum anti-HBcAb) and 5%–15% suffer from chronic infection (defined by the presence of serum HBsAg).(Omland et al, 2008) As a consequence, 2–4 million of the 33 million people living with HIV globally are also co-infected with chronic hepatitis B.(Lacombe et al, 2010).Conditions associated with hepatitis B and C are currently among the leading causes of hospital admission and recent studies have shown increasing rates of liver disease and related death among those with HIV. (Kenneth et al, 2007)

The impact of HIV and HBV co-infection is especially apparent in regions with widespread use of highly active antiretroviral therapy (HAART).The introduction of HAART has led to the emergence of liver related disease and mortality as HBV infection increases HAART related hepatotoxicity(Peters, 2007 ; Puoti et al, 2002) .

## *1.2. Statement of the problem*

Since the introduction of highly active antiretroviral treatment (HAART), morbidity and mortality have decreased greatly in HIV-infected individuals. The management of other non HIV associated chronic diseases in HIV patients has become increasingly important. In these regard HBV co infection with HIV is becoming a major challenge

In acknowledging this problem, an international forum was convened in Jackson Hole, Wyoming in September 2006, recommending the search of treatment options for HIV and HBV co –infected patients. A key topic of conversation was the development of new agents for treating viral hepatitis in patients with HIV though Challenges including the risk of hepatic injury and low patient tolerance, which limits compliance, will accompany the upcoming treatment. (Sherman et al, 2006)

Thereafter, some therapeutic drugs designed to slow HIV replication has been known to slow the replication of HBV as well in patients co-infected with HIV and HBV (EurekAlert and U. S. Medical center, 2006)

In the treatment of HIV/HBV co-infection, a number of treatment options are recommended.

Since there is not a 'cure' at this time for hepatitis B, the main goal of treating HBV/HIV-co-infection is to stop or slow down HBV viral activity as much as possible and for as long as possible and prevention of HIV and HBV reverse transcriptase resistance mutations. Several nucleosides and nucleotides used as part of a combination antiretroviral regimen have activity against HBV.

Treatment with regimens containing tenofovir (TDF) with either lamivudin (3TC) or emtricitabine (FTC) has been found to be effective. (Hoffmann, 2007).In HIV-HBV co-infected patients; anti-retroviral (ARV) drugs should be selected and monitored to minimize the risk of HBV and HIV resistance.

Despite these great upcoming challenge there is limited information regarding the prevalence of hepatitis co-infection amongst HIV positive individuals in Africa (Nelson, 2008). In Ethiopia, previous population-based surveys have reported medium to high endemicity of HBV infection. However, the magnitude of the infection in different risk groups including people living with HIV/AIDS is barely stated. (Techalew et al, 2008; Tesga et al, 1986; Abebe et al, 2003).

The rationale behind the current study was that Hepatitis B virus infection in immunocompromised individuals is inconceivable and unpredictable as inactive infection could spontaneously recur and result in end stage liver disease like cirrhosis and hepatocellular carcinoma.

Moreover, the association of HIV and HBV co-infection in the setting of HAART, Whether HBV co infection has any effect on HAART or vice versa? is not explored at large in Ethiopia context In consideration of the above discussion, this research is intended to answer the following questions

- ✓ What is the prevalence of HBV/HIV co infection among HIV patients attending care and treatment services?
- ✓ Is there any immune recovery difference(CD4 count) between antiretroviral therapy started patients who are co infected with HBV and those without chronic HBV infection?
- ✓ Does frequency and severity of hepatotoxicity to drugs differ between patients co infected with HBV and those without chronic HBV infection?

## *1.3. Literature review*

## Hepatitis B virus

It is estimated that 40% of the world's population has had contact with or are carriers of the hepatitis B virus (HBV). This corresponds to an estimated 350 million HBV carriers (Peters, 2007; Fix et al, 2007)

In 1965, Blumberg and colleagues - in search of tools to identify and track genetic differences in different human populations – found a novel antigen present in sera of Australian Aborigines (Blumberg et al, 1965). This antigen was preliminarily named Australia antigen now called as Hepatitis B surface antigen (HBsAg) (Murray et al, 2005) and was associated with a clinical course of hepatitis in the following years.

**Natural history and clinical manifestation**

HBV can be transmitted by transfusion of infected blood and carrying out healthcare procedures using contaminated instruments and other unsafe practices. Perinatal and sexual exposures to HBV are also highly efficient modes of transmission (Alter, 2006; Techalew et al, 2008)

**Acute Hepatitis**

After HBV transmission, the incubation period lasts from one to four months. A prodromal phase may appear before acute hepatitis develops. During this period a serum sickness-like syndrome with fever, skin rash, arthralgia and arthritis may develop without liver damage but elevated liver enzymes level. It will usually cease with the onset of hepatitis, which is manifested with right upper quadrant discomfort, nausea, jaundice and other unspecific constitutional symptoms. At least 70% of patients will then have subclinical or mild hepatitis, while less than 30% will develop icteric hepatitis. In case of co-infection with other hepatitis viruses or other underlying liver disease the clinical course may be more severe. The symptoms including jaundice generally disappear after one to three months, but some patients have prolonged fatigue even after normalisation of serum aminotransferase concentrations (Mauss et al, 2009)

Concentrations of alanine and aspartate aminotransferase levels (ALT and AST) may rise to 1000-2000 IU/L in the acute phase. ALT is typically higher than AST. Bilirubin concentration may be normal in a substantial portion of patients. In patients who recover, normalisation of serum aminotransferases usually occurs within one to four months.

Persistent elevation of serum ALT for more than six months indicates progression to chronic hepatitis (Mauss et al, 2009)

The rate of progression from acute to chronic hepatitis B is primarily determined by the age at infection and T-cell response (Murray et al, 2005). In adult-acquired infection the chronicity rate is 5% or less, whereas it is higher if acquired at younger ages. It is estimated to be approximately 90% for perinatally-acquired infection, and 20-50% for infections between the ages of one and five years. Patients who recover from acute hepatitis may carry HBV DNA for long periods of time even in presence of anti-HBs and anti-HBc. Complete eradication rarely occurs.

This is an important finding, as immunosuppression can lead to reactivation of the virus. Antiviral treatment of patients with acute hepatitis B usually is not recommended (Cornberg et al, 2007). The likelihood of fulminant hepatitis B is less than 1%, and the likelihood of progression to chronic hepatitis B is less than 5% in adults. Therefore, treatment of acute hepatitis B is mainly supportive in the majority of patients. Treatment can be considered in certain subsets of patients, e.g., patients with a severe or prolonged course of hepatitis B, patients co infected with other hepatitis viruses or underlying liver diseases, patients with immunosuppression

**Chronic Hepatitis**

Persistent Hepatitis B surface antigen for more than 6 months defines HBV chronic infection. Chronic active infection requires active HBV viral replication with presence of HBV DNA. Presence of HBsAg without detectable HBV DNA or HBeAg defines chronic carrier state. These patients usually have anti-HBeAg and normal liver chemistries. Small amounts of HBV DNA might be detected as long as HBsAg antigens are present (Brooks et al, 2007). This indicates that presence of HBsAg could diagnose chronic infection without determining the presence or absence of HBV DNA, HBeAg or anti-HBeAg.

Most patients with chronic hepatitis B are clinically asymptomatic. Some may have nonspecific symptoms such as fatigue. In most instances, significant clinical symptoms will develop only if liver disease progresses to decompensated cirrhosis with jaundice, ascites, peripheral oedema, and encephalopathy accompany it (Mauss et al, 2009)

Accordingly, physical examination will be normal in most instances. In advanced liver disease there may be stigmata of chronic liver disease such as splenomegaly, spider angiomata, Caput medusae, palmar erythema, testicular atrophy, gynecomastia, etc.

Laboratory testing shows mild to moderate elevation in serum AST and ALT in most patients, whereas normal transaminases occur rarely. In exacerbations of hepatitis B, ALT concentrations as high as 1000 mg/L may be observed. There are two different states that are distinguished in chronic HBV infection: firstly, a high-replicative state with active liver disease and elevated serum ALT. HBV DNA and HBeAg are present. Secondly, a low or non-replicative phase, where serum ALT may normalize, HBeAg disappears, and anti-HBe antibodies appear. In some patients, virus replication stops completely although they remain HBsAg-positive. These patients have undetectable HBV DNA in serum and normal ALT concentrations. No sign of ongoing liver damage or inflammation is found on liver biopsy. This state is called inactive carrier state. A small percentage of patients continue to have moderate levels of HBV replication and active liver disease (elevated serum ALT and chronic inflammation on liver biopsies). The first high-replicative phase may switch into the nonreplicative phase spontaneously or upon antiviral treatment. Conversely, the non-replicative phase may reactivate to the high-replicative phase either spontaneously or with immunosuppression (e.g., in HIV infection or with chemotherapy). Very few patients with chronic HBV infection become HBsAg negative in the natural course of infection. The annual rate of HBsAg clearance has been estimated to be less than 2%/year in Western patients and even lower (0.1 - 0.8%) in patients of Asian and African origin (Mauss et al, 2009)

**Diagnosis**

Over the last three decades, laboratory diagnostics of viral infections have become influenced more and more by molecular biology, the field of technology that has grown the fastest in this same period of time. Classical serologic and virology tests have advanced and sometimes been replaced by novel detection methods that rely on genome amplification procedures like PCR (Mauss et al, 2009)

After serologic screening is completed and a replicative HBV infection is assumed, the more expensive molecular methods such as HBV DNA are performed. This is generally to decide on whether to start treatment, to monitor treatment efficacy and treatment adherence, to identify resistant strains, and to identify pre-core mutant strains of HBV (Mauss et al, 2009)

The HBV genome is translated in overlapping ORFs, which limits the number of mutations that can be tolerated. As a result most mutant strains are produced in times of treatment or HBV vaccination to confer resistance.

These drug resistant mutant strains are less replication efficient and are mostly related to Asian ethinicity (Puoti et al, 2002 ). In addition HBV core mutations have not been proven to result in loss of immune recognition (WHO, 2002)

**Treatment**

Despite the availability of a prophylactic vaccine, with more than 350 million chronically infected individuals worldwide, chronic hepatitis B virus (HBV) infection remains a major global health concern. Chronically infected individuals carry a significantly increased risk of life-threatening liver complications such as hepatic decompensation, liver cirrhosis and hepatocellular carcinoma (HCC). Recent studies have shown that the level of serum HBV DNA for more than a decade correlates with the risk of developing cirrhosis and HCC (Chen et al, 2006)

Therefore, suppressing the replication of HBV to levels below the limit of detection of sensitive HBV DNA diagnostic tests has become a major goal in HBV treatment. For the treatment of chronic HBV infection two classes of agents are approved. The nucleoside analogues, Lamivudine, Telbivudine and Entecavir. The acyclic nucleotide analogues; Adefovir and Tenofovir which directly inhibit HBV DNA replication. Additionally, interferon -based therapies which modulate host immune response as well as viral replication are approved (Mauss et al, 2009)

## HIV/HBV co-infection

A person who is infected with both the HIV and the hepatitis B viruses is said to have a **HIV/ HBV Co-infection**. Infection with HIV and hepatitis B virus (HBV) are often found in the same individual because of shared routes of transmission. (Sexual intercourse and blood transfusion are the most important routes).

Globally an estimated 350–400 million people are chronically infected with hepatitis B virus (HBV) and 40 million are living with HIV infection today (Landes et al, 2008; Chang et al, 2009)

90% of HIV-infected persons have biological signs of prior HBV infection (defined by the presence of serum anti-HBcAb) and 5%–15% suffer from chronic infection (defined by the presence of serum HBsAg) (Hoffmann and Thio, 2007; Lacombe et al, 2010). Consequently, 2–4 million of the 33 million people living with HIV globally are also co-infected with chronic hepatitis B (Lacombe et al, 2010)

In the United States, up to 10% of all HIV-infected individuals have HBV co infection. (Fix et al, 2007). In Europe according to Euro-Sida cohort the prevalence of co infection is found to be 8.7% in hetero sexual and 5-10% in injection drug users (Peters, 2007)

In Africa the prevalence of co-infection according to small cohorts done in Africa is 9% in Tanzania, 16.5% in Malawi, 25.5% in Nigeria (Peters, 2007). In another comparative study from cote d'Ivoire, Malawi and Tanzania, the prevalence of co infection is found to be similar between HIV-uninfected and HIV-infected individuals (6.0-14.4% and 9.0-13.9%,respectively) (Hoffmann and Thio, 2007)

In Ethiopia even though a number of studies explore the prevalence of HIV and HBV independently, the prevalence of HBV co-infection among HIV patients is not well studied. One cross-sectional study done among people attending VCT clinic of St Paul's General Specialised Hospital in Addis Ababa found the prevalence to be 4.5% in HIV patients who are naïve for ART based on the HBsAg serological marker in serum(Techalew et al, 2008)

The prevalence of co infection in HIV patients who start ART is also fresh bread to touch. In a cohort study done in Thailand, a chronic HBV prevalence of 8.7% was reported among patients receiving ART. In Ethiopia the above study reported 2.9% prevalence (Hoffmann and Thio, 2007; Techalew et al, 2008)

**Diagnosis of HIV-HBV co-infection**

Diagnosis of HBV co-infection in HIV patients should be made based on HBsAg-positive, anti-HBc total positive, anti-HBs-positive status. If patients are HBsAg-positive, hepatitis B envelope antigen (HBeAg), anti-HBe, and HBV DNA should be measured. If patients are HBsAg negative, they should receive HBV vaccination (Peters, 2007; Puoti et al, 2002). But unlike the HBsAg which is an indicator of acute as well as chronic infection, the other testes are indicators of either patient infectivity or help management during treatment (HBV DNA), viral load (HBeAg) or overall prevalence (anti-HBc).

**Impact of co-infection on natural history of HIV and HBV**

Early studies done before effective HIV therapy is introduced suggested that HBV may be more mild in those co infected with HIV (Gilson et al, 1997; Bodsworth et al, 1989). These conclusions however, were based on lower serum aminotransferase levels and not on liver histology. In HIV-HBV co infected patients, HBV increase HIV replication, ART related hepatotoxicity and delay immune recovery ($CD_4^+$cell count) (Peters, 2007).

A study on extra-chromosomal sequences of HBV-DNA in peripheral mononuclear cells found that extra-chromosomal sequences of HBV-DNA are more prevalent among AIDS patients than among asymptomatic HIV carriers. Another *in vitro* study demonstrated that HBV-X protein (HBx) super-induces ongoing HIV replication and HIV-1 long-terminal repeat (LTR) transcription. These findings suggest that HBV could alter the course of HIV infection, inducing faster progression to AIDS (Gomez-Gonzalo et al, 1976). HIV in turn increase HBV carriage rates, increased replication, hepatitis flares, progression to chronic HBV infection and end stage liver disease (cirrhosis and hepatocellular carcinoma). It also increases reactivation episodes. (Soriano et al, 2010). Cirrhosis due to HBV is more common in co-infected patients than those mono infected by HBV despite low ALT levels (DiMartino, 2002; Ameeta and Wong, 2009).The annual risk of developing cirrhosis in HBV appears to be much higher in those co infected with HIV. This may be especially true in those with low CD4 counts (Sterling, 2003). HIV also reduce efficacy of anti–HBV therapy, including the risk of lamuvidine resistance and decreased response to interferone alfa (Peters, 2007).Furthermore, several recent reports have shown higher HBV DNA levels in patients co infected with HIV (Sterling, 2003). The impact of co infection is especially important in regions with widespread use of ART.

The introduction of HAART has led to the emergence of liver related disease and mortality as HBV infection increases HAART related hepatotoxicity (Peters, 2007;Puoti et al, 2002)

It is unclear at present if the risk of hepatocellular carcinoma (HCC) is increased, but there is some evidence that HIV infected individuals with lower CD4 counts are at greater risk of developing HCC (Clifford et al, 2008)

In a co-infection cohort study of 4967 homosexual men, patients had a 19 fold higher risk of liver death than those mono infected with HBV alone and they were >8times more likely to die of liver disease than those infected with HIV alone with associated increase with alcohol consumption, low nadir $CD_4^+$ count and ART. Rates of liver-related mortality by 1000 person-years were 14.1 with HIV and HBV co-infection, 1.7 with HIV mono infection, 0.8 with HBV mono infection, and 0 with neither HBV nor HIV infection (Thio et al, 2002; Peters, 2007)

In another study Among 5728 HIV-infected individuals tested for HBsAg, 498 (8.7%) were positive; there was a 3.6-fold higher risk of liver-related deaths in them compared with HBsAg-negative individuals (Soriano et al, 2010)

**Impact of hepatitis B infection on response to HAART**

There are conflicting results about the effect of chronic hepatitis B on response to HAART ($CD_4^+$ cell count and HIV viral load, HIV RNA copies/ml). In a cohort study in Italy showed increasing divergence of mean CD4 lymphocyte count up to 36 weeks after HAART initiation between patients with and without chronic hepatitis B, with those with chronic hepatitis B having lesser CD4 increase (p=0·03) (deLuca et al, 2002). In a cohort study in Denmark HBV infection sero-status is observed to have no effect on response to HAART in terms of HIV viral load suppression and $CD_4^+$ cell count (Omland et al, 2008) similarly in Nigeria, HIV RNA suppression and absolute CD4 rise was similar between HBsAg-positive and negative patients started on HAART (Idoko et al, 2007) But Hepatotoxicity is a concern that occurs in 5-15% of patients (Nunez et al, 2001; Martinez et al, 2001; Bonfanti et al, 2001) HIV co infection with HBV has been associated with increased hepatotoxicity to HAART (Sterling, 2003)It increase the risk three to five fold (Hoffmann and Thio, 2007) HBsAg reactivity has independently been associated with a higher incidence of severe liver damage during HAART. The Liver damage related to several factors: drugs toxicities, immune restoration of anti- HBV immune response, HBV flare after lamivudine withdrawal, occurrence of lamivudine HBV resistant strains or other co-infections or super-infections by hepatotropic viruses (HAV and/or HCV and/or HDV). HAART is associated with immune mediated HBV specific liver damage after HAART reconstitute cell-mediated immunity (Puoti et al, 2002; Soriano et al, 2010) A nationwide cohort done in Denmark showed that HBV-specific treatment did not have effect in initial HAART regimen on mortality in the HBV positive HIV patients (Omland et al, 2008) As the potential of chronic hepatitis B to blunt immune recovery after initiation of HAART is an area of special relevance to low-income settings in Africa and Asia with high HBV endemicity, more studies needed to characterise the effect of chronic hepatitis B co-infection on CD4 lymphocyte recovery during antiretroviral therapy. If reduced immune recovery is found to occur in co-infected populations in Asia and Africa, current WHO guidelines for antiretroviral monitoring may not be optimal because of delayed CD4 recovery (Hoffmann and Thio, 2007)

A careful definition of the aetiology of ALT flares occurring during HAART is mandatory before stopping or changing an antiretroviral regimen.

