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**PREVALENCE OF HEPATITIS B VIRUS IN PATIENTS WITH  
DIABETES MELLITUS: A COMPARATIVE CROSS  
SECTIONAL STUDY AT WOLDIYA GENERAL HOSPITAL,  
ETHIOPIA.**

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## List of Abbreviations

AAU	-----	Addis Ababa University
AOR	-----	Adjusted Odds Ratio
CDC	-----	center for Disease control
CHB	-----	Chronic Hepatitis B
CHC	-----	Chronic Hepatitis C
CKD	-----	Chronic Kidney Disease
DM	-----	Diabetes Mellitus
DMIP	-----	Department of Microbiology, Immunology and Parasitology
DNA	-----	Deoxy Ribonucleic Acid
ELISA	-----	Enzyme Linked Immunosorbent Assay
HBcAg	-----	Hepatitis B Core Antigen
HBeAg	-----	Hepatitis B e Antigen
HBIG	-----	hepatitis B immunoglobulin
HBsAg	-----	Hepatitis B Surface Antigen
HBV	-----	Hepatitis B Virus
HCC	-----	hepatocellular carcinoma
HCV	-----	Hepatitis C Virus
HGV	-----	Hepatitis G Virus
HLA	-----	Human Leukocyte Antigen
IDDM	-----	Insulin Dependent Diabetes Mellitus
IFCC	-----	International Federation for Clinical Chemistry
IV	-----	Intravenous
Lab.	-----	Laboratory
LDH	-----	Lactate Dehydrogenase
LFT	-----	Liver Function Tests
MDH	-----	Malate Dehydrogenase
NAFLD	-----	Non Alcoholic Fatty Liver Disease
NASH	-----	Non Alcoholic Steatohepatitis
NIDDM	-----	Non Insulin Dependent Diabetes Mellitus

PCR-----Polymerase Chain Reaction

PHC----- Primary Hepatocellular Carcinoma

PLP-----pyridoxial Phosphate

RNA -----Ribonucleic Acid

SES-----Socioeconomics Status

SGOT/AST/---Serum Glutamate Oxaloacetate Transaminase/ Aspartate Aminotransferase /

SGPT/ALT/----Serum Glutamate Pyruvate Transaminase /Alanine Aminotransferase/

SOP-----Standard Operation Procedure

SPSS----- Statistical Package for the Social Sciences

VCT-----Voluntary Counseling and Testing

Vs-----Versus

WHO----- World Health Organization

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## Abstract

**Background:** *Hepatitis B Virus* (HBV) infection and its sequelae (cirrhosis and liver cancer) are major global health problems. Overall prevalence in Ethiopia varies from 4.7-16.8% for HBsAg and 70-76.4% for at least one marker positive. During recent years evidence has accumulated that in patients with diabetes mellitus, phagocytosis by polymorpho nuclear leukocytes is impaired and disturbances in cell-mediated immune responses can be demonstrated. Cell-mediated immunity is involved in the defense against viruses as well as in that against *Mycobacterium* and *Fungi*. However, no increased incidence of viral infections like HBV has yet been reported in patients with diabetes.

**Objective:** To determine the prevalence of HBsAg in patients with diabetes mellitus and to compare those with the non diabetes. It also asses associated factors and liver function tests (LFT); and compare Diabetic and non Diabetic subjects; and HBV positive and negative participants.

**Methods:** The study was a comparative cross sectional study design conducted at Woldiya General Hospital using 108 consented study populations from each study group during the period November, 2010 through January, 2011. A convenience sampling method was used. A total of 216 samples were tested for HBsAg serostatus using VISITECT HBsAg rapid test kit and LFT tests using Humastat 80 chemistry analyzer. Data entered to SPSS-16 and then analyzed using the same soft ware. Multivariate logistic regression was used to see the association of HBV with clinical history of participants and sociodemographic variables. All tests were two-sided with  $\alpha$ -level of 0.05 and 80% power.

**Results:** prevalence of HBsAg was equal between diabetes and non diabetes, 3.7% indicating that there was no difference between the two groups. No any Sociodemographic and clinical history of participants were associated with HBV infection ( $p>0.05$ ) except chronic liver disease.

**Conclusion:** In this study a positive relation was not indicated between HBV and Diabetes and DM in the study area did not predisposed to HBV infection than the rest of the population.