Causes of liver enzyme elevations in HIV/HBV-co infected patients following initiation of HAART could be direct drug-related liver injury, immune reconstitution in HBsAg+ patients, seroconversion from HBeAg+ and/or HBsAg+ to anti-HBeAg and/or anti-HBsAg, HBV reactivation in inactive carriers and occasionally in those with resolved HBV infection;

Emergence of drug resistant HBV, development of other viral hepatitides [acute hepatitis A virus (HAV), HCV, HDV]. These causes identified with a through history and serological testing. Clinicians must bear all these possibilities in mind before misinterpreting hepatic flares as drug injury (Soriano et al, 2010; Hoffmann and Thio, 2007)

**Treatment of HIV/HBV co-infection**

The impact of HBV on HAART tolerability and on the occurrence of end stage liver disease makes mandatory the search for an effective strategy for HBV prevention and treatment in HIV-sero positive individuals (Puoti et al, 2002)

HBsAg sero conversion is the ultimate, but elusive, goal of chronic hepatitis B therapy. A more achievable objective is to halt the progression of chronic hepatitis B-associated liver disease in light of this treatment is usually prolonged and may need to be continued indefinitely to maintain benefit through persistent HBV suppression. Treatment is most beneficial and efficacious for those in the immunoactive phase.

When considering management of chronic hepatitis B in HIV/HBV co-infected patients in Low-income countries, modifications in management recommendations are required to account for limited availability of anti-HBV agents and diagnostics. Of the seven agents used for treating chronic hepatitis B in the USA, only one, the nucleoside analogue lamivudine, is widely available throughout most of Africa and Asia.

Management options recommended for use in regions with limited resources include, First, HBsAg and liver enzymes test before starting HAART. Second, routine monitoring of liver enzymes once or twice during the first 3 months of HAART when CD4 or HIV RNA is assayed. The presence of HBsAg and repeatedly elevated liver enzymes suggest active disease with necro-inflammatory activity and the need for anti-HBV therapy (Hoffmann and Thio, 2007)

For management of both chronic hepatitis B and HIV in most low and middle-income countries includes, lamivudine or tenofovir disoproxil fumarate-containing HAART (Omland et al, 2008; Hoffmann and Thio, 2007; Sterling, 2003)

In this study, determining the magnitude of HIV/HBV co-infection in the setting of HAART is the main goal.

## *1.4. Significance of the study*

HBV Co-infection with HIV is becoming a major challenge in treatment in HIV infected population. Though replication of HIV is successfully being controlled using antiretroviral therapies, HBV co-infected patients are showing hepatotoxicity due to antiretroviral treatment which result in increased frequency of Non-AIDS related and AIDS related end stage liver diseases including cirrhosis and hepatocellular carcinoma.

In HIV/HBV co-infection, HIV modifies the course of HBV infection by increasing rates of chronicity, prolonging HBV viremia and increasing liver related morbidity.HBV in turn aggravate the drug toxicity or adverse effects the ART impose on liver forcing to change treatment regime. Co-infection also increases horizontal and vertical transmission of HBV in areas of the world with a high prevalence of HIV/HBV co-infection.

The study showed the prevalence of Hepatitis B virus in HIV infected individuals as well as in antiretroviral treatment initiated and naïve individuals. It rings a siren for health care workers especially laboratory workers as the magnitude of co infection is a bit unpredicted and different from our expectations. It also determined the extent of immune recovery and level of hepatotoxicity in HIV/HBV co infected individuals for whom it's temporal diminished relation on delaying immune recovery gives them a gleam of hope and relief beside the alert of its progression to end stage disease in latter times unnoticed.

It also suggested possible ways of HBV/HIV Co-infection management for current and future cases as well as inform higher bodies to give attention for the virus.

The ultimate significance that the current study offered is that it showed the current status of Hepatitis B virus in HIV infected individuals after treating them for more than 6 years with first line regimens all of which contained a drug recommended to treat hepatitis B infection.

# 2. Objectives

## 2.1. General objective

To determine the magnitude of Hepatitis B virus infection in HIV patients

## 2.2. Specific objectives

1. To determine the prevalence of Hepatitis B virus infection in HIV infected individuals those attending care and treatment services

2.To Compare immune recovery differences between HBsAg positive and HBsAg negative HIV infected individuals

3. To compare incidence of hepatotoxicity among individuals taking antiretroviral therapy and Co-infected with HBV.

# 3. Materials and Methods

## 3.1. Study design and study period

A cohort study was conducted in North Shoa zone from November 2010 to May 2011.

The design is cohort in that patients are grouped according to the exposure status of antiretroviral initiation.

## 3.2. Study area

The study was conducted in North shoa zone of Amhara region, Ethiopia. The administrative capital is DebreBerhan. It is located 130km north east of Addis Ababa, Ethiopia. The zone resides in 15,954.54 square kilometres and is divided in to 23 Woredas and estimated to have a total population of 1,907,392 based on a report of Amhara national regional state bureau of finance and economic development. (BoFED, 2009; Esubalew, 2007) At the time the study the zone had 3 hospitals and 72 health centers. The study included one referral hospital and six health centers who deliver care and treatment services for HIV infected individuals. The institutions were DebreBerhan hospital, Shewarobit, Debresina, Mendida, Deneba, Enewary and DebreBerhan health centres. The health centers send blood samples of HIV infected individuals to DebreBerhan hospital for CD4 cell count and measurement of levels of ALT and AST as a part of the care and treatment follow up service.

## 3.3. Study variables

**Dependent variables**: - Hepatitis B virus sero status (HBV status)

- ALT and AST Levels
- $CD4^+$ cell count

**Independent Variables**: - age, sex, income, occupation, Place of Residence, Educational level, HAART and other clinical variables

## 3.4. Sampling technique and Sample size determination

Purposive sampling technique was used to collect data from study subjects.As the study population is uniformely distributed, all patients visiting the hospital during the study period were included in the study untill the required sample size is achived.

The total sample size of the study was 812 calculated using the Epi Info statistical software. The sample size for each group will be 406.

The assumptions were -

P1=proportion of HIV patients recieving HAART and with chronic HBV infection taken as 9% (Hoffmann and Thio, 2007)

P2=proportion of HIV patients naive for HAART and with chronic HBV infection taken as 14% (Hoffmann and Thio, 2007))

Confidence level(α/2)-95%

Power (1- β) = 80%

r (Ratio of exposed: nonexposed)=1:1

Formula;

$$n_1 = \frac{\left[ z_{\alpha/2}\sqrt{(r+1)\bar{p}\bar{q}} + z_{1-\beta}\sqrt{rp_1q_1 + p_2q_2} \right]^2}{r(p_1 - p_2)^2}, \quad n_2 = r \times n_1$$

Where:

| Variable | Cohort |
|---|---|
| $n_1$ | number of exposed |
| $n_2$ | number of unexposed |
| $z_{\alpha/2}$ | z-score for two-tailed test based on α level |
| $z_{1-\beta}$ | z-score for one-tailed test based on β level |
| r | unexposed : exposed |
| $p_1$ | proportion of exposed with disease |
| $q_1$ | $1 - p_1$ |
| $p_2$ | proportion of unexposed with disease |
| $q_2$ | $1 - p_2$ |

## 3.5. Study population

All adult HIV infected individuals (above 15years old) who started HAART and those on Pre-ART follow up visiting DebreBerhan Zonal hospital and 6 health centers selected during the time of the data collection.

## 3.6. Eligibility criteria

Exposed: Confirmed HIV infected individuals who are currently taking HAART

Unexposed: HIV infected individuals who were on Pre-ART follow up

Inclusion criteria: HIV infected individuals who had at least three month follow up

Exclusion criteria: HIV infected individuals with less than three months follow up and HIV infected individuals less than 15 years old.

## 3.7. Materials and equipments

For the study the following materials were used

- ✓ Test kits ;Hepatitis B surface antigen kit (SD HBsAg rapid kit; SD BIOLINE, Standard Diagnostics, inc. south Korea), HBsAg confirmatory reagent(AxSYM HBsAg(V2); Abbott AXSYM System, ABBOTT Diagnostic division, Germany) ALT and AST reagents; HUMAN )
- ✓ Laboratory equipment; (AXSYM Machine, FACS, Micropipettes, Vacutainer tube, Centrifuge, etc...)
- ✓ Stationary materials

## 3.8. Data collection, processing and analysis

**Data collection**

Socio-demographic data was collected with a structured questionnaire from the study participants supplemented with patient intake form, follow up form and medical record review. Following informed consent, all participants were interviewed on Sex, age marital status, residence, and previous history related to HIV transmission and other opportunistic infections.
Clinical data, including baseline CD4 count, mean CD4 lymphocytes count, mean ALT and AST, type of first line regimens they started, adverse reactions to ARV, the duration of antiretroviral therapy were recorded from the follow up forms in their clinical chart.
Blood sample were collected from each study participants with standard operational procedure (Annex II). Serological testes for determining HBV infection were done by SD HBsAg rapid test kit (SD Company, Korea) at DebreBerhan Hospital and positive results were confirmed with HBV confirmatory reagent AxSYM HBsAg(V2) (Abbott AXSYM System, ABBOTT Diagnostic division, Germany ) according to the manufacturer's manual.(Annex III)

**Data Entry and analysis**

Data entry was done using Epi Info statistical software Version 3.5. The analysis consists of basic summaries of patient characteristics, univariate binary logistic analysis of the relation between HBsAg serostatus and various factors, and multivariate logistic regression analysis to adjust values of the dependent variable for the influence of the likely confounding explanatory variables (covariates).

The primary outcome variable was HBsAg sero status.HBV sero prevalence distribution is described using frequency and crosstab commands. The magnitude of the association between the different variables in relation to HBsAg sero-status was measured through Relative Risk (RR) and their 95% confidence interval (CI).

Comparisons for which P-values were less than 0.05 were considered statistically significant

## *3.9. Quality control*

Standard operational procedure (SOPS) was followed for blood collection, serological and clinical chemistry tests.

Chemistry analyzer instruments and HBsAg and confirmatory tests were checked for validity using positive and negative controls according to the manufacturer's manual.

Data quality was assured by prior training of Data collectors and daily checking of results.

## *3.10. Operational Definitions*

***U/L- International unit (U) of activity:*** quantity of enzyme able to convert one micromole (1 _mol) of substrate per minute to product; often expressed as units per liter (U/L) or microunits per liter (mU/L). The formula for determining U/L is;

$$\frac{\Delta A/\text{min} \times ([1/\text{micromolar absorptivity of product}] \times \text{total volume [mL]})}{\text{Volume of sample (mL)}}$$

**Baseline CD4**- the number of CD4 cells an individual had when starting ART

**Previous** –any time throughout their enrollment in HIV care and treatment.

**CD4 cell count**- the average numbers of CD4 cell count of an individual for the period of time in follow up calculated by adding all counts in patients follow up chart and divide by the number of counts

**ALT/AST level**- the average level of either ALT or AST of an individual for the period of time in follow up calculated by adding all counts in patients follow up chart and divide by the number of counts

**WHO stages**- Clinical stages of an HIV infected individual defined by the WHO.

**Duration in Chronic care**-The total number of months an HIV infected individual in Pre-ART being followed before starting ART

**Duration of initiated ART**- The total number of months an HIV infected individual on ART being followed after starting ART

**Follow up**-the continual psychological and clinical support HIV infected individuals' pursued after knowing their status

**Pre –ART individuals**- HIV infected individuals who are in Pre-ART follow up

**ART Individuals**-HIV infected individuals who are on ART

## 3.11. Ethical considerations

The research protocol together with consent form was submitted to Addis Ababa University, Department of Medical Microbiology, Immunology and Parasitology ethical committee to get approval. Written consent was obtained from each patient in writing form after explaining about the purpose, confidentiality, protection and anonymity of data. In this study, blood collected for patient follow up of Liver function and CD4 count was used. The participants were free to with draw from the study at any time without losing any of the benefits they were supposed to obtain from the hospitals under study.

The results will be communicated with their physician for better management of the patients in the second phase of the study

# 4. Results

## 4.1. Socio-demographic characteristics of study population

All the respondents who provided informed written consent were above 15 years of age, diagnosed with HIV. Of the total number of participants 760(94%) were included in the study. 6% of participants were excluded from analysis due to incompleteness of data obtained.

The majority of the respondents were Females, 468(61.6%) and urban residents, 605(79.6%). Their age distribution indicates that 46.6% study participants were between the ages of 25-34Years old. Concerning their marital status 20.6% were either widows or separated. Their average educational attainment was relatively high with 63% attended school **(Table1).** 39.9% of the participants did not have secured source of income but either supported by pension or plead for bread.16.1% were employed and working at the time of the study, 18.6% were farmers with 35.4% reporting to have less than 500 birr average monthly income and 44.1% unpredictable and variable income.

**Table 1. Socio-demographic Characteristics of Respondents, North Shoa zone, 2011**

| *Characteristics* | *Frequency(N=760)* | *Percent (%)* |
|---|---|---|
| **Sex** | | |
| Male | 292 | 38.4 |
| Female | 468 | 61.6 |
| **Age interval(year)** | | |
| 15-24 | 109 | 14.3 |
| 25-34 | 354 | 46.6 |
| 35-44 | 216 | 28.4 |
| >=45 | 81 | 10.7 |
| **Educational Level** | | |
| Illitrate | 282 | 37.1 |
| Elementary school | 302 | 39.8 |
| Secondary school | 133 | 17.5 |
| Higher Education | 43 | 5.7 |
| **Marital Status** | | |
| Single | 122 | 16.1 |
| Married | 348 | 45.8 |
| Divorced | 133 | 17.5 |
| Widowed | 112 | 14.7 |
| Separated | 45 | 5.9 |
| **Place of Residence** | | |
| Urban | 605 | 79.6 |
| Rural | 155 | 20.4 |
| **Average monthly income ETB (N=425)** | | |
| <=500 | 269 | 35.4 |
| 501-999 | 62 | 8.2 |
| >=1000 | 94 | 12.4 |
| Unstated | 335 | 44.1 |

## HIV related History of study participants

**Table 2. HIV related clinical Characteristics of study participants, North Shoa zone, 2011**

| Characteristics | Frequency (N=760) | Percent of study population (%) |
|---|---|---|
| **How do you contract HIV** | | |
| Heterosexual | 301 | 39.6 |
| Parenteral | 32 | 4.2 |
| Undisclosed | 427 | 56.2 |
| **Had Habit of Alcohol Drinking** | | |
| Yes | 97 | 12.8 |
| No | 663 | 87.2 |
| **Had Treatment for TB** | | |
| Yes | 182 | 23.9 |
| No | 578 | 76.1 |
| **Had History of Liver disease** | | |
| Yes | 44 | 5.8 |
| No | 716 | 94.2 |
| **Had Previous Opportunistic infection** | | |
| Yes | 372 | 48.9 |
| No | 388 | 51.1 |
| **Type of opportunistic disease (N=372)** | | |
| Pulmonary Tuberclosis | 132 | 35.1 |
| Bacterial pneumonia | 13 | 3.5 |
| All or either of Oral candidiasis, zoster, diarrhoea | 169 | 44.9 |
| TB, Diarrhoea, candidiasis and zoster combined | 50 | 13.3 |
| Other(Toxoplasmosis, syphilis, PCP*) | 12 | 3.2 |
| **ART status** | | |
| Pre-ART | 380 | 50% |
| ART started | 380 | 50% |

***PCP-Pneumocystis pneumonia**

According to Table 2, 92.7% of individuals lived with HIV for 5 or fewer years, 56.2% did not remember or refused to disclose how they contracted HIV. From those who did, 301(39.6%) report heterosexuality to be the mode of transmission. 182(23.9%) had been treated for TB. This indicates that the prevalence of Pulmonary Tuberculosis in HIV patients is 23.9%.As a result one out of 5HIV infected individuals develop Pulmonary Tuberculosis. 372(48.9%) developed opportunistic infection, of which 132(35.1%) was pulmonary Tuberculosis, 44.9% of patients developed either or all of oral candidiasis, Herpes zoster and diarrhoea.

## Clinical characteristics of HIV infected individuals on Pre-ART Follow up

Pre-ART individuals comprised a total of 380(50%). Figure 3 describes the clinical characteristics of pre ART individuals which is collected from each individual follow up charts. Accordingly 177 (46.6%) patients had CD4 cell count greater or equal to 350cells/mm$^3$ which enable them stay in pre-ART follow up for some months before starting ART. The majority of pre ART individuals (54.2%) had been enrolled in chronic HIV care for average of less than or equal to 6 months, ranging from one month to 6 years. Measured levels of ALT/AST were found only from 32 patients from whom approximately 40% of them had abnormal ALT/AST levels (>40U/L) (**Fig.1**)

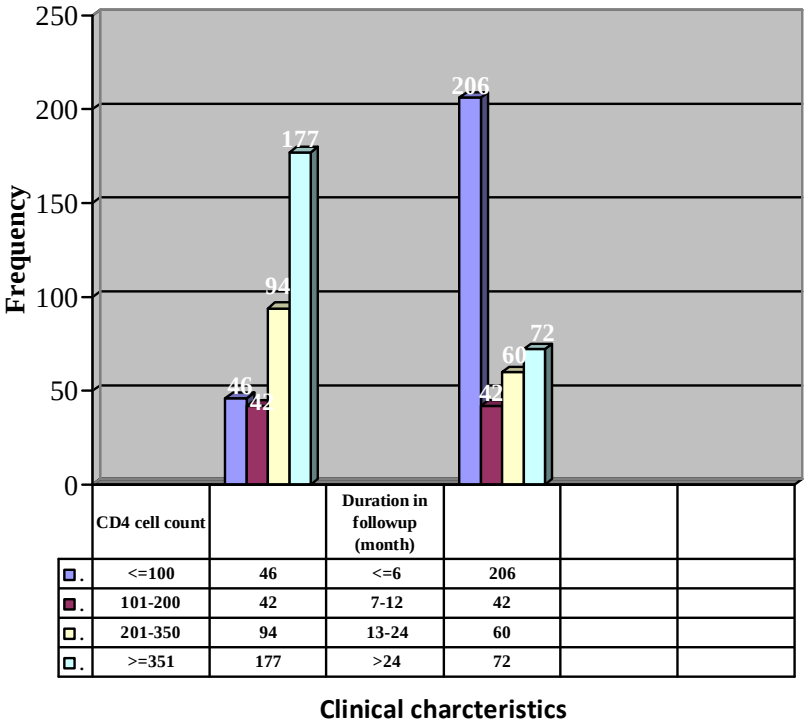


**Fig.1 Clinical Characteristics of HIV infected individuals on Pre-ART follow up, North shoa zone, 2011**

## Clinical characteristics of HIV infected individuals who started ART

HIV infected individuals attending treatment services were the second group of study participants comprising of 380 individuals. They were followed in ART on average for 28±17.4 months ranging from one month to 7 years. As shown in **figure 2**, half of these individuals, 200(52.6%) had been on ART for more than 24 months (2 years)

17.6% of individuals started ART at very low baseline CD4 cell count, <50cells/mm$^3$. But 40% started at the right time, baseline CD4 count of 101-200cells/mm$^3$. **(Figure 2)**

At the time of the study 44.2% of individuals were at WHO stage of III and half of them (49.5%) either at Stage I or II.347 (91.3%) were actively working.

Their follow up record showed that 93.2% of individuals had Good adherence to their respective prescribed ARV regimen.

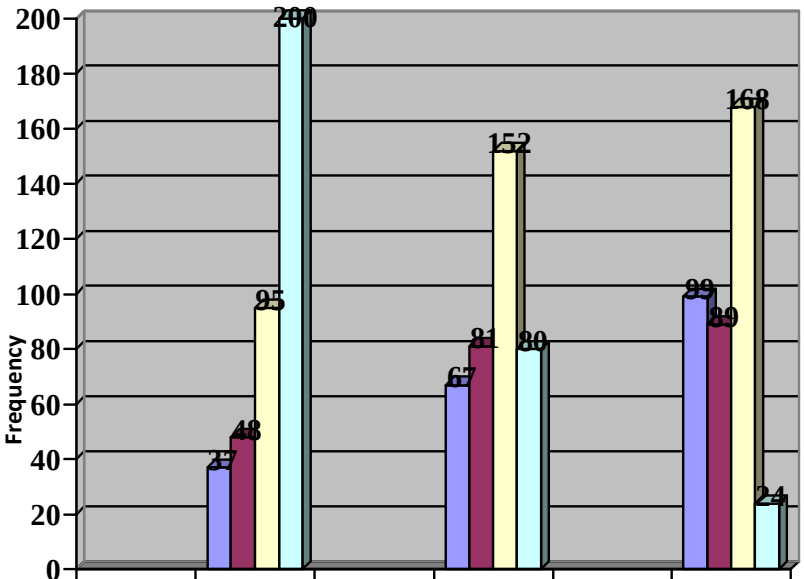


**Fig.2 Duration of initiated ART and baseline CD4cell count and WHO stage of ART individuals, North Shoa zone, 2011**

The Liver function test results showed that from a total of 118 individuals in which records of Liver function enzymes levels recorded, approximately one third (35.6%) of individuals had abnormal ALT/AST levels for the whole duration of initiated ART. (Figure 3)

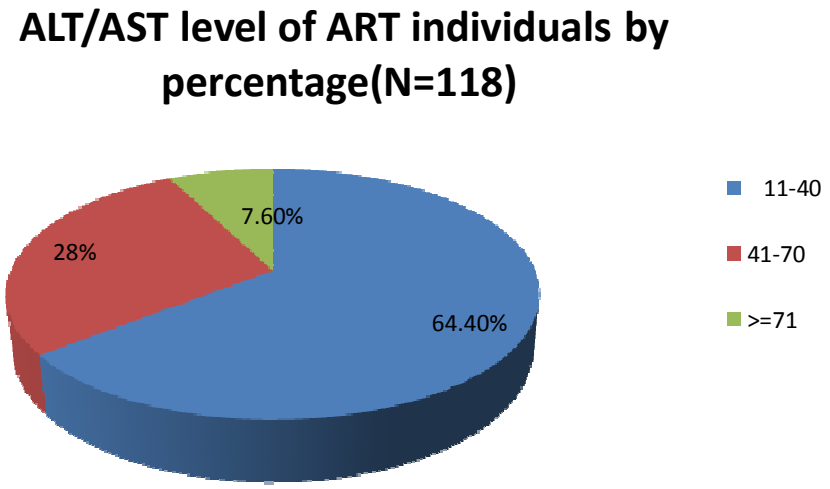


**Fig. 3.ALT/AST levels of HIV ART individuals (N=119), North Shoa zone, 2011**

During the study period CD4 lymphocytes count of all study participants has been recorded. The CD4 count of the respective indiviuals for the whole duration of initiated ART indicated that 151(39.7%) had a CD4 cell count ranging from 201-350cells/mm$^3$. (**Figure 4**)

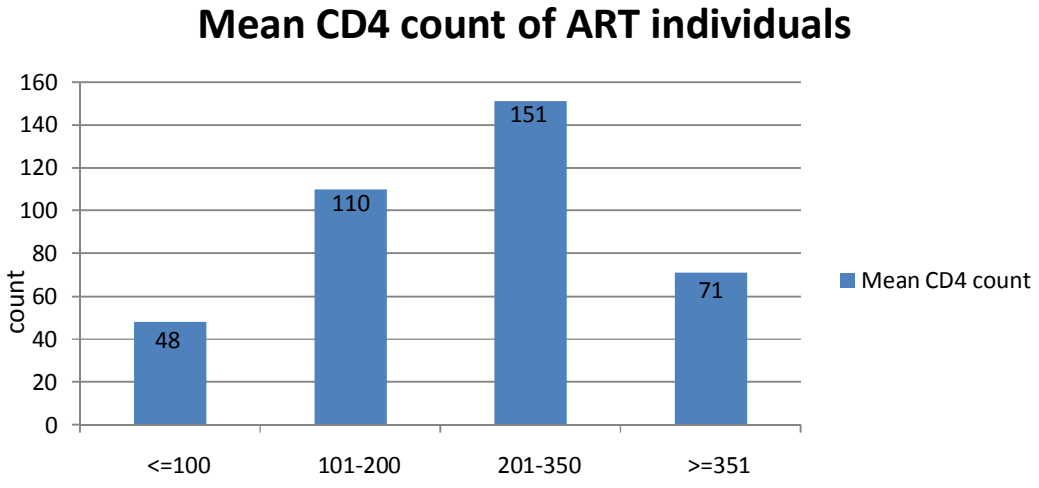


**Fig.4 CD4 cell count of ART individuals (N=380), North Shoa Zone, 2011**

From the 380 individuals currently taking ART, all had been taking first line regimens of which approximately 1/3rd(30%) had started with **1a regimen,** a combined ARV containing Stavudine (d4t), Lamivudine(3TC),NRTIs and Nevirapine(NVP) a NNRTI's.88 (23.2%)had started with **1c regimen** which differ from 1a by Zidovudin(AZT) instead of d4t. **(Figure5).**

During their course of treatment 52 (13.7%) patients had been forced to substitute the first regimen they started due to different reasons of which drug toxicity or side effect accounts 82.7%.

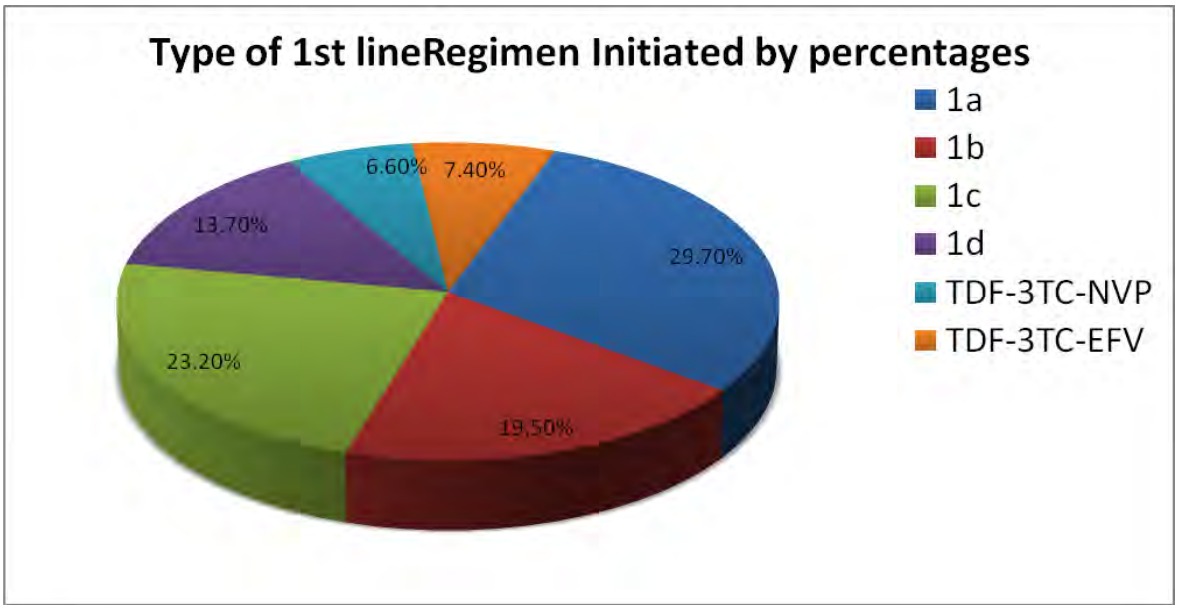


**Figure5. Types of first line regimens prescribed for ART individuals at initiation of ART, North Shoa Zone, 2011**

## *4.2. Prevalence of Hepatitis B virus among HIV infected individuals attending Pre-ART and ART follow ups*

The prevalence of Hepatitis B virus was determined by the presence of Hepatitis B surface Antigen in individual's plasma or serum using an immune chromatographic one step assay, and confirmed by a micro particle Enzyme immunoassay (MIEA) technology. All samples with a positive rapid test result were found to be strong positives in the confirmatory test (all with a value of >100, a positive result is interpreted at cut off value of >1) (Annex III) In this study the cumulative prevalence of HBsAg was found to be 3.9%.Among the men, 5.8% were infected with Hepatitis B virus, while the proportion of female with HBV was 4% lower (2.8%)

The age groups 25-34years and 35-45years had the highest proportion of males and females with HBV (93%) (**Table 3**)

The prevalence of HBV significantly increased with sex($X^2$=4.39; P<0.05).This indicated a significant difference in hepatitis B virus infection between male and female of which males were found to be 2times more likely to contract HBV than female. (RR=2.09, 95%CI (1.03, 4.3) (**Table 4**) Insignificant increase in Hepatitis B virus infection was also observed in old adults of the age 25-45, p=0.09.

**Table3. Age and gender distribution of study subjects by HBV infection, North Shoa Zone, 2011 (n = 760)**

| *Characteristics* | *HBsAg sero status* | | | | | |
|---|---|---|---|---|---|---|
| | *Men* | | *Women* | | *Total* | |
| **Age** | **Positive N (%)** | **Negative N (%)** | **Positive N (%)** | **Negative N (%)** | **Positive N (%)** | **Negative N (%)** |
| **15-24** | 0(0) | 22(100) | **2(2.3)** | 85(97.7) | **2(1.8)** | 107(98.2) |
| **25-34** | **7(7.1)** | **92(92.9)** | **6(2.9)** | 199(97.1) | **13(4.3)** | 291(95.7) |
| **35-45** | **10(9.5)** | 95(90.5) | **5(3.8)** | 127(96.2) | **15(6.3)** | 222(93.7) |
| **>45** | 0(0) | 66(100) | 0(0) | 44(100) | 0(0) | 110(100) |
| **Total** | **17(5.8)** | 275(94.2) | **13(2.8)** | 455(97.2) | **30(3.9)** | 730(96.1) |

Other socio-demographic variables including marital status, place of residence and source of income were not found to be significantly associated with prevalence of Hepatitis B virus at P< 0.05**. (Table 4)** There is also no statistically significant association between prevalence of Hepatitis B virus and variables related to previous clinical history of individuals (previous TB treatment, history of liver disease and previous opportunistic).Even though increased prevalence of HBV was observed in individuals who did not develop any opportunistic, it was not statistically significant. (P=0.53)

The immunoassay result of the HBsAg in the two study groups revealed that the prevalence in ART individuals is twice that of the pre-ART ones, 5.3% and 2.6%, respectively.

This increment with initiation of ART implied that Pre-ART individuals are less likely to have a positive HBsAg test result, but the current sample is not enough to describe it statistically. (P=0.06, RR=0.50, 95%CI (0.24, 1.05) **(Table 5)**

**Table 4.Bivariate analysis of distributions of HBsAg sero prevalence by socio demographic characteristics,North Shoa zone,2011**

| *Characteristics* | *HBsAg sero status* | | *$X^2$* | *P* | *RR(95%CI)* |
|---|---|---|---|---|---|
| | **Positive** | **Negative** | | | |
| **Sex** | | | | | |
| Male | 17 | 275 | **4.39** | **0.036** | **2.096 (1.03, 4.3)** |
| Female | 13 | 455 | | | |
| **Age(years)** | | | | | |
| 15-24 | 2 | 107 | | | |
| >=25 | 28 | 623 | 2.05 | 0.22 | 0.42 (0.1-1.70) |
| **Marital Status** | | | | | |
| Married | 10 | 338 | 1.95 | 0.16 | 0.59 (0.28, 1.25) |
| Single | 20 | 392 | | | |
| **Place of Residence** | | | | | |
| Urban | 25 | 580 | 0.27 | 0.65 | 1.30 (0.5-3.4) |
| Rural | 5 | 150 | | | |
| **Source of income** | | | | | |
| Secure | 17 | 440 | 0.16 | 0.69 | 0.87 (0.43,1.76) |
| Unsecure | 13 | 290 | | | |
| **Habit of alcohol drinking** | | | | | |
| Yes | 1 | 96 | 2.49 | 0.08 | 0.24(0.03,1.71) |
| No | 29 | 634 | | | |

**Table 5. Distribution of HBsAg sero status by ART status, North Shoa Zone, 2011 (n = 760)**

| | HBV Sero-status | | |
|---|---|---|---|
| | **Positive** | **Negative** | |
| **ART status** | **No (%)** | **No (%)** | **Significance** |
| **Pre-ART** | 10(2.6) | 370(97.4) | P=0.06 |
| **ART Initiated** | 20(5.3) | 360(94.7) | RR=0.50(0.24,1.05) |
| **Total** | 30(3.9) | 730(96.1) | |

## Comparisons of prevalence of Hepatitis B Virus among Pre-ART individuals

Bivariate analysis of socio demographic and clinical variables of pre-ART individuals showed that, only previous treatment for tuberculosis (p=0.02) and immune recovery (P=0.01) were found to be significantly associated with HBsAg sero prevalence (p =0.01) **(Table 6)**
Accordingly the likelihood of an HBsAg positive test result is 4 times higher in those with a CD4 cell count <= 200cells/mm$^3$ than those with >200cells/mm$^3$. (RR=4.62 (1.33-15.99) other variables including duration of follow up in chronic care, previous opportunistic and socio demographic variables did not show any significant associations with the prevalence of HBsAg