**Key word:** hepatitis B virus, prevalence, association ,diabetes millitus,non diabetics.

# 1. Introduction

## 1.1 Hepatitis B virus

Hepatitis B virus is first identified in 1960s by Baruch Samuel Blumberg from an Australian Aborigine. HBV, the Dane particle, is a spherical lipid-containing structure with an outer diameter of 42 to 47 nm. Nucleocapsids contain a single copy of the partially double-stranded DNA genome, which is covalently linked to the viral reverse transcriptase (RT) at the 5' end of the complete minus strand. It is classified in the family Hepadnaviridae and genus orthohepadnaviruses. The only DNA viruses of animals that are known to replicate their DNA by reverse transcription of a viral RNA (David and Peter, 2007).

The natural history of HBV is complex and is influenced by many factors, including viral factors (HBV genotype, viral mutations, level of HBV replication), host factors (gender, age, and immune status), and exogenous factors such as concurrent infection with other hepatotropic viruses like HCV and HDV or alcohol (Lok and McMahon, 2009; Sharma *et al*, 2005).

HBV is classified by the World Health Organization (WHO) as the world's second greatest carcinogen after tobacco (British liver trust, 2009; WHO, 2002) and divide the world into three areas where the prevalence of chronic HBV infection is: high (>8% like South-east Asia, Pacific Basin, Sub-Saharan Africa, the Amazon Basin and parts of the middle east), intermediate (2-8% mainly Europe and southern America), and low (<2% , Northern America) (Abbot Diagnostics, 2006; Lok and McMahon, 2009; WHO, 2002).

HBV infection and its sequelae (cirrhosis and liver cancer) are major global health problems. It has been estimated that up to 2 billion individuals have evidence of exposure to HBV (British liver trust, 2009) and an estimated 350 million persons worldwide are chronically infected with HBV (Lok and McMahon, 2009; British liver trust, 2009). Most of these came from East Asia and sub-Saharan Africa (Edmunds *et al*, 1996; WHO, 2002). Approximately 470 million inhabitants of Africa are infected with this virus at some time during their lives and about 10% remain infected. HBV induced diseases, especially hepatocellular carcinoma (HCC), cause more than 230,000 deaths in Africa each year (Kew, 1996). Overall prevalence in Ethiopia varies from 4.7-16.8% for HBsAg and 70-76.38% for at least one marker positive (Abebe *et al*, 2003;

Kefene *et al*, 2005; Pasquini *et al*, 1988; Shimelis *et al*, 2007; Tessema *et al*, 2010; Negero *et al*, 2011).

A community-based sero epidemiological survey was conducted in 2003 in Addis Ababa using venous blood from 4736 individuals less than 50 years of age. In this study HBsAg prevalence was found to be 7%, higher in males than females (Abebe *et al*, 2003). HBsAg, anti-HBc and anti-HBs were determined using 432 Hospital employees in Addis Ababa by the Hepanostika micro enzyme linked immunoassay method. The overall prevalence rate was 9.02% for HBsAg, 46.25% for anti-HBs, 73.6% for anti-HBc and 76.38% for "at least one marker positive (Kefene *et al*, 1989). A study done at Saint Paul's General Specialized Hospital, Addis Ababa; the prevalence of HBsAg and anti-HBc antibody in VCT clients were found to be 5.7% and 44.8%, respectively (Shimelis *et al*, 2007). A nationwide seroepidemiological study of hepatitis B markers prevalence was conducted in Ethiopia on 5,270 young males from all regions of the country. Overall prevalence rates were 10.8% for HBsAg and 73.3% for "at least one marker positive (Kefene *et al*, 2005). Institution based cross-sectional study was performed from November to December, 2008 and 384 VCT clients were investigated. The prevalence of HBsAg in this study group was 5.7% (Negero *et al*, 2011). A study from Gondar University by Tessema *et al*, (2010) from the total of 6361 consecutive blood donors. The overall seroprevalence of HBV was 4.7% for HBsAg.

While most persons recover from hepatitis B infection, 90% of infants, 30% of children, and 10% of adults become chronically infected (Lok and McMahon, 2009; CDC, 2010). In addition, immunosuppressed persons are more likely to develop chronic HBV infection after acute infection (Lok and McMahon, 2009) and impairment of immune system is well demonstrated in patients with DM (Demir *et al*, 2008). Persons who are chronically infected with hepatitis B are at an increased risk for the development of cirrhosis and HCC (Lok and McMahon, 2009; CDC, 2010). Studies performed in mice suggest that HBeAg may cross the placenta during pregnancy and induce HBV-specific immune tolerance in the fetus to epitopes of the viral core protein. This finding was interpreted to indicate that when the newborn of an HBeAg-positive mother is exposed to HBV at birth, the risk of chronic infection becomes very high because the potential to react to a major viral antigen has been reduced. Injection of hyper immune globulin to HBsAg

(HBIG) and vaccination of the newborns at birth can reduce the risk of chronic infection from HBeAg-positive mothers by >90% (David and Peter, 2007).