**Table 6.Bivariate Analysis of distributions of HBsAg seropositivity among Pre-ART individuals,NorthShoa Zone, 2011**

| *Characteristics* | *HbsAgserostatus Pre-ART individuals* | | *$X^2$* | *P* | *RR(95%CI)* |
|---|---|---|---|---|---|
| | **Positive** | **Negative** | | | |
| **Had treatment for TB** | | | | | |
| Yes | 4 | 42 | 7.51 | **0.02** | **4.84(1.42,16.51)** |
| No | 6 | 328 | | | |
| **Had prev. opportunistic** | | | | | |
| Yes | 5 | 111 | 1.84 | 0.15* | 2.28(0.67,7.71) |
| No | 5 | 259 | | | |
| **CD4 cell count on Pre ART(N=359)** | | | | | |
| <=200 | 6 | 82 | 7.00 | 0.01 | **4.62 (1.33-15.99)** |
| > 200 | 4 | 267 | | | |
| **ALT/AST level on Pre ART (N=32)** | | | | | |
| 11-40 | 1 | 18 | 0.078 | 0.65 | 0.68 (0.05-9.98) |
| >=41 | 1 | 12 | | | |

## Comparisons of prevalence of Hepatitis B Virus among ART individuals

A significant increment in the prevalence of HBsAg sero-positivity was observed in males($X^2$=5.40; P<0.03) Insignificant increment was also observed in individuals at WHO stage of I and II, with previous history of liver disease and a CD4 cell count of >200.In other variables, the prevalence was equal in the different sub groups. **(Table 7)**

**Table 7. Bivariate Analysis of distributions of HBsAg seropositivity among ART individuals,NorthShoa Zone, 2011**

| *Characteristics* | *HBsAg sero status ART individuals* | | $X^2$ | P | *RR(95%CI)* |
|---|---|---|---|---|---|
| | **Positive** | **Negative** | | | |
| **Sex** | | | | | |
| Male | 13 | 140 | 5.40 | **0.02** | **2.75 (1.13, 6.75)** |
| Female | 7 | 220 | | | |
| **Had Previous Liver disease** | | | | | |
| Yes | 3 | 25 | 1.80 | 0.18 | 2.22(0.69, 7.12) |
| No | 17 | 335 | | | |
| **Had TB treatment** | | | | | |
| Yes | 4 | 132 | 2.29 | 0.13 | 0.45(0.15,1.31) |
| No | 16 | 228 | | | |
| **WHO stage** | | | | | |
| Stage 1&2 | 13 | 175 | 2.03 | 0.11 | 1.89 (0.77-4.65) |
| Stage 3&4 | 7 | 185 | | | |
| **Are you taking INH Prophylaxis** | | | | | |
| Yes | 8 | 69 | 5.09 | **0.03** | **2.62(1.11-6.19)** |
| No | 12 | 291 | | | |
| **Duartion of Initiated ART** | | | | | |
| <=6 months | 2 | 34 | 0.00 | 0.58 | 1.06(0.26-4.39) |
| >6months | 18 | 326 | | | |
| **CD4 cell count** | | | | | |
| <=200 | 6 | 152 | 0.10 | 0.47 | 1.22(0.37-4.01) |
| >200 | 14 | 208 | | | |
| **ALT/AST level (n=119)** | | | | | |
| 11-40 | 1 | 76 | 0.18 | 0.58 | 0.54(0.03,8.50) |
| > 40 | 1 | 41 | | | |
| **1st line regimen initiated** | | | | | |
| AZT/TDF based | 9 | 184 | 0.28 | 0.59 | 0.79(0.34,1.87) |
| D4t based | 11 | 176 | | | |

### *4.3 Immune recovery differences between HBsAg positive and negative individuals*

According to the second objective of the current study, the immune recovery difference between HBV/HIV co infected and HIV mono infected individuals was determined. Figure 6 describes the HBsAg sero status in each of the study groups by mean CD4 cell count changes calculated. The mean CD4 cell difference is also explained in each group. Though comparison between pre-ART and ART groups is difficult, with respect to mean CD4 cell recovery, results within each group were comparable.

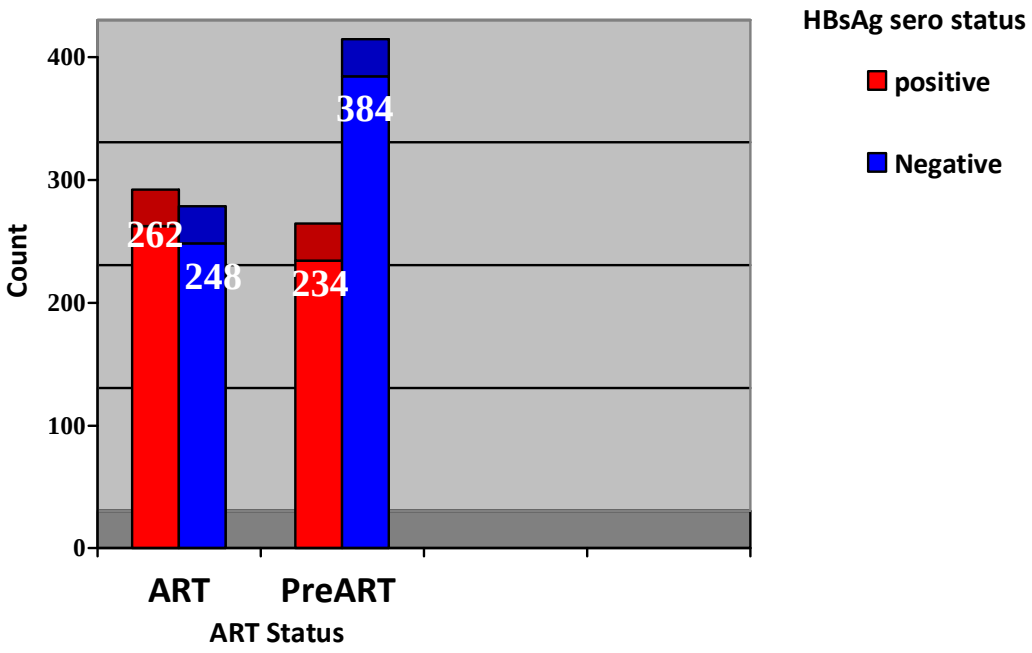


**Figure6. Mean CD4 cell count by HBsAg sero status of HIV infected individuals, North Shoa Zone, 2011**

#### CD4 cell count differences among Pre-ART individuals

CD4 cell count records were found from 359 individuals. The remaining 21 individuals had no previous records because of instrument failure or undetectable number of CD4 cells

A CD4 cell count of <=200cells/mm$^3$ was positively associated with HBsAg sero positivity. Individuals co-infected with HBV had experienced delayed recovery of immune cells (CD4 cell count). In those individuals, the likelihood of a delayed recovery, <=200cells/mm$^3$, is 2.55 times higher in those with a positive HBsAg test result (RR=2.55, 95% CI: 1.49-4.38)

The meanCD4 cell count of HBsAg positive and negative Pre-ART individuals also showed that the mean CD4 cell count of HBsAg negative study subjects is higher than that of HBsAg positive individuals, 384/mm3 and 234/mm3 respectively. **(Figure 6)**

The result of analysis for statistical significance at p=0.05 of the mean CD4 cell count of the two groups indicated that there is statistically significant difference in the mean CD4 cell count when analysed with one way ANOVA (P<0.05**).** The true population mean was estimated to be 380±12, 95% CI: 229, 531)

### CD4 cell count differences among ART individuals

Point estimate of the bivariate analysis of CD4 cell count of ART individuals with prevalence of HBsAg showed that there is no statistically significant difference in immune recovery (CD4 cell count) between HBsAg positive and negative ART individuals. (P>0.05)

The mean CD4 cell count of ART individuals was 249 cells/$mm^3$ ranging from19 to 1068 cells/$mm^3$.Individuals with a positive HBsAg test had relatively a higher mean CD4 cell count than those with negative HBsAg test result , 262 cells/$mm^3$ and 248 cells/$mm^3$ respectively. **(Figure 6)**

Analysis of the mean CD4 cell count of the two HBsAg groups for statistical significance difference showed that there is no statistically significant difference in the mean CD4 cell count of the two individuals.(P>0.05**).** The true population mean was estimated to be 249±8, 95% CI: 170, 326

## *4.4. Incidence of hepatotoxicity among ART individuals co infected with HBV*

The incidence of Hepatotoxicity among ART initiated individuals was measured using the levels of ALT/AST which could be recorded from the follow up charts of each study subjects. As the population of ART individuals whose ALT/AST levels measured were small (n=119), drug substitution due to toxicity as a possible associate of Hepatitis B infection is also analyzed. The small number of records is attributed to malfunctioning of the HUMAN chemistry analyzer that occurred approximately every week.

Point estimate of the bivariate analysis of ALT/AST levels of the 119 ART individuals with prevalence of HBsAg showed that those individuals with a positive HBsAg test result were equally likely to have an ALT/AST level fallen outside the normal range (>40 U/L) as those with a negative HBsAg test result. (P>0.05)

Analysis of the mean ALT/AST levels of HBsAg positive and negative ART study subjects was also made. The results were shown in table 8 below. Accordingly the mean ALT/AST levels of study subjects (n=119) was 39 U/L ranging from 10 U/L to 146 U/L. individuals with a positive HBsAg test had slightly higher mean ALT/AST level than those with a negative HBsAg test result , 41.50 U/L and 39.10U/L, respectively. But the difference is not statistically significant when analysed using one way ANOVA (P= 0.87)

Association between HBsAg sero positivity and drug substitution was also assessed. It was found that HBsAg positivity is not associated with drug substitution (P=0.46)

**Table8. Descriptive statistics of ALT/AST levels of ART individuals, North Shoa Zone, 2011 (n=119)**

| HBsAg sero status | Observed | Mean ALT/AST level (IU/L) |
|---|---|---|
| **Positive** | 2 | 41.5 |
| **Negative** | 117 | 39.1 |

**Adjusted Analysis of Variables associated with outcome variables; a Multivariate Logistic regression Analysis.**

Variables associated with HBsAg sero prevalence in the univariate binary logistic regression analysis were also re-evaluated again separately with HBsAg sero prevalence as a dependent variable and controlling for the effect of socio-demographic and clinical variables (age, sex, residence, marital status, educational status, income, previous history, etc.)

The "step up" procedure is used to build the logistic regression model.

Some variables associated with HBsAg sero prevalence; CD4 recovery and level of hepatotoxicity on the bivariate analysis remained significantly associated on the multivariate analysis. Associations with p<0.25 were included in the regression model (**Table 9 through 12**)

**Table9. Crude and adjusted RR's for variables identified as correlates of prevalence of HBsAg, North Shoa Zone, 2011 (n=760)**

| *Characteristics* | *HBsAg sero status* | | *P* | *Crude RR(95%CI)* | *P* | *Adjusted RR(95%CI)* |
|---|---|---|---|---|---|---|
| **Socio demographic variables** | **Positive** | **Negative** | | | | |
| **Sex** | | | | | | |
| Male | 17 | 275 | **0.03** | **2.096 (1.03, 4.3)** | **0.02** | **2.51(1.16,5.41)** |
| Female | 13 | 455 | | | | |
| **Age interval(year)** | | | | | | |
| 15-24 | 2 | 107 | | | | |
| >=25 | 28 | 623 | 0.22 | 0.42 (0.1-1.70) | 0.46 | 1.76(0.40,7.81) |
| **Marital Status** | | | | | | |
| Married | 10 | 338 | | | | |
| Single | 20 | 392 | 0.16 | 0.59 (0.28, 1.28) | 0.08 | 2.04(0.92,4.56) |
| **Habit of alcohol drinking** | | | | | | |
| Yes | 1 | 96 | 0.08 | 0.24(0.03,1.71) | 0.14 | 0.22(0.03,1.67) |
| No | 29 | 634 | | | | |
| **ART Status** | | | | | | |
| Pre-ART | 10 | 370 | 0.06 | 0.50(0.24,1.05) | 0.19 | 0.59(0.26,1.30) |
| ART Initiated | 20 | 360 | | | | |

**Table10. Crude and adjusted RR's for variables identified as correlates of prevalence of HBsAg in specific groups (Pre-ART and ART patients), North Shoa Zone, 2011**

| *Characteristics* | *HBsAg sero status among patients* | | *P* | *Crude RR(95%CI)* | *P* | *Adjusted RR(95%CI)* |
|---|---|---|---|---|---|---|
| *Pre-ART individuals* | **Positive** | **Negative** | | | | |
| **CD4 cell count(n=359)** | | | | | | |
| <=200 | 6 | 82 | 0.01* | **4.60(1.33,16.01)** | 0.04 | **3.98(1.02,15.48)** |
| >200 | 4 | 267 | | | | |
| **Marital status** | | | | | | |
| Married | 3 | 187 | 0.19 | 0.40(0.11,1.63) | 0.33 | 0.49(0.12,2.04) |
| Single | 7 | 183 | | | | |
| **Income source** | | | | | | |
| Secure | 4 | 223 | 0.16* | 0.45(0.13,1.56) | 0.14 | 0.36(0.09,1.40) |
| Unsecure | 6 | 147 | | | | |
| **Had treatment for TB** | | | | | | |
| Yes | 4 | 42 | **0.02** | **4.84(1.42,16.51)** | **0. 04** | **4.13(1.02,16.71)** |
| No | 6 | 328 | | | | |
| *ART patients* | | | | | | |
| **Sex** | | | | | | |
| Male | 13 | 140 | **0.02** | **2.75(1.12,6.75)** | **0.01** | **3.65(1.36,9.76)** |
| Female | 7 | 220 | | | | |
| **Had TB treatment** | | | | | | |
| Yes | 4 | 132 | 0.13 | 0.45(0.15,1.31) | 0.15 | 0.41(0.12,1.38) |
| No | 16 | 228 | | | | |
| **Previous Liver disease** | | | | | | |
| Yes | 3 | 25 | 0.17* | 2.22(0.69,7.11) | **0.03** | **4.67(1.13,19.38)** |
| No | 17 | 335 | | | | |
| **WHO stage** | | | | | | |
| Stage1/2 | 13 | 175 | 0.15 | 1.90(0.77,4.65) | 0.17 | **2.00(0.74,5.46)** |
| Stage3/4 | 7 | 185 | | | | |
| **Are you taking INH Prophylaxis** | | | | | | |
| Yes | 8 | 69 | **0.03** | **2.62(1.11-6.19)** | 0.14 | 2.09(0.77,5.69) |
| No | 12 | 291 | | | | |

***the P-value is fishers exact test**

**Table11.Multivariate logistic regression analysis of Variables associated with immune recovery (CD4cell count) in Pre-ART and ART individuals, North Shoa Zone, 2011**

| *Characteristics* | *CD4 count among patients* | | *P* | *Crude RR(95%CI)* | *P* | *Adjusted RR(95%CI)* |
|---|---|---|---|---|---|---|
| ***Pre-ART patients*** | **<=200 cells/mm$^3$** | **>200 cells/mm$^3$** | | | | |
| **HBsAg sero-status** | | | | | | |
| **Positive** | 6 | 82 | 0.00 | **2.55(1.49,4.38)** | **0.02** | **5.97(1.32,27.04)** |
| **Negative** | 4 | 267 | | | | |
| **Sex** | | | | | | |
| **Male** | 39 | 93 | 0.09 | 1.37(0.95,1.96) | 0.36 | 1.28(0.75,2.20) |
| **Female** | 49 | 178 | | | | |
| **Age** | | | | | | |
| **15-25** | 11 | 84 | | | | |
| **>25** | 77 | 188 | **0.00** | **2.52(1.40,4.52)** | **0.02** | **2.47(1.14,5.36)** |
| **Residence** | | | | | | |
| **Urban** | 61 | 226 | | | | |
| **Rural** | 27 | 45 | **0.00** | **1.76(1.22,2.56)** | **0.00** | **2.58(1.41,4.73)** |
| **Had prev. opportunistic** | | | | | | |
| **Yes** | 43 | 63 | **0.00** | **2.28(1.60,3.24)** | **0.00** | **2.81(1.64,4.81)** |
| **No** | 45 | 208 | | | | |
| **Are you taking INH Prophylaxis** | | | | | | |
| **Yes** | 1 | 45 | **0.00** | **0.23(0.07,0.69)** | **0.01** | **0.20(0.06,0.71)** |
| **No** | 85 | 226 | | | | |
| ***ART individuals*** | | | | | | |
| **HBsAg Sero-status** | | | | | | |
| **Positive** | 6 | 151 | 0.24 | 0.71(0.36,1.41) | 0.19 | 0.48(0.16,1.43) |
| **Negative** | 14 | 209 | | | | |
| **Sex** | | | | | | |
| **Male** | 71 | 82 | 0.13 | 1.22(0.96,1.55) | 0.97 | 1.01(0.62,1.64) |
| **Female** | 86 | 141 | | | | |
| **Age** | | | | | | |
| **15-24** | 8 | 19 | | | | |
| **>=25** | 150 | 203 | 0.19 | 1.43(0.79,2.60) | 0.04 | **3.03(1.07,8.56)** |
| **Residence** | | | | | | |
| **Urban** | 118 | 187 | **0.02** | **0.72(0.56,0.94)** | 0.70 | 1.12(0.62,2.04) |
| **Rural** | 40 | 35 | | | | |
| **Duration on ART** | | | | | | |
| **<=2years** | 103 | 77 | | | | |
| **>2 years** | 54 | 146 | 0.00 | **0.48(0.39,0.60)** | 0.00 | **0.32(0.19,0.54)** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Baseline CD4** | | | | | | |
| **<50** | 45 | 18 | | | | |
| **>=50** | 112 | 205 | **0.00** | **0.47(0.38,0.58)** | 0.00 | **0.14(0.07,0.29)** |
| **First line regimen started** | | | | | | |
| **TDF /AZT based** | 93 | 100 | 0.00 | **1.38(1.08,1.77)** | 0.10 | 1.52(0.92,2.53) |
| **D4t based** | 65 | 122 | | | | |
| **Are you takeing Cotrimoxazole** | | | | | | |
| **Yes** | 150 | 158 | **0.00** | **4.38(2.25,8.51)** | **0.00** | **6.51(2.73,15.55)** |
| **No** | 8 | 64 | | | | |
| **Is there substitution in the 1st line regimen** | | | | | | |
| **Yes** | 13 | 39 | **0.00** | **0.57(0.35,0.92)** | **0.02** | **0.41(0.19,0.88)** |
| **No** | 145 | 183 | | | | |

**Table12. Multivariate logistic regression analysis of Variables associated with level of hepatotoxicity ART individuals, North Shoa Zone, 2011 (n=119)**

| *Characteristics* | *ALT/AST levels of ART patients* | | *P* | *Crude RR(95%CI)* | *P* | *Adjusted RR(95%CI)* |
|---|---|---|---|---|---|---|
| | <=40 IU/L | >40 IU/L | | | | |
| **HBsAg sero-status** | | | | | | |
| Positive | **1** | **1** | **0.58*** | 0.77(0.19,3.10) | **0.74** | 0.62(0.04,10.50) |
| Negative | **76** | **41** | | | | |
| **CD4 cell count** | | | | | | |
| <=200 | **26** | **10** | **0.22** | **1.18(0.92,1.54)** | **0.09** | 2.27(0.88,5.85) |
| >200 | **51** | **32** | | | | |
| **Initiated first line regimen** | | | | | | |
| TDF/AZT based | **45** | **20** | **0.24** | 1.17(0.89,1.54) | 0.28 | 1.58(0.69,3.62) |
| D4t based | **32** | **22** | | | | |
| **TB treatment** | | | | | | |
| Yes | **19** | **20** | **0.01** | **0.67(0.47,0.95)** | **0.04** | **0.40(0.16,0.95)** |
| No | **58** | **22** | | | | |
| **INH prophylaxis** | | | | | | |
| Yes | **21** | **2** | **0.00** | **1.56(1.27,1.93)** | **0.02** | **5.88(1.25,27.64)** |
| No | **56** | **40** | | | | |

# 5. Discussion

In this study, the prevalence of HBV, immune recovery difference and incidence of hepatotoxicity among HIV infected adults was determined. The study comprised of two groups, namely adults on Pre-ART follow up and those on ART.