Liver is the primary site of hormone and glucose metabolism, and intercommunication between liver and diabetes has long been recognized. Majority of patients with cirrhosis have glucose intolerance (60%) or overt DM (20%) (Chen *et al*, 2006).

## 1.2 Diabetes Mellitus

DM refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production (Fauci *et al*, 2008).

Diabetes in a patient with cirrhosis is frequently caused by hemochromatosis (excessive deposition of iron in tissues, especially in the liver and pancreas), since iron deposits compromise the production of insulin by the islets of Langerhans in the pancreas (Digestive system disease, 2010; Blonski *et al*; 2010). Increased body mass index and diabetes with subsequent development of non-alcoholic steatohepatitis (NASH) represent significant risk factors for HCC (Blonski *et al*, 2010).

A study to estimate global prevalence of diabetes for the year 2000 and projections for 2030 showed that the prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% for 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Fauci *et al*, 2008; Wild *et al*, 2004). In 2000, the prevalence of diabetes in the WHO African Region was estimated to be 7.02 million people, out of whom about 10% people had type I diabetes and 90% had type II diabetes (Kirigia *et al*, 2009; Levitt, 2008). WHO estimated the number of diabetic cases in Ethiopia to be 800,000 by the year 2000, and the number is expected to increase to 1.8 million by 2030 (Yemane *et al*, 2007).

A study by Yemane *et al*, (2007) in Jimma Town showed that, the blood glucose level of 28 out of 526 participants was in diabetic range making the prevalence of Type II diabetes to be 5.3%.

### 1.3 HBV and DM association

Viruses are involved in the pathogenesis of type I DM in at least two distinct ways: (a) by directly destroying insulin-producing pancreatic  $\beta$ -cells by cytolytic infection, and (b) by triggering or somehow contributing to  $\beta$ -cell-specific autoimmunity, leading to the development of type I DM (Won Yoon *et al*, 2004). Viral infections have been implicated in pancreatic islet destruction but are an extremely rare cause of DM. A form of acute onset of type I diabetes, termed fulminant diabetes, has been noted in Japan and may be related to viral infection of islets (Fauci *et al*, 2008).

Recently evidences have accumulated that in patients with untreated or poorly controlled diabetes mellitus, phagocytosis by polymorpho nuclear leukocytes is impaired and disturbances in cell-mediated immune responses can be demonstrated (Kew *et al*, 2001). Cell-mediated immunity is involved in the defense against viruses as well as in that against mycobacterium and fungi. No increased incidence of viral infections has as yet been reported in patients with diabetes, either in the stable or in the uncontrolled state (Kew *et al*, 2001).

There are discrepancies among studies and it still remains unclear whether hepatitis virus infection causes the development of DM or if some diabetic patients are more liable than others to acquire HBV or HCV infections (Kew *et al*, 2001; Won Yoon *et al*, 2004). A study from Taiwan, South Africa, Turkey and Nigeria showed that the prevalence of HBV is higher among diabetic than the general population, but not statistical significant. In another study from China, Turkey, Poland and Athens showed different outcome association between HBV and DM.

To our knowledge, there is no research that was done in Ethiopia and in the study area in particular on the prevalence of HBV in diabetic patients. But there are some researches that were done on the prevalence of HBV among diabetic patients out side Ethiopia.

A study by Chen *et al* (2003) in Taiwan using 820 consecutive type II diabetic patients and 905 control subjects who came for medical check-ups; they determined HBsAg and anti-HCV in both groups, using enzyme immunoassay. No significant difference was found between type II DM patients and the control group for seropositivity of HBsAg (13.5% versus 12.4%; odds ratio [OR] = 1.09; 95% CI: 0.77–1.55; p = 0.441).

A study conducted in South Africa by Kew *et al* (2001); the prevalence of HBsAg and anti-HBs was determined in 531 white and 519 black diabetic outpatients and in appropriate white and black control inpatients and outpatients attending the same hospitals as the diabetic and suffering from diseases other than diabetes mellitus and voluntary blood donors. HBsAg was detected in the serum by solid-phase radioimmunoassay. Blood sugar levels were measured in the diabetic and control subjects. There was no difference between the prevalence of either HBsAg (4.6% vs. 4.3%) or anti-HBs (44.7% vs 44.4%), in either the white or black diabetes or that in the white and black controls.