The majority of the study participants were females (61.6 %).The male to female ratio was 0.60:1. This difference in part indicated that more females were seeking care and treatment services than does the men. This is may be the very reason that females visit health institutions for maternal services like family planning, antenatal care and delivery. These occasions will help them to have a voluntary counseling and testing and ultimately enrollment in the care and treatment service. Other researchers also described similar outcomes. In a study conducted in Addis Ababa to assess use of VCT service, reported those out of the total users 61% were females (Dawit, 2006; Techalew et al, 2008)

Of the participants who stated their monthly income, approximately two third reported a monthly income <500Birr.Whether this reflect a difference in the care and treatment seeking behavior differences between people with low income and those with high income group or it is the reflection of the economic status of the general population is undisclosed. A baseline assessment for mobile VCT conducted by USAID reported that the risk level for HIV/AIDS and the ultimate care and treatment seeking behaviour for the service in DebreBerhan town is the result of poverty (especially among women) (USAID, 2007) In addition according to the observations of data collectors, people with low income welcomed care and treatment service s expecting extra financial aid once established to attract HIV infected individuals to the service.

More than half of the study participants (56%) reported that they don't know how they contract HIV. These showed that discloser of their sero-status, which greatly enhance the prevention and control measures for the HIV pandemic, is still waiting its age.

Half of the participants developed opportunistic which is inevitable as their CD4cells count varied gradually in the course of time. Pulmonary Tuberculosis is the most common opportunistic study participants developed (23.9 %).This is consistent with previous studies and government reports which stated prevalence of TB-HIV co-infection. The ministry of health of Ethiopia reported 20-50% co infection prevalence in Ethiopia (MOH, 2008)

The clinical characteristics of HIV infected Pre ART study participants showed that 46.6% of individuals had an average CD4 count >= 350 cells/$mm^3$after 12 months of chronic care

follow up. As a result a significant number of individuals don't require initiation of treatment for some months to come.

Among the second group of the study participants who were on ART, half of them were on ART for more than 2 years. According to the WHO, it was an adequate period of follow up for determining any associations related with ARV treatments and to evaluate immune recovery. WHO recommended that the first six months on ART are critical during which clinical and immunological improvement are expected and should manifest. (WHO, 2006)
In this study 38% of the individuals started ART at CD4 cell counts below 100cells/$mm^3$ of which 18% started below a critical level of 50cells/mm3. It is described that starting ART at a CD4 cell count less than 50cells/mm3 impairs effectiveness of treatment and resulted poor immune recovery and in most cases results in treatment failure and death (WHO, 2006; Mascolini, 2010)

WHO recommends that to experience the benefit of ARV regimens as well as to assess any associations related to treatment, patients has to adhere to the specific drug for more than 95% of the times (WHO, 2006) In this study 95.5% of individuals had a good adherence level which resulted in the greater number of individuals to be actively working.

The immune recovery of each individual was measured by recording the average number of CD4 cells over the respective period of follow up. This measurement was in line with the WHO recommendation which stated that serial measurements should be used to evaluate immune recovery as absolute CD4 cell counts fluctuate greatly between individuals (WHO, 2006) accordingly, the immune recovery pattern showed that study participants on ART had a mean CD4cell count of 284cells/$mm^3$. The mean CD4 cell count for Pre-ART is greater than the ART groups, 380cells/$mm^3$

The first objective of the current study is exploring the prevalence of HBV/HIV co-infection in the two study groups. Accordingly, the results showed that the cumulative prevalence of HBV in HIV infected adults was 3.9% (95% CI: 2.7-5.7%)This implied that 3.9% of HIV infected adults have persistence of HBs-antigen with or without replicative hepatitis B which should be defined by the presence of HBV DNA. Although direct comparison is difficult because of methodological differences, the prevalence we reported appears to be similar to a previous reports of HBV infection rate in Addis Ababa (3.9% HBsAg)(Techalew et al, 2008) the slight increment was attributed to the small number of ART initiated participants involved in the above research (n=103) It is also consistent with the rate reported in women attending

clinics in Addis Ababa (5% HBsAg,) (Duncan et al, 1995) and pregnant women in Jimma (south west part of Ethiopia) (3.7% HBsAg) (Awole and GebreSelassie, 2005).In the other way, this prevalence was different from the prevalence in the general population. A cross sectional household based study done in Addis Ababa reported a prevalence of 6.2% in the general population (Abebe et al, 2003). The higher occurrence of occult HBV infection (presence of HBV DNA in the absence of HBsAg) in HIV-positive people may attribute to the lower rate of HBsAg in HIV infected population. (Burnett et al, 2005)In addition in the above study 38% of the prevalence is attributed to the age group less than 15 years old who were excluded in the current study.

The prevalence is significantly different between males and females where male are 2.5 times more likely to be exposed to HBV infection than females. ($P<0.03$ Adjusted RR: 2.21 (1.16, 5.41). The result is similar with a study done in the United States and in Addis Ababa which reported a 6 times and a 1.5 times extra risk in males, respectively (Kennard, 2006; Techalew et al, 2008) Mark Mascolini also reported higher risk of male gender for HBV infection at the 17th Conference on Retroviruses and Opportunistic Infections in 2010 ($P=0.046$) (Mascolini, 2010). Although the reason for these is unclear it may be in part due to males' involvement in extra field work and so increased exposure for the different routes of HBV transmission or there may be genetic explanation that invited further exploration. Of course, there may be differences in risk behaviour by gender in the early years of life. (Abebe et al, 2003 and Techalew et al, 2008)

Moreover, the rate of HBsAg sero -positivity increased with age, higher in the age group 25-34(4.3%) and 35-45(6.3%) higher than the cumulative prevalence. A similar research from neighbouring country, Kenya also reported older age a significant associate of HIV/HBV co infection. $P <0.05$ (Nelson, 2008) this may be due to the increased risk of exposure to HBV infection with time. In addition infection in early age may reactivate at later age due to immunesuppression in HIV/AIDS or immunosuppressive drugs resulting in detectable Hepatitis B surface antigens. (Mauss, 2009)

In the current study the prevalence of HBsAg sero prevalence was also compared between ART initiated and Pre-ART HIV infected people. Accordingly the prevalence of HBsAg is 5.3% and 2.6%, respectively. The prevalence found among ART initiated individuals is comparable with the prevalence in the general population. Similar result was reported from a study done in Thailand, where a chronic HBV prevalence of 8.7% was reported among patients receiving ART consistent with the population prevalence of Thailand (5-10%)

(Hoffmann and Thio, 2007) The prevalence we reported was higher among ART initiated group than Pre-ART ones. Whether or not the difference is significant was also determined. In the crude estimate, HBsAg positivity rate was lower in Pre-ART HIV infected people as compared to those on ART though the current sample was small to prove it statistically. Accordingly the risk of developing a positive HBsAg test result was 50% lower in pre-ART groups. When adjusted for socio-demographic and clinical variable (sex, age, marital status, history of previous TB and other opportunistic infection), the risk remained similar but the significance declined (Adjusted P=0.19) this in turn implies that if the sample size could be increased, the significance will be uncovered. The current result contrasted with the higher rate of HBsAg positivity in Pre-ART groups instead of ART initiated groups reported in a study done in similar populations in Addis Ababa(4.5% in Pre ART patients and 2.9% in ART initiated individuals) (Techalew et al, 2008). The difference could be attributed to the small sample size used in the previous study as well as involvement of patients who were on ART for short period of time. (N=305 versus N=760) It was also different from the expectations and reported results from other studies. The presence of lamivudine as part of the combination therapy in HAART was expected to halt the multiplication of HBV. (Mauss et al, 2009; Techalew et al, 2008; WHO, 2006)

Though it is difficult to explain the higher rate of HBsAg positivity among ART initiated individuals, it may be explained by the fact that a nucleoside anti HBV drug like lamivudine, requires a long term treatment to achieve HBsAg clearance or seroconversion to anti-HBsAg. After short term treatment clearance could be achieved only in <5% of patients. (Mauss et al, 2009) So if complete suppression was not achieved during treatment, rather quick resistance commences. It is reported that within the first year of treatment, 20% patients on lamivudine may develop mutation resulting in loss of activity on HBV. (Chang et al, 2005) In addition HIV could also reduce efficacy of anti–HBV therapy, including the risk of lamuvidine resistance and decreased response to Interferon (Peters, 2007).In the current study, since the mean duration of ART follow up was 28months (more than 2 years) it was possible to explain emergence of mutant strains. But emergence of resistant mutation should be decided after a detectable HBV viral load measured as HBV DNA copies in serum or plasma. In addition the HBV genome is translated in overlapping ORFs, which limits the number of mutations that can be tolerated. As a result most mutant strains are produced in times of treatment or HBV vaccination to confer resistance. Another study also reported the incidence of HBV resistance in patients treated with lamivudine after two years as about 50% in HIV/HBV co-infected patients. (Benhamou et al, 1999; Puoti et al, 2002)In contrast the increased prevalence could

by the result of HBeAg sero conversion to anti-HBeAg and the subsequent suppression of HBV replication by anti-retroviral like Lamivudine. In these patients HBsAg remains detectable and transaminases are within normal range. (Mauss et al, 2009) For this reason the levels of HBV DNA and HBeAg should be determined to determine the actual cause.

A relevant but unexpected consequence in particular of lamivudine resistance is the induction of conformational changes in the HBs-antigen due to an overlapping reading frame in the genetic sequence of the HBV polymerase and the HBs-antigen. This in turn may induce replication of HBV and so increased prevalence of HBsAg in lamivudine treated patients.

The low prevalence in pre-ART individuals may also be due to presence of occult HBV infection in which simultaneous infection with HIV reduces immune control of previous HBV infection and as a result inactive HBV reactivate and the HBV DNA starts to replicate without presence of detectable HBsAg (Soriano, 2005)

In ART individuals' previous liver disease was found to be a strong predictor of HBsAg positivity (Adjusted RR, 4.67; 95%CI 1.13,19.38) followed by male sex (Adjusted RR, 3.65; 95%CI 1.36,9.76) The possible explanation for increase risk associated with previous liver disease is that people with previous liver disease are expected to be chronic carriers of HBV and less effective immune recovery induced by ART initiation reactivate HBV and result in increased replication and risk to end stage liver disease, liver cirrhosis and hepatocellular carcinoma. (Mauss et al, 2009)

In this study, relation between CD4 cell count and HBsAg sero positivity was also assessed in the two specific study groups. Consequently in Pre-ART individuals, statistically significant associations were sought between CD4 cell count and HBsAg sero positivity. In those groups, lower rate of CD4 cell recovery was recorded in HBsAg positive individuals (Adjusted P=0.04) In those individuals, poor CD4 recovery was a strong predictor of HbsAg positivity (Adjusted RR: 3.98; 95%CI: 1.02, 15.48) other studies also reported similar results. Doctor Peter Marion reported in the international AIDS society conference that HBV infection independently reduce CD4 cell recovery (Peters, 2007; Thio et al, 2002) In contrast two longitudinal studies from Britain did not show any impact of HBV co-infection on CD4 depletion, progression to full-blown AIDS, or AIDS induced mortality but these studies suffer from small sample size and lack of baseline CD4 cell count (Puoti et al, 2002)

In ART initiated individuals though a slight increase in mean CD4 cell count was observed in HBsAg sero positive individuals, the difference was not statistically significant(P=0.19)The

result is similar with other studies. In the study of Chang and colleagues, HBV was found to have no effect on CD4 cell loss(Chang et al, 2005)

In Thailand a cohort study showed that CD4 lymphocyte increases were similar regardless of hepatitis B status. In Nigeria also found that absolute CD4 cell rise was similar between HBsAg positive and negative individuals who are started HAART (Hoffmann and Thio, 2007; Crane et al, 2010; Puoti et al, 2002; Thio et al, 2002) The repression of HBV effect on CD4 recovery after initiation of ART could be the result of treatment in boosting immunity and consequently overrun the HBV suppressive action.

Other clinical variables including long duration of follow up on ART, type of first line regimen initiated, and regimen substitution in the first line regimen were not found to be significant associates of HBsAg positivity. P>0.05 but we have said that long duration in ART indirectly contribute to drug resistance. Similarly type of first line regimen lacks association in contrary to the expected effect of Lamivudine on HBV clearance which strengthens our argument of development of drug resistant mutants in our study groups. In addition we have found that though only 25(6%) of the total 380 ART initiated study groups were taking TDF-3TC combined regimen, they accounted for 10% of HBsAg positive individuals, high proportion than any other regimen. As we recalled otherwise this falsify the recommendation of combination therapy containing tenofovir and lamivudine as part of combination antiretroviral treatment as it is superior in terms of HBV DNA suppression than was tenofovir or lamivudine administered alone (Levy and Robert, 2006; Hoffmann and Thio, 2007; Nunez et al, 2001; Soriano, 2010).

According to the second objective of the study, factors associated Immune recovery (mean number of CD4 cells), were assessed. Accordingly positive HBsAg test, older age, previous opportunistic and living in rural were found to be independent risk factors for low CD4 recovery in Pre-ART individuals. P < 0.05.Instead Isoniazide preventive therapy was found to boost CD4 cells. The association of HBsAg with CD4 cell recovery was already discussed above. HbsAg positivity was a strong predictor of CD4 repression.HBV could affect CD4 recovery either directly or by increasing HIV replication (Peters, 2007) The consequence of older age could be explained as decrement of memory T-cells and lower naïve CD4 cell production in the course of time (Wilkin, 2010)

In ART initiated group, taking Cotrimoxazole, duration on ART for less than 2 years and a very low baseline CD4 count(<50) were independent risk factors for poor CD4 recovery.

Accordingly a unit increase in baseline CD4 cell count will result in a 0.56 increase in each CD4 cell count after initiation of ART (Correlation coefficient, r=0.56, P<0.00)

No association was seen between the specific antiretrovirals used and immune recovery. P=0.10.Other studies reported similar findings (Wilkin, 2010; Mascolini, 2010) Studies also showed that in the presence of Hepatitis B virus infection, changes in CD4 cell count will not be affected. (Thio et al, 2002; Puoti et al, 2002)

Incidence of Hepatotoxicity, measured by the serum levels of ALT and AST, is also assessed among ART initiated participants. The result showed that incidence of hepatotoxicity is not different between HBsAg positive and negative ART initiated individuals (P=0.74) even though the mean levels of ALT/AST is slightly higher in HBsAg positive individuals (42U/L versus 39U/L).Normal value of ALT/AST is 11-40U/L. This may also attributed to the small proportion of HBV induced liver toxicity that occur in individuals at ART (5-15%) (Puoti et al, 2002) Other studies also reported that HIV/HBV co infection brought no change or reduced levels of ALT (Colin et al, 1999) In contrast a study done by Judy Chang and colleagues reported that individuals co infection with HBV are in increased risk of worsening liver function following antiviral therapy and of more rapid HBV disease progression (Chang et al, 2005) This difference could be ascribed to the small number of individuals with a recorded levels of ALT/AST and the absence of clinical diagnosis of liver including liver histology .