Kilic *et al* (2007) in Turkey investigate possible relationships between chronic HBV infection and NIDDM in 88 patients with type II diabetes and 89 gender- and age-matched non-diabetic controls. HBsAg positivity rates in diabetic patients and controls were 3.4% and 2.2% ( $p=0.657$ ) respectively. Differences were not found in the rates of HBV positivity between patients with type II diabetes and control groups.

A study from Lagos by Onyekwere *et al* (2002) using 100 outpatient diabetic and 80 non-diabetic controls at the medical outpatient department between January and July 1992. Twenty diabetic patients [20%] and 14 controls [17.5%] had serological markers (HbsAg and anti-HBc) indicating ongoing chronic HBV infection. The difference between diabetics and non-diabetic controls was not statistically significant ( $P>0.05$ ).

Gulcan *et al* (2008) in Turkey aimed to investigate the risk factors and seroprevalence of hepatitis B and C in type I and type II diabetic patients using 630 diabetic and 314 non diabetic patients. Serologic testing for anti-HCV and HBsAg was done using ELISA. HBsAg and anti-HCV seropositivity rates were 5.1% and 3.2% in diabetic patients and were 3.8% and 1.3% in control group, respectively. There was no statistically significant difference between the two groups with respect to either marker.

A recent study in Turkey by Demir *et al* (2008) aimed to investigate the prevalence of occult HBV infection among 100 HbcAb+/-, anti-HBs positive type II DM patients and 100 age and sex matched, HbcAb+/-anti-HBs negative blood donors. HBV DNA was detected in 11% of the diabetic patients ( $1 \times 10^{-5}$  to  $10$  copies/ml) and in 3% of the controls ( $4 \times 10^{-1}$  to  $10$  copies/ml).

The difference between groups was statistically significant ( $P < 0.05$ ). These data suggest that the prevalence of occult HBV infection is higher in diabetics compared with healthy controls.

Khuri *et al* (1985) using 395 healthy control subjects who were hospital personnel and 100 diabetic patients; significant difference was found in the prevalence of HBV markers (mainly HB surface antibody) between the diabetic group and the controls (51% versus 25%,  $P < 0.001$ ).

Huo *et al* in (2003) in Taiwan aimed to investigate the outcome in HCC patients undergoing resection with and without DM and the interaction with HBV and HCV. A total of 239 HCC patients were included. Survival and tumor recurrence were analyzed according to the status of DM and viral hepatitis. DM does not affect the long-term survival in HCV-related HCC but is a recurrence-independent poor prognostic factor for HBV-related HCC.

Another study in China by Cheng *et al* (2006), examined the association between chronic HBV infection and clinical outcomes in a consecutive cohort of Chinese patients with type II diabetes. Between 1995 and 1999, 2,838 type II diabetes patients underwent comprehensive assessments and blood screening for HBsAg. The risk of occurrence of cardiovascular events and end-stage renal disease was compared between HBsAg-positive and HBsAg-negative groups. In type II diabetes patients, chronic HBV infection was associated with increased risk of end-stage renal disease.

In China by Lao *et al* (2007) retrospective cohort study was performed to examine the relationship between maternal HBV infections, as indicated by the surface antigen status, with the development of gestational diabetes mellitus in 13,683 a normal-risk Chinese obstetric population. The result confirmed the independent association between hepatitis B infection with gestational diabetes mellitus.

A report on the 37th Annual Meeting of the European Association for the Study of the Liver in Spain, by Drakoulis *et al* (2002) showed the frequency of Type II diabetes in patients with CHB or CHC among 850 patients. 98 were found with CHB and 117 with CHC. The overall frequency of Type II diabetes in both CHB and CHC patients is greater than that of the general population. This result showed a slightly higher incidence of diabetes mellitus in CHB patients, although it is not statistically significant.

The recent article by Huang *et al* (2010), a ten year cohort of 1233 adults who received health examinations in 1997–1998 and in 2000–2001 were enrolled. Among them, 483 subjects who received a third health examination in 2006–2008 were further sampled. The prevalence and incidence of diabetes between asymptomatic HBV carriers and non-HBV controls were compared using the  $\chi^2$ -test and logistic regression. There was no significant correlation between asymptomatic HBV infection and the presence of diabetes in subjects examined in 1997–1998, 2000–2001, or 2006–2008.

A recent study in Tehran by Alavian and Tabatabaei (2010) aimed to evaluate the immunological response to HBV vaccine in diabetic patients with chronic kidney disease (CKD) by conducting a meta-analysis of the current literature involving 15,073 unique patients with CKD. Meta-analysis determined that HBV vaccination's seroprotection rate in diabetic CKD patients is significantly lower than that in non-diabetic CKD patients.