In the current study, we tried to explore the type and phases of HBV infection in the HBsAg positive study participants. Clinically, concentrations of Alanine and Aspartate Aminotransferase levels (ALT and AST) may rise to 1000-2000 IU/L in acute phase. Most patients with chronic hepatitis B are clinically asymptomatic though dependant on the specific phase of chronic state. Some may have nonspecific symptoms such as fatigue. In most instances, significant clinical symptoms will develop only if there is active replication of HBV DNA revealed by level of HBV DNA greater than 100,000copies/ml recorded for six months and liver disease progresses to decompensated cirrhosis revealed by liver histology.In advanced liver disease there may be stigmata of chronic liver disease. In patients with decompensated cirrhosis jaundice, ascites, peripheral edema, and encephalopathy may be present. Laboratory testing shows mild to moderate elevation in serum AST and ALT in most patients (Mauss et al, 2009) According to the above clinical definition in the current study the mean ALT/AST levels was appreciably low or normal and 93% of HBsAg positive individuals were actively working and no clinical diagnosis of liver related disease.

Hence these HBsAg positive individuals may be in the chronic Hepatitis stage. The phase of chronic Hepatitis should however be identified by assessing the level of HBV DNA which is the ideal diagnosis to determine active replication. HBeAg detection could also aid in determining the chronic phase in these patients though it is unreliable viral load indicator and up to 30% of long term infected individuals are HBeAg negative but high HBV DNA replication (Mauss et al, 2009) After determining HBV DNA, if HBV DNA is low or undetectable and normal ALT levels, one can describe these HBsAg positive subjects as inactive HBsAg carriers.

The recommendation of WHO appealing early diagnosis of chronic hepatitis B by HBS antigen (HBsAg) screening in high-risk groups as a crucial step in the management of HBV infection pays a great deal of value for our research. Afterwards other serological markers could be used to determine success of treatment hence anti-HBs test will help to show HBsAg clearance which is the ultimate goal of treatment, and HBeAg to anti-HBeAg sero conversion test, though unreliable due to sero-reversion, to indicate viral load and low infectivity of carriers, respectively.

In addition we argued that this magnitude of HBsAg we reported explained the overall prevalence of chronic HBV infection in HIV co infected individuals as HBsAg clearance is difficult to achieve whether the virus is replicating or at its inactive state. In addition HBV has a nuclear accumulated episomal covalently closed circular DNA (cccDNA) in all infected individuals in hepatocytes. This extrachromosomal DNA induces reactivation of HBV infection whenever immunosupression occurred and makes complete clearance or sero conversion of HBsAg inconceivable. (Rehermann et al, 1996; Mauss et al, 2009)

In generally in Pre-ART individuals, after adjustment for socio-demographic and clinical history variables, poor CD4 cell recovery and previous TB treatment were independent correlates of HBsAg sero prevalence at $P<0.05$. Correspondingly male sex and previous liver disease were independent predictors of HBsAg positivity in ART initiated individuals.

In turn HBV infection, age, residence and previous opportunistic were identified as risk factors for delayed CD4 cell recovery observed in HIV infected Pre-ART individuals. Similarly people early age, high baseline CD4 cell count, long duration on ART were protective variables for delayed immune recovery during ART. In contrast cotrimoxazole treatment was found an independent risk factor for poor CD4 cell recovery after a period of ART treatment. Furthermore according to the current study incidence of hepatotoxicity for prescribed ARV drugs was not affected by Hepatitis B infection.

# 6. Limitation and strength of the study

## Strength of the study

- ✓ Hepatitis B surface antigen is detected using antigen detecting tests including microparticle enzyme immunoassay.
- ✓ The study is done relatively in large sample size than previous studies done in the country.
- ✓ In our study we have utilized a combination of methodologies, which has helped us to cover wider concepts related to ARVs adherence and complement our findings in each method by the other.
- ✓ In addition inclusion of several variables related to the outcome variables enables us to present results in adjusted regression.

## Limitation of the study

- ✓ HBV co infection was determined based on HBsAg positivity which leads to inclusion of active infections, chronic infections and carriers.
- ✓ Levels HBV DNA which is an important marker of active replication and occurrence of resistance mutation was not determined.
- ✓ Other tests of HBV infection including tests of markers like HBeAg, anti-HBcAg, and anti-HBsAg was not done

# 7. Conclusion

The current study showed increased prevalence of HBsAg among ART initiated than Pre-ART individuals. HBsAg sero positivity did not affect immune recovery after HAART for more than 2 years of treatment

Cotrimoxazole treatment, duration on ART for less than 2 years and a very low baseline CD4 count (<50) were independent risk factors for poor CD4 cell recovery.

Before starting ART, HIV/HBV co infected individuals were at increased risk of sharp drop in their CD4 cells forcing them to ART initiation quickly. Those individuals were also at greater risk of reactivating HBV infection if their CD4 dropped below 200cells/$mm^3$

All first line regimens were found to have similar actions in boosting CD4 cells after ART initiation but none of them were found to decrease the risk of clearing hepatitis B surface antigen from the blood.

Greater prevalence of hepatitis B surface antigen was seen in individuals taking TDF-3TC based combination regimens.

We presented a possible emergence of drug resistance Hepatitis B mutant strains. But the needs to be proven by anti-viral resistance test by genotyping.

Incidence of hepatotoxicity was not affected by HBV infection in ART initiated individuals

According to the current findings, development of viral resistance to polymerase inhibitors like Lamuvidine and tenovofir may result in rapid replication of HBV and rapid development of end stage liver disease in ART initiated HIV/HBV co infected individuals. So unless otherwise alternative treatments options are designed, continuation of lamivudine leads to development of compensatory mutations that could potentially limit future treatment options.

# 8. Recommendations

According to the findings of the study the following recommendation is forwarded

- ✓ As patients at the chronic inactive Hepatitis B carrier state are the first candidates for treatment, especially in situations when there is a significant level of HBV replication, further testes including HBV DNA isolation and determination of viral load should be done to decide on whether to start treatment or to select the treatment of choice. But inactive chronic HBsAg carriers, characterised by negative HBeAg status (anti- HBeAg-positivity), low HBV DNA levels (<10,000 copies/mL) together with normal serum aminotransferase levels and normal liver histology can be excluded from antiviral therapy.
- ✓ As recommended by the WHO patients who are eligible to start treatment(HBV DNA >2000 IU/ml and a positive liver fibrosis and elevated ALT level) should be treated with a combination therapy containing Tenofovir (TDF) and Lamivudine (3TC) whether or not the individual is ART naïve or previously treated with Lamivudine containing ARV but with close follow ups
- ✓ Those patients with an HBV DNA <2000 IU/ml and no relevant liver fibrosis no specific antiretroviral regimen is recommended .Though the activity of the HBV infection in these patients is minimal, it should be assessed at least every six months as part of routine monitoring of the HIV infection including an ultrasound and HBsAg and ALT and AST levels due to the slightly increased risk of hepatocellular carcinoma.
- ✓ Effectiveness of treatment for HBV/HIV co- infection should be evaluated by the absence of HBsAg from serum or presence of anti-HBsAg antibodies which shows immune control of infection
- ✓ Further studies should be conducted in different population groups in the form of long-term follow up analyses to determine the different factors associated with HIV/HBV co-infection
- ✓ All laboratory and other professionals working in HIV care and treatment services should be screened and HBsAg negative ones get vaccinated.

# 9. References


Abebe A, Nokes J, Dejene A, et al. (2003) Seroepidemiology of hepatitis B virus in Addis Ababa, Ethiopia: transmission patterns and vaccine control. Epidemiol. Infect. 131: 757-770.

Addis A (2007) Assessment of factors associated with HIV/AIDS among women seeking post abortion care in public hospitals. School of public health, Addis Ababa University: 13.

Alter MJ (2006) Epidemiology of viral hepatitis and HIV co-infection. *J Hepatol* 44: 56-9.

Ameeta E and Wong T (2009) HIV and hepatitis B co-infection: Background document. WHO.

Arneson W and Brickell J (2007) Clinical Chemistry: A Laboratory Perspective, p.92. F. A. Davis Company.

Awole M and GebreSelassie S (2005) Seroprevalence of HBsAg and its risk factors among pregnant women in Jimma, Southwest Ethiopia. *Ethiopian Journal of Health Development* 19: 45-50.

Benhamou Y, Bochet M, and Thibault V (1999) Long-term incidence of hepatitis B virus resistance to lamivudine in human immunodeficiency virus-infected patients. Hepatology 30(5): 1302-6.

Blumberg BS, Alter HJ, and Visnich S (1965) A New Antigen in Leukemia Sera. JAMA 191: 541-546.

Bodsworth N, Donovan B and Nightingale BN (1989) The effect of concurrent human immunodeficiency virus infection on chronic hepatitis B: a study of 150 homosexual men. J Infect Disease 160(4): 577-582.

BoFED (2009) The population report of Amhara region by zone:Reports for North shoa zone. Bahirdar. Available from http://www.amharabofed.gov.et/population_report.html

Bonfanti P, Landonio S and Ricci E (2001) Risk factors for hepatotoxicity in patients treated with highly active antiretroviral therapy. J AIDS 27: 316-8.

Brooks GF, Butel JS and Morse SA (2007) Jawetz, Melnick, & Adelberg's Medical Microbiology, the McGraw-Hill Companies, Inc.

Burnett RJ, Francois G and Kew M (2005) Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa: a call for further investigation. Liver Int. 25: 201-24.

Chang JJ, Sirivichayakul S, and Avihingsanon A (2009) Impaired Quality of the Hepatitis B Virus (HBV)-Specific T-Cell Response in Human Immunodeficiency Virus Type 1-HBV Coinfection. Journal of Virology 83(15): 7649-7658

Chang JJ, Wightman F, Bartholomeusz A, et al. (2005) Reduced Hepatitis B Virus (HBV)-Specific CD4_ T-Cell Responses in Human Immunodeficiency Virus Type 1-HBV-Coinfected Individuals Receiving HBV-Active Antiretroviral Therapy. Journal of virology 79(5): 3038-3051

Chen CJ, Yang HI, Su J, et al. (2006) Reveal-HBV Study Group:Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 295(1): 65-73.

Chen MT, Billaud JN, Sallberg M, et al. (2004) Function of the hepatitis B virus precore protein is to regulate the immune response to the core antigen. Proc Nat Acad. Sci. U SA 101(41): 14913-14918.

Clifford GM, Rickenbach M and Polesel J (2008) Influence of HIV-related immunodeficiency on the risk of hepatocellular carcinoma. AIDS 22: 2135-41.

Colin J, Cazals-Hatem D, Loriot MA, et al. (1999) Influence of HIV infection on chronic hepatitis B in homosexual men. Hepatology 29: 1306-1310.

Cornberg M, Protzer U and Dollinger MM (2007) Prophylaxis, diagnosis and therapy of hepatitis B virus (HBV) infection: the German guidelines for the management of HBV infection. Gastroenterol 45(12): 1281.

Crane M, Sirivichayakul S and Chang JJ (2010) No Increase in Hepatitis B Virus (HBV)-Specific CD8-T Cells in Patients with HIV-1–HBV Co-infections following HBV-Active HAART. Journal of Virology 84(6): 2657-2665.

Dawit A (2006) Assessment of Quality of Voluntary HIV Counselling and Testing Services in Addis Ababa, Ethiopia, Addis Ababa University. A thesis submitted to the school of graduate studies Addis Ababa University: 29.

deLuca A, Bugarini R and Lepri AC (2002) Co-infection with hepatitis viruses and outcome of initial antiretroviral regimens in previously naive HIV-infected subjects. Arch Intern Med 162: 2125-2132.

DiMartino V (2002) Influence of HIV infection on the response to interferon therapy and the long term outcome of chronic hepatitis B. Gastroenterol 123: 1812-22.

Duncan M, Tibaus G and Pelzer A (1995) Prevalence and significance of sexually transmitted diseases among women attending clinics in Addis Ababa. Ethiopian Journal of Health Development 9: 31-40.

Esubalew T (2007) Assessment of health care waste generation rate and evaluation of health care waste management in Debre Birhan zonal hospital. A thesis submitted to the school of graduate studies. Addis Ababa University: 12.

EurekAlert and U. S. Medicalcenter (2006) Researchers Refocus Studies on Patients with HIV, Hepatitis. ScienceDaily, from http://www.eurekalert.org.

Fix OK, Peters MG and Locarnini SA (2007) virology and Clinical Management of Hepatitis B and HIV Co-infection, from http://www.prn.org

Gilson RJ, Hawkins AE, Beecham MR, et al. (1997) Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. AIDS 11(5): 597-606.

Gomez-Gonzalo M, Carretero M and Rullas J (1976) The Hepatitis B virus X protein induces HIV-1 replication and transcription in synergy with T-cell activation signals. J Biol Chem 38: 35435-43.

HepatitisBfoundation (2009) HBV/HIV co-infection, from http://www.hepb.org/

Hoffmann CJ and Thio CL (2007) Clinical implications of HIV and hepatitis B co-infection in Asia and Africa. The Lancet Infectious Diseases 7(6): 402 - 409.

Idoko J, Meloni S and Muazu M (2007) Hepatitis B virus co-infection impacts baseline HIV parameters and HAART-related hepatotoxicity risk in an HIV-infected Nigerian cohort. 14th Conference on Retroviruses and Opportunistic Infections; Los Angeles, CA, USA.

Kenard J (2006) Hepatitis B: Men More at Risk of Hepatitis B. Health's Disease and Condition content, from http//:www.About.com.

Kenneth S, Marion P and Margaret K (2007) HIV and Liver Disease Forum: Conference Proceedings." Hepatology.

Lacombe K, Bottero J, Lemoine M, et al. (2010) HIV/hepatitis B virus co-infection: current challenges and new strategies. J Antimicrob Chemother 65: 10-17.

Landes M, Newell ML and Barlow P (2008) Hepatitis B or hepatitis C coinfection in HIV-infected pregnant women in Europe. HIV Medicine 9: 526-534.

Levy V and Robert M (2006) Antiretroviral Therapy for Hepatitis B Virus-HIV Co infected Patients: Promises and Pitfalls. Clinical Infectious Diseases 43: 904-910.

Martinez E, Blanco G and Arnaiz A (2001) Hepatotoxicity in HIV-1-infected patients receiving nevirapine-containing antiretroviral therapy. AIDS 15: 1261-8.

Mascolini M (2010) Older Age, Lower CD4 Nadir, NNRTI Use Predict Slow CD4 Recovery in Athena. 17th Conference on Retroviruses and Opportunistic Infections, San Francisco CA.

Mauss S, Berg T, Rockstroh J, et al. (2009) Hepatology: A clinical textbook. 1st ed. Germany.

Moges F, Kebede Y, Kassu A, et al. (2006) Seroprevalence of HIV,Hepatitis B infection and syphilis among street dwellers in Gondar city, Northwest Ethiopia. Ethiop.J.Health Dev 20(3): 160-165.

MOH (2008) Guidelines for management of opportunistic infections in Adults and Adolescents in Ethiopia. Federal HAPCO. Addis Ababa: 10.

Murray PR, Rosental KS and Pfaller MA (2005). Medical Microbiology, Elsevier Inc.

Nelson M (2008) HIV, hepatitis B and hepatitis C co infection in Kenya. Fourth International Workshop on HIV and Hepatitis Co-infection. Madrid.

Nunez M, Lana R and Mendoza J (2001) Risk factors for severe hepatic injury after introduction of highly active antiretroviral therapy. J AIDS 27: 426-31.

Omland HL, Weis N, Skinh JP, et al. (2008) Impact of hepatitis B virus co-infection on response to highly active antiretroviral treatment and outcome in HIV-infected individuals: a nationwide cohort study. British HIV Association HIV Medicine 9: 300-306.

Peters MG (2007) Diagnosis and Management of Hepatitis B Virus and HIV Coinfection. Top HIV Med 15(5): 163-166

Puoti M , Airoldi M, Bruno R, et al. (2002) Hepatitis B Virus Co-infection in Human Immunodeficiency Virus-infected Subjects. *AIDS Rev 4*: *27-35*.

Rehermann B, Ferrari C, , Pasquinelli C, et al. (1996) The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med 2(10): 1104-8.

Sherman KE, Neff GW, Eghtesad B, et al. (2006) HIV and Liver Disease Forum: When HIV and Liver Disease Co-Exist. Conference Proceedings, Wyoming. Hepatology.

Soriano V, Puoti M, Bonacini M, et al. (2010) Care of HIV patients with chronic hepatitis B: updated recommendations from the HIV-Hepatitis B Virus International Panel. HIV-Hepatitis B virus international panel, from www.HIV and Hepatitis.com.

Sterling R K. (2003) Hepatitis B Virus in the Setting of HIV Infection: A Clinical Challenge. Medical Writers' Circle, from http://www.hcvadvocate.org/hcsp/board.asp.

Techalew S, Torben W, Medhin G, et al. (2008) Hepatitis B virus infection among people attending the voluntary counselling and testing centre and anti-retroviral therapy clinic of St. Paul's General Specialised Hospital. Sexually Transmitted Infections 84: 37-41.

Tesga E, Mengesha B, Hanson BG, et al. (1986) Hepatitis A, B, and Delta infection in Ethiopia: a serologic survey with demographic data. Am J Epidemiol 123: 345-51

Thio CL, Seaberg EC, J. Skolasky R, et al. (2002) HIV-1, hepatitis B virus, and risk of liver related mortality in the Multicenter Cohort Study (MACS). *Lancet* 360: 1921-1926.

USAID (2007) Identifying at risk populations and HIV/AIDS referral services, United States agency for international development: 7.

WHO (2002) Hepatitis B, World Health Organization, Department of communicable diseases surveillance and response: 18.

WHO (2006) AntiRetroviral Therapy for HIV infection in Adults and Adolescents: Recommendations for a public health approach, WHO: 26.

Wilkin T (2010) What Causes Poor CD4-Cell Recovery in the Setting of Suppressive Antiretroviral Therapy? Journal Watch HIV/AIDS Clinical Care from http://aids-clinical-care.jwatch.org.

# 10. Annexes

## *Annex I. Q uestionnaire*

**Unique ART Number_____**

QUESTIONNAIRE

The following questionnaire consists of different questions related to the study with 3 parts. The Questions will be filled from **Patient interview, Pre-ART/ART register, Intake and follow up forms and laboratory records**

ይህ መጠይቅ ለዚህ ጥናት የሚረዱ የተለያዩ ጥያቄዎችን በ3 ክፍል ይዟል፣ጥያቄዎቹ ተሳታፊዎችን በመጠየቅ፣ ከኤ.አር.ቲ፣ ከቅድመ ኤ.አር.ቲ መዝገብ ፣ከቅበላና ከክትትል ፎርሞች ነው

**ውድ የጥናቱ ተሳታፊ**፡-እኛ የምናጠናው የሔፓታይተስ ቢ ቫይረስ ስርጭትን ኤች አይቪ በደማቸው ባለባቸው ፀረ ኤች አይቪ መድሃኒት በሚወስዱና ባልጀመሩ ሰዎች ላይ ነው፣የዚህ ጥናት ዋና አላማም የሔፓታይተስ ቢ ቫይረስ ስርጭትን በማጥናት ቫይረሱ ያለባቸውን ሰዎች በተሻለ መንገድ የሚታ ከሙበትን መንገድ መጠቆም ነው

በጥናቱ ውስጥ ከእርስዎ የምናገኛቸው ማንኛውንም መረጃ ሚስጥራዊነቱ የ ተጠበቀ ነው፣ በጥናቱ ውስጥ የምንጠቀ መው የሚስጥር የፀረ ኤች አይቪ መድሃኒት መጠ ቀሚያ ቁጥርዎን በመሆኑ እርስዎን የሚገልጽ ምንም አይነት መለያ አይኖርም።የእርስዎን ማንነትም በጥናቱ ውጤት ላይም ይሁን በማንኛውም ሁኔታ አይገለጽም

የተሳታፊው ፊርማ_____

የሆስፒታሉ/የጤና ጣቢያው ስም _____ የ መጠይቅ ቁጥር_____

**PART ONE:-PATIENT INTERVIEW**

| S. No | Variables | Answer | code | Skip |
|---|---|---|---|---|
| 001 | Sex | Male | 1 | |
| | | female | 2 | |
| 002 | Age | / ..... / Year | | |
| 003 | Educational status | Not read and write | 1 | |
| | | Read &write only | 2 | |
| | | Grade level | ___ | |

| | | | | |
|---|---|---|---|---|
| 004 | Marital status | Single | 1 | |
| | | Married | 2 | |
| | | Divorced | 3 | |
| | | Widowed | 4 | |
| | | Separated | 5 | |
| 005 | Place of Residence | Urban | 1 | |
| | | Rural | 2 | |
| 006 | Do you possess the following properties | Radio | 1 | |
| | | TV | 2 | |
| | | Telephone | 3 | |
| | | Car | 4 | |
| | | Refrigerator | 5 | |
| | | Electricity | 6 | |
| | | An electric 'Mitad' | 7 | |
| 007 | Main source of income | Agriculture | 1 | |
| | | Trade | 2 | |
| | | Salary | 3 | |
| | | Others(specify) | 4 | |
| 008 | What is the average amount of monthly income of the family (in Birr or kind) | _____ | | |
| 009 | Occupation (What do you work) | _____ | | |
| 010 | Mode of HIV transmission (How does HIV transmitted to you) | Heterosexual | 1 | |
| | | IDU(Intravenus drug | 2 | |

| | | | | |
|---|---|---|---|---|
| | | use) | | |
| | | Unknown | 3 | |
| 011 | How many years is it after you know your HIV status | /..... /Year | | |
| 012 | Do you have a habit of Alcohol drink | Yes | 1 | If No go to 014 |
| | | No | 2 | |
| 013 | If Yes, How often? | Once a day | 1 | |
| | | More than 2per week | 2 | |
| | | Every week | 3 | |
| | | Twice in a month | 4 | |
| 014 | Have you ever take treatments for TB | Yes | 1 | f No go to 016 |
| | | No | 2 | |
| 015 | Are you taking drugs for TB now | Yes | 1 | |
| | | No | 2 | |
| 016 | Do you have a history of Liver disease (History of jaundice/ecteric sclera) | Yes | 1 | |
| | | No | 2 | |
| 017 | Do you had Previous opportunistic infections(Example:- tuberclosis,Diarrhea,Herpes zoster) | Yes | 1 | |
| | | No | 2 | |
| 018 | Patient Adherence by self report | Previous Day | Yes | |
| | | | No | |
| | | | Yes | |

| | | | | |
|---|---|---|---|---|
| | | Past 3 days | No | |
| | | Past 7 Days | Yes | |
| | | | No | |
| 019 | Do you Take additional medicines with HAART | Yes | 1 | |
| | | No | 2 | |
| 020 | If yes, which drugs are you taking or for what disease | _____ | | |
| 021 | Do you take Suboptimal Anti retroviral treatment including PMTCT previous to HAART | Yes | 1 | |
| | | No | 2 | |
| 022 | ART status | Pre- ART | 1 | |
| | | ART initiated | 2 | |

**To the data collector**:- The following questions should be filled from ART/Pre-ART Registers, Intake and follow up forms

**ለሚጃ ሰብሳቢ:-ከዚህ ቀጥሎ ያሉት ጥያቄዎች የሚሞሉት ከኤአርቲ/ከቅድመ ኤአርቲ፤ ከቅበላና ከክትትል ፎርሞች ነው**

**PART TWO:- FOR PRE-ART PATIENTS(ኤአርቲ ሳይጀምር ክትትል ላይ ላለ ሰው የሚሞላ)**

| S. No | Variables | Answer | code | Skip |
|---|---|---|---|---|
| 101 | Duration of enrolment in chronic HIV care | /...../Months | | |
| 102 | Mean CD4 count for the whole Pre-ART visits | /...../ per l | | |
| 103 | Mean ALT and AST level for the whole Pre-ART visits | /...../ per l | | |
| 104 | Is the patient taking Cotrimoxazole | Yes | 1 | |
| | | No | 2 | |
| 105 | Is the patient taking INH | Yes | 1 | |
| | | No | 2 | |
| 106 | Other hepatitis infection(Hepatitis C,D,A,E) | _____ | | |

**PART THREE:- FOR ART PATIENTS (ኤአርቲ ለጀመረ ሰው የሚሞላ)**

| | | | | |
|---|---|---|---|---|
| 201 | ART start date | _____ | | |
| 202 | WHO Stage of the patient | _____ | | |
| 203 | Is the patient taking Cotrimoxazole | Yes | 1 | |
| | | No | 2 | |
| 204 | Is the patient taking INH | Yes | 1 | |
| | | No | 2 | |
| 205 | Baseline CD4 count | /...../ per l | | |
| 206 | Duration of Initiated ART | /...../months | | |
| 207 | Functional status | On bed | 1 | |

| | | | | |
|---|---|---|---|---|
| | | Walking with help | 2 | |
| | | Working | 3 | |
| 208 | Mean CD4 count for the whole ART duration | /...../ per l | | |
| 209 | Mean ALT and AST level for the whole ART duration | /...../per l | | |
| 210 | Adherence level | Good | 1 | |
| | | Fair | 2 | |
| | | Poor | 3 | |
| 211 | Other hepatitis infection (Hepatitis C,D,A,E) | _____ | | |
| 212 | HAART therapy | 1st line Regimen | 1 | |
| | | 2nd line Regimen | 2 | If 2 goto 217 |
| 213 | 1st line Regimen(write the codes) | _____ | | |
| 214 | Is there any substitution to the first line regimen | Yes | 1 | |
| | | No | 2 | |
| 215 | If yes,<br>a. which regimen is substituted for (write the codes) | _____ | | |
| | b. what is the reason for substitution | _____ | | |
| 216 | 2nd line Regimen (write the codes) | _____ | | |
| 217 | Is there any substitution to the second line regimen | Yes | 1 | |
| | | No | 2 | |
| 218 | If yes,<br>a. which regimen is substituted for (write the codes) | _____ | | |
| | b. what is the reason for substitution | _____ | | |

## *Annex II Venous blood collection procedure*

1. Introduce yourself and identify the patient
2. Explain the procedure to the patient
3. Wash hands and wear gloves
4. Prepare materials (syringes, needles, test tubes etc.)
5. Prepare the patient and apply tourniquet
6. Disinfect the draw site
7. Collect 5ml of blood with either vacutainer tubes or syringe and needle.
8. Exit the pain, apply pressure and check the patient.
11. Discard the needle in safety box
12. Label the specimen
13. Allow the specimen for 30minutes (to facilitate clotting) and centrifuge with medium speed for 5minutes
14. Separate serum from the blood by Pasteur pipette
15. Perform lab. Test according to the manufacturers manual and store the remaining serum at -20 $^{o}$c .

## *Annex III Principle of the test*

ONE STEP HBsAg Test

**SD BIO LINE HBsAg**

### 1. Explanation of the test

The SD BIOLINE HBsAg test is an in-vitro immunochromatographic, one step assay designed for qualitative determination of HBsAg in human serum or plasma. This test cassette contains a membrane strip, which is pre-coated with mouse monoclonal anti-HBs capture antibody on test band region. The mouse monoclonal anti-HBs-colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the antibody-antigen-antibody gold particle complex forms.
The SD BIOLINE HBsAg test cassette has a letter of T and C as "Test Line" and "Control Line" on the surface of the cassette. Both the Test Line and Control Line in result window are not visible before applying any samples. The Control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the reagents of control line are working. The SD BIOLINE HBsAg can identify HBsAg in plasma or serum specimens with a high degree of sensitivity.

### 2. Materials provided

SD BIOLINE HBsAg kit contains following items to perform the assay.
1) SD BIOLINE HBsAg test device.
2) Instructions for use

### 3. Precaution

The SD BIOLINE HBsAg should be stored at room temperature. The test device is sensitive to humidity and as well as to heat. Perform the test immediately after removing the test device from the foil pouch. Do not use it beyond the expiration.

### 4. Specimen collection and storage

1) The test may be performed using human plasma or serum.
2) If specimens are not immediately tested, they should be refrigerated at 2 – 8℃. For storage periods greater than three days, freezing is recommended.
3) Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

### 5. Warnings

1) For in vitro diagnostic use only.
2) Do not eat or smoke while handling specimens.
3) Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
4) Avoid splashing or aerosol formation.
5) Clean up spills thoroughly using an appropriate disinfectant.
6) Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
7) Do not use the test kit if the pouch is damaged or the seal is broken.

### 6. Procedure of the test

1) Remove the test device from the foil pouch, and place it on a flat, dry surface.
2) Add 100㎕ of specimen into the sample well (Figure 1).
3) As the test begins to work, you will see purple color move across the Result Window in the center of the test device.
4) Interpret test results at 20 minutes.
5) A positive result will not change once it has been established at 20 minutes. However, in order to prevent any incorrect results, the test result should not be interpreted after 30 minutes.

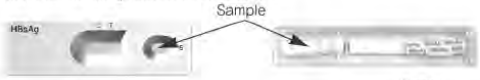

Figure 1

**Caution** : The above interpreting time is based on reading the test results at room temperature of 15 to 30℃. If your room temperature is significantly lower than 15℃, then the interpreting time should be proper increased to 30 minutes.

### 7. Interpretation of the test

1) A color band will appear at left section of the Results Window to show that the test is working properly. This band is the Control Band.
2) The right section of the Results Window indicates the test results. If another color band appears at the right section of the Results Window, this band is the Test Band.

**Negative Result** : The presence of only one purple color band within the Results Window indicates a negative result (Figure 2).

Figure 2

**Positive Result** : The presence of two color bands ("T" band and "C" band) within the Results Window no matter which band appears first indicates a positive result (Figure 3).

Figure 3

**Invalid Result** : After performing the test and no purple color band is visible within the Results Window, the result is considered invalid (Figure 4). The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.

Figure 4

### 8. Limitations of the test

A negative result does not preclude the possibility of infection with HBV. Other clinically available tests are required if questionable results are obtained. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

### 9. Comparison study

To comparison of SD BIOLINE HBsAg results with reference results, we used 276 samples consist of as followings

| Origin | HBsAg positive specimens | HBsAg negative specimens | Total |
|---|---|---|---|
| Africa | 16 | 43 | 59 |
| Asia | 30 | 30 | 60 |
| Europe | 30 | 67 | 97 |
| Latin America | 22 | 38 | 60 |
| Total | 98 | 178 | 276 |

In this multi-site evaluation of 276 samples, we found the relative sensitivity is 100% (98/98), the relative specificity is 100% (178/178). The results are summarized in the following tables.

| Results | | SD BIOLINE HBsAg | | |
|---|---|---|---|---|
| | | Positive | Negative | Total Results |
| Reference | Positive (98) | 98 | 0 | 98 |
| | Negative (178) | 0 | 178 | 178 |
| Total | | 98 | 178 | 276 |

### 10. Bibliography of suggested reading


1) Blumberg, B.S. The Discovery of Australian Antigen and its relation to viral hepatitis. Vitro. 1971; 7: 223
2) Sato K., Ichiyama S., Iinuma Y., Nada T., Shimokata K., Nakashima N., Evaluation of immunochromatographic assay systems for rapid detection of hepatitis B surface antigen and antibody. Dainascreen HBsAg and Dainascreen Ausab, J Clin Microbiol, 1996; 34(6) : 1420-2.
3) Epidemiologic Notes and Reports. Hepatitis B Contamination in a Clinical Laboratory-Colorado, MMWR 29 :459, 1980
4) David D.S., Present W., Martinls J., Wang R., Batholomew R., Desmond W. and Sevier E.D. : Monoclonal antibodies in the detection of hepatitis infection. Med. Lab. Sci. 38: 341-348, 1981



Date Issued : 2007. 01
01FK10-02-5, 01FK11-02- 5



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**BIOLOGICAL PRINCIPLES OF ABBOTT, AXSYM HBV CONFIRMATORY TEST**

AxSYM HBsAg (V2) is based on the Microparticle Enzyme Immunoassay (MEIA) technology. Sample and all AxSYM HBsAg (V2) reagents required for one test are pipette by the Sampling Probe into various wells of a reaction vessel (RV) in the Sampling Center. The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Probe.

The reactions occur in the following sequence:

- Sample, Anti-HBs Coated Microparticles and Biotinylated Anti-HBs are combined in one RV well.
- When HBsAg is present in the sample, it binds to the Anti-HBs Coated Microparticles and Biotinylated Anti-HBs, forming an antibody-antigenantibody complex in the reaction mixture.
- A portion of the reaction mixture is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix.
- The Anti-Biotin:Alkaline Phosphatase Conjugate is dispensed onto the matrix cell and binds with any microparticle-bound antibody-antigen antibody complex.
- The matrix cell is washed to remove materials not bound to the microparticles.
- The substrate, 4-Methylumbelliferyl Phosphate, is added. The alkaline phosphatise labeled conjugate catalyzes the removal of a phosphate group from the substrate, yielding the fluorescent product, 4-Methylumbelliferone. This fluorescent product is measured by the MEIA optical assembly.

The presence or absence of HBsAg in the sample is determined by comparing the rate of formation of fluorescent product to a cut off rate determined from a previous AxSYM HBsAg (V2) Index Calibration. If the rate of formation of fluorescent product in the test sample is greater than or equal to the cut off rate, the sample is considered reactive for HBsAg.

INTERPRETATION OF RESULTS

The Cutoff rate is determined by multiplying the Index Calibrator mean rate by 2.

**Cutoff Rate (CO) = Index Calibrator mean rate x 2**

## S/N

The AxSYM HBsAg (V2) assay calculates a result based on the ratio of the sample rate to the stored Index Calibrator mean rate for each sample and control.

$$\text{S/N} = \frac{\text{Sample Rate}}{\text{Index Calibrator Mean Rate}}$$

## S/CO

The AxSYM HBsAg (V2) assay also calculates a result based on the ratio of the sample rate to the Cut off rate (CO) for each sample and control.

$$\text{S/CO} = \frac{\text{Sample Rate}}{\text{Cut off Rate (CO)}}$$

• Samples with S/N less than 2.00 are negative by the AxSYM HBsAg (V2) assay and need not be tested further.

• Samples with S/N values greater than or equal to 2.00 are considered reactive.

• Samples with S/CO values less than 1.00 are negative by the AxSYM HBsAg (V2) assay and need not be tested further.

• Samples with S/CO values greater than or equal to 1.00 are considered reactive.

• All samples that are reactive on initial testing should be retested in duplicate using the AxSYM HBsAg (V2) assay. If neither of the retests is reactive, the sample must be considered negative for HBsAg. If the sample is reactive in either of the repeated replicates, the sample must be considered repeatedly reactive.

• Repeatedly reactive samples should be tested by a neutralizing confirmatory test, such as the AxSYM HBsAg Confirmatory assay.Samples which are confirmed by neutralization with human anti-HBs must be considered positive for HBsAg.