A study by El-ottol *et al* (2010) in Palestine on the prevalence and risk factors of HBV among hemodialysis patients showed the overall prevalence of HBV among the four Haemodialysis centers and it was found to be 8.1%. The main risk factors were Haemodialysis centers (HD) ( $p = 0.05$ ) and history of blood transfusion ( $p < 0.01$ ). On the other hand, no statistically significant relationship was found between HBV infection and the level of education ( $p = 0.9$ ); smoking ( $p = 0.51$ ); blood transfusion abroad ( $p = 0.7$ ); and surgical operation abroad ( $p = 0.7$ ).

## **1.4 Statement of the Problem**

Infectious and communicable diseases account for about 60-80% of the health problems in Ethiopia (WHO Regional office for Africa, 2009). Information on the prevalence of HBV among diabetic patient and the association between the two is none in Ethiopia. With 75% of the global population currently living in areas of high infection, it is clear that hepatitis B is a major international health problem (British Liver trust, 2009). It is one of the world's most common and serious infectious diseases. About 5% of the populations are chronic carrier of HBV, and nearly 25% of all carriers develop serious liver diseases such as chronic hepatitis, cirrhosis, and primary HCC (WHO, 2002). Every 30-45 seconds, one person dies from this vaccine-preventable disease (Physician's Guide, 2007).

HBV is present in many body fluids of infected individuals. Because viral load is very high and may reach  $10^{10}$  copies per ml of serum, the rate of transmission may be very high compared to other viruses such as HIV and HCV. The main routes of infection are perinatal transmission, blood, percutaneous transmission, and sexual transmission (David and Peter, 2007; Lok and McMahon, 2009; WHO, 2002). HBV is 50 to 100 times more infectious than HIV. Morbidity and mortality statistics are significant. About 350 million live with chronic infection (British Liver trust, 2009). An estimated 600,000 die each year due to the acute or chronic consequences of hepatitis B (British Liver trust, 2009). The high rate of chronicity in many parts of Africa is attributed to horizontal spread to young children from playmates, adults involved in their care (David and Peter, 2007) and perinatal infection of infants from infected mother (Lok and McMahon, 2009; WHO, 2002).

Patients suffering from type I DM incur high risk of infection with hepatotropic viruses because of frequent hospitalization and blood tests (Halota *et al*, 2002). A well-documented, yet under-acknowledged risk associated with blood glucose monitoring is the transmission of blood borne viral pathogens such as HBV (Thompson and Perz, 2009).

Nosocomial transmission of HBV has previously been associated with unsafe injection practices, including contamination of multi-dose-multi-patient vials and finger stick blood sampling devices with reusable components (Gotz *et al*, 2008; Thompson and Perz, 2009). Incidences of hepatitis B transmission linked to blood glucose monitoring (Dreesman *et al*, 2006; Thompson and Perz, 2009) in care homes have been reported since the early 1990s. A review of infection control procedures at the home revealed that the glucose monitoring apparatus (glucometer) and spring-loaded barrel of the finger stick device are not cleaned between uses, although a new end cap and lancet are used each time. Insulin and other multi-dose medications were also not labeled with patient names or the dates when the vials were opened. An anonymous staff survey also revealed that some staff members had observed others re-using needles or failing to change their gloves between sampling different patients' blood (Schrijver *et al*, 2005).

Frequent Immunosuppressed persons (e.g., hemodialysis patients and persons with HIV infection) are at increased risk for chronic infection (CDC, 2008; Lok and McMahon, 2009). The impairment of the immune system is well demonstrated in diabetes. The prevalence of occult

HBV infection is relatively frequent among patients with immune suppression (Demir *et al*, 2008).

Having the above points in mind, economic hardship, overcrowding and poor hygienic practices making diabetic patient at increased risk of infections (Abebe *et al*, 2003; Kefene *et al*, 2005; Shimelis *et al*, 2007 and Tsega *et al*, 1986). Thus, the aim of this study was to determine the prevalence of HBsAg among diabetic patients and to compare with the general population if there is a difference.

## **1.5 Significance of the Study**

About one million deaths each year are attributable to HCC and cirrhosis, related to chronic hepatitis B virus infection (Mathaw, 2008). HBV induced diseases, especially HCC; cause more than 230 000 deaths in Africans each year (Kew, 1996). Clinically, hepatitis B can result in a spectrum of clinical problems