## *Annex IV Laboratory data collection format*

| Unique ART Number | HBsAg* | $CD4^{+}$** count | ALT level | AST level | Remark |
|---|---|---|---|---|---|
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***HBsAg is Hepatitis B virus Surface Antigen determined by the INSTANTHBsAg test kit**

**** Current or last recorded $Cd_4^{+}$ count**

## *Annex V:Amharic version of information sheet,consent form and questionnaire*

ስለ ጥናቱ መረጃ እና የተሳትፎ ፍቃድ መጠየቂያ ቅጽ

**አላማው:–** እኛ የምናጠናው የሔፓታይተስ ቢ ቫይረስ ስርጭትን ኤች አይቪ በደማቸው ባለባቸው ፀረ ኤች አይቪ መድሃኒት በሚወስዱና ባልጀመሩ ሰዎች ላይ ነው፣ሔፓታይተስ ቢ ቫይረስ ኤች አይቪ ባለባቸው ሰዎች ላይ ኤች አይቪን ወደ ኤድስነት መቀየር ከማፋጠኑም በላይ በፀረ ኤች አይቪ መድሃኒት ምክንያት የሚመጣውን የጉበት ችግርም ያባብሳል፣ የፀረ ኤች አይቪ መድሃኒት የሚጨምረውን የ$CD4^+$ መጠንንም በፍጥነት እንዳይጨምር እንቅፋት ይሆናል

የዚህ ጥናት ዋና አላማም የሔፓታይተስ ቢ ቫይረስ ስርጭትን በማጥናት ቫይረሱ ያለባቸውን ሰዎች በተሻለ መንገድ የሚታ ከሙበትን መንገድ መጠቆም ነው

**ተ ሳት ፎ :–** የጥናቱ አባላት እርሶና ሌሎችን በፍቃደኝነት በጥናቱ እንድትሳተፉ እንጠይቆታለን፣ ከእርስዎ የሚጠበቀው ለአምስት ደቂቃ ያህል መጠይቆችን መመለስና አምስት ሚሊ ሊትር ደም መስጠት ነው፣ የሚሰጡት ደም የሚሰበሰበው ከብክለት በጸዳ መንገድ ነው

**ጉ ዳ ቶች:–**አምስት ሚሊ ሊትር ደም መውሰድ ለጤናዎ የ ከፋ ችግር አያመጣም፣ ነገርግን ደም የተቀዳበት ቦታ ለተወሰኑ ደቂ ቃዎች ሊያሳምሞ ይችላል፣ይህ ለክፉ ጉዳት አይሰጥም ሆኖም ችግር ካጋጠመ አስፈላጊውን ህክምና እንዲያገኙ እናደርጋለን

**ጥቅ ሞች:–** በጥናቱ ተሳትፈው ቫይረሱ በሰውነትዎ ውስጥ ቢገኝ አስፈላጊውን ህክምና እንዲያገኙ ለህኪምዎና ለሆስፒታሉ እናሳውቃለን

**ሚስጥራዊ ነት:–** በጥናቱ ውስጥ ከእርስዎ የምናገኛቸው ማንኛውንም መረጃ ሚስጥራዊነቱ የ ተጠበቀ ነው፣ በጥናቱ ውስጥ የምንጠቀ መው የሚስጥር የፀረ ኤች አይቪ መድሃኒት መጠ ቀሚያ ቁጥርዎን በመሆኑ እርስዎን የሚገልጽ ምንም አይነት መለያ አይኖርም የእርስ ዎን ማንነትም በጥናቱ ውጤት ላይም ይሁን በማንኛውም ሁኔታ አይገለጽም

**የጥናቱን ውጤት ስለማካፈል:–** የጥናቱ አባላት የጥናቱን ውጤት ለሚመለከታቸው አካላት ገለጻ እናደርጋለን፣ ሪፖርቱ የእርስ ዎን የግል ጉዳይ እንደማይነካ እናረጋግጥልዎታለን፣ የእርስዎን የደም ውጤቶዎን ለሪፖርት እና ጥናቱን ለማሳተም እንድንጠቀም በትፍቃድ እንዲሰጡን እንጠይቅዎታለን፣

**በጥናቱ ያለመሳተፍ መብት:–** በዚህ ጥናት መሳተፍ የእርስዎን ሙሉ ፈቃድ እንደ መጠየቁ መጠን በማንኛውም ጊዜ በጥናቱአ ለመሳተፍ መብትዎ የተጠበቀ ነው፣ በጥናቱ አለመሳተፍ በህይወትዎ የሚያመጣው ምንም አይነት ጫና የለም፣

ጌታዬ/እመቤቴ ስለ ጥናቱ ጥያቄ አለዎ? በጥናቱለ መሳተፍ ፍቃደኛ ነዎት? ከተስማሙ የፍቃድ ማረጋገጫ ቅጹን ይሙሉልን፣

**<u>የስምምነት መግለጫ ፎርም</u>**

እኔ ስሜ ከዚህ በታች የተገለጸው የዚህን የጥናት ዓላማና የሚያስከትለውን የጎንዮሽ ጉዳት በመረዳትና በማንኛውም ጊዜ ካልፈለኩኝ ከጥናቱ ራሴን ማግለል እንደምችል መስማማቴን አረጋግጣለሁኝ፣

እኔ ___ _____ ከዚህ በኋላ ፍቃዴን ለአቶ/ ወ/ ሮ/ ወ/ት _____ በጥናቱ ውስጥ ለመሳተፍ ሰጥቻ ለሁኝ፣ስለጥናቱ አስፈላጊውን መረጃ በሚገባኝ ቋንቋ ተሰጥ ቶኛል፣እንዲሁም በማን ኛውም ጊዜፍቃ ዴንማንሳት እንደምችልና ይህም በመሆኑ ምንም ችግር እንደማይ ገጥመኝ ማረጋገጫ ተሰጥቶኛል፣

የተሳታፊው ስም_____

የተሳታፊው ፊርማ_____

የፍቃድ ተቀባይ ስም አቶ/ ወ/ ሮ/ ወ/ት _____

የፍቃድ ተቀባይ ፊርማ _____

ቀን _____

ምስክር _____

## <u>መጠይቅ</u>

ይህ መጠይቅ ለዚህ ጥናት የሚረዱ የተለያዩ ጥያቄዎችን በ3 ክፍል ይዟል፣ጥያቄዎቹ ተሳታፊዎችን በመጠየቅ፣ ከኤ.አር.ቲ፣ከቅድመ ኤ.አር.ቲ መዝገብ ፣ከቅበላና ከክትትል ፎርሞች ነው

ውድ የጥናቱ ተሳታፊ፤ የዚህ ጥናት ዋና አላማ ኤችአይቪ በደማቸው ባለባቸው ሰዎች ውስጥ የሔፓታይተስ ቢ ቫይረስ ስርጭትን በማጥናት ቫይረሱ ያለባቸውን ሰዎች በተሻለ መንገድ የሚታ ከሙበትን መንገድ መጠቆም ነው

**<u>ውድ የጥናቱ ተሳታፊ፡-</u>**አኛ የምናጠናው የሔፓታይተስ ቢ ቫይረስ ስርጭትን ኤች አይቪ በደማቸው ባለባቸው ፀረ ኤች አይቪ መድሃኒት በሚወስዱና ባልጀመሩ ሰዎች ላይ ነው፣የዚህ ጥናት ዋና አላማም የሔፓታይተስ ቢ ቫይረስ ስርጭትን በማጥናት ቫይረሱ ያለባቸውን ሰዎች በተሻለ መንገድ የሚታ ከሙበትን መንገድ መጠቆም ነው

በጥናቱ ውስጥ ከእርስዎ የምናገኛቸው ማንኛውንም መረጃ ሚስጥራዊነቱ የ ተጠበቀ ነው፣ በጥናቱ ውስጥ የምንጠቀ መው የሚስጥር የፀረ ኤች አይቪ መድሃኒት መጠ ቀሚያ ቁጥርዎን በመሆኑ እርስዎን የሚገልጽ ምንም አይነት መለያ አይኖርም የእርስ ዎን ማንነትም በጥናቱ ውጤት ላይም ይሁን በማንኛውም ሁኔታ አይገለጽም

የተሳታፊው ፊርማ_____

የሆስፒታሉ/የጤና ጣቢያው ስም _____

የ መጠይቅ ቁጥር_____

የሚስጥር የፀረ ኤችአይቪ

መድሃኒት መጠቀሚያ ቁጥር _____

## ክፍል አንድ:-የበሽተኛ መጠይቅ

| ተራ ቁጥር | ጥያቄ | መልስ | ኮድ | ዝለል |
|---|---|---|---|---|
| 001 | ፆታ | ወንድ | 1 | |
| | | ሴት | 2 | |
| 002 | እድሜ | / / አመት | | |
| 003 | የትምህርት ደረጃ | ማንበብና መፃፍ የማይችል | 1 | |
| | | ማንበብና መፃፍ ብቻ | 2 | |
| | | የትምህርት ደረጃ | 3 | |
| 004 | የጋብቻ ሁኔታ | ያላገባ | 1 | |
| | | ያገባ | 2 | |
| | | የተፋታ | 3 | |
| | | የሞተበት/ ባት | 4 | |
| | | ተለያይተው የሚኖሩ | | |
| 005 | የሚኖሩበት ቦታ | ከተማ | 1 | |
| | | ገጠር | 2 | |
| 006 | የሚከተሉት ንብረቶች አለዎት | ሬዲዎ | 1 | |
| | | ቴሌቪዥን | 2 | |
| | | ስልክ | 3 | |
| | | መኪ ና | 4 | |
| | | ፍሪጅ | 5 | |
| | | የኤሌክትሪክ ምጣድ | 6 | |
| 007 | ዋና የገቢ ምንጭዎ ምንድን ነው | ግብርና | 1 | |
| | | ንግድ | 2 | |
| | | የመንግስት ደሞዝ | 3 | |
| | | ሌላ ካለ ይገለጽ | _____ | |
| 008 | የቤተሰብዎ የወር ገቢ ምን ያህል ነው | _____ | | |
| 009 | ስራዎ ምንድን ነው | _____ | | |
| 010 | ኤች አይቪ በምን መንገድ ያዘዎት | በግብረ ስጋ ግንኙነት | 1 | |

| | | | | |
|---|---|---|---|---|
| | | በክንድ በሚሰጥ እፅ | 2 | |
| | | አላውቀውም | 3 | |
| 011 | ከኤች አይቪ ጋር ስንት አመት ኖሩ | /...../አመት | | |
| 012 | ጠላ፤አረቄ፤ቢራ፤ድራፍት...ይ ጠጣሉ | አዎ | 1 | የለምከመረጡ ወደ014 |
| | | የለም | 2 | |
| 013 | መልስዎ አዎ ከሆነ በየስንት ጊዜ | በቀን አንዴ | 1 | |
| | | በሳምንት ከሁለት ጊዜ በላይ | 2 | |
| | | በየ ሳምንቱ | 3 | |
| | | በወር ሁለቴ | 4 | |
| 014 | የቲቪ መድሃኒት ወስደው ያውቃሉ | አዎ | 1 | የለምከመረጡ ወደ016 |
| | | የለም | 2 | |
| 015 | መልስዎ አዎ ከሆነ ከሆነ አሁን ይወስዳሉ | አዎ | 1 | |
| | | የለም | 2 | |
| 016 | የጉበት በሽታ(የወፍ በሽታ ) አምዎት ያውቃል | አዎ | 1 | |
| | | የለም | 2 | |
| 017 | ምቹ ጊዜ ጠባቂ በሽታዎች አ ሞዎት ያውቃል ለምሳሌ፡-በአፍ እና በቆዳ ላይ የሚታዩ የፈንገስ በሽታዎች፣ የሆድ እቃና የአንጀት መታወክ፣ተቅማጥ፣አልማዝ ባላጭራ ( ታመው ተኝተው ያውቃሉ) | አዎ | 1 | |
| | | የለም | 2 | |
| 018 | የፀረ-ኤችአይቪ መድሃኒቱን በትክክል ይጠቀማሉ ( የለም ከሆነ ምን ያህል) | ትናንት | አዎ | |
| | | | የለም | |
| | | ባለፉት 3 ቀናት | አዎ | |
| | | | የለም | |
| | | | አዎ | |

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|---|---|---|---|---|
| | | ባለፉት 7 ቀናት | የለም | |
| 019 | ከፀረ ኤችአይቪ መድሃኒቱ ሌላ ተጨማሪ መድሃኒት ይ ወስዳሉ | አወ | 1 | የለም ከመረጡ ወደ021 |
| | | የለም | 2 | |
| 020 | መልስዎ አዎ ከሆነ የመድ ሃኒቱን ስም ወይም በሽ ታውን ይግለፁ | _____ | | |
| 021 | ከአሁኑ በፊት የነበሩ የፀረ ኤችአይቪ መድሃኒቶችን ይጠቀሙ ነበር | አወ | 1 | |
| | | የለም | 2 | |
| 022 | የፀረ-ኤችአይቪ መድሃኒት ሁኔታ | ቅድመ ፀረ ኤችአይቪ | 1 | |
| | | ፀረ-ኤችአይቪ መድሃኒት የጀመረ | 2 | |
| | | | | |

**<u>ለመረጃ ሰብሳቢ፡-ከዚህ ቀጥሎ ያሉት ጥያቄዎች የሚሞሉት ከኤአርቲ/ከቅድመ ኤአርቲ፤ከቅበላና ከክትትል ፎርሞች ነው</u>**

## <u>ክፍል ሁለት፡-ኤአርቲ ሳይጀምር ከትትል ላይ ላለ ሰው የሚሞላ</u>

**የሚስጥር የፀረ ኤችአይቪ መድሃኒት መጠቀሚያ ቁጥር _____**

| ተራ ቁጥር | ጥያቄ | መልስ | ኮድ | ዝለል |
|---|---|---|---|---|
| 101 | በኤችአይቪ ህክምና ከትትል የቆየበት ጊዜ | /...../months | | |
| 102 | የፀረ-ኤችአይቪ መድሃኒት ከጀመሩ አንስቶ ያለው አማካይ የ"$CD_4^+$" መጠን | /...../ per l | | |
| 103 | የፀረ-ኤችአይቪ መድሃኒት ከጀመሩ አንስቶ ያለው አማካይ የ"ALT" እና "AST" መጠን | /...../ per l | | |
| 104 | Cotrimoxazole ይወስዳሉ | አወ | 1 | |
| | | የለም | 2 | |
| 105 | INH ይወስዳሉ | አወ | 1 | |
| | | የለም | 2 | |

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| 106 | በሽተኛው ሌላ አይነት ሄፓታይቲስ አለበት(ሄፓታይቲስ ሲ፣ዲ፣ኤ፣ኢ) | _____ | | |

## ክፍል ሶስት፦ ኤአርቲ ለጀመረ ሰው የሚሞላ

| | | | | |
|---|---|---|---|---|
| 201 | የፀረ-ኤችአይቪ መድሃኒት የጀመረበት ቀን | _____ | | |
| 202 | የአለም የጤና ድርጅት የበሽተኛው የጤና ደረጃ | _____ | | |
| 203 | Cotrimoxazole ይወስዳሉ | አወ | 1 | |
| | | የለም | 2 | |
| 204 | INH ይወስዳሉ | አወ | 1 | |
| | | የለም | 2 | |
| 205 | መድሃኒቱን ሲጀምሩ የነበረው የ “CD4+” ቁጥር | /...../ per l | | |
| 206 | ፀረ ኤችአይቪ መድሃኒት እየወሰዱ የቆዩበት ጊዜ | /...../ ወራት | | |
| 207 | አሁን ያሉበት የጤና ሁኔታ | አልጋ ላይ ያሉ | 1 | |
| | | በድጋፍ የሚንቀሳቀሱ | 2 | |
| | | ስራ የሚሰሩ | 3 | |
| 208 | የፀረ-ኤችአይቪመድሃኒት ከጀመሩ አንስቶ ያለው አማካይ የ“CD4+” መጠን | /...../per l | | |
| 209 | የፀረ-ኤችአይቪ መድሃኒት ከጀመሩ አንስቶ ያለው አማካይ የ“ALT” እና “AST” መጠን | /...../per l | | |
| 210 | የፀረ-ኤችአይቪ መድሃኒቱን በትክክል የመውሰድ ደረጃ | ጥሩ | 1 | |
| | | ደህና | 2 | |
| | | ደካማ | 3 | |
| 211 | በሽተኛው ሌላ አይነት ሄፓታይቲስ አለበት(ሄፓታይቲስ ሲ፣ዲ፣ኤ፣ኢ) | _____ | | |
| 212 | የሚሰጠው የፀረ ኤችአይቪ መድ ሃኒት | መጀመሪያ ደረጃ መድሃኒት | 1 | 2 ከሆነ ወደ 216 |
| | | ሁለተኛ ደረጃ መድሃኒት | 2 | |

| | | | | |
|---|---|---|---|---|
| 213 | የመጀመሪያ ደረጃ መድሃኒትከሆነ<br>( የመድሃኒቱ ኮድ ይጠቀስ) | _____ | | |
| 214 | የመጀመሪያ ደረጃ መድሃኒት ውስጥ የተቀየረ መድሃኒት አለ | አ ወ | 1 | የለም ከሆነ ጨርስ |
| | | የለም | 2 | |
| 215 | ካለ,<br>a. የትኛው መድሃኒት | _____ | | |
| | b. የተቀየረበት ምክንያት | _____ | | |
| 216 | ሁለተኛ ደረጃ መድ ሃኒት ከሆነ<br>(የመድሃኒቱ ኮድ ይጠቀስ) | _____ | | |
| 217 | የሁለተኛ ደረጃ መድሃኒት ውስጥ የተቀየረ መድሃኒት አለ | አ ወ | 1 | የለም ከሆነ ጨርስ |
| | | የለም | 2 | |
| 218 | ካለ,<br>a. የትኛው መድሃኒት | _____ | | |
| | b.የተቀየረበት ምክንያት | _____ | | |
| ለመረጃ ሰብሳቢ፡- የሚጨምሩት ነገር ካለ | | | | |

**DECLARATION**

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

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Signature: _____

Place: Addis Ababa

Date of Submission: May 15, 2011

Advisor's Name Signature_____

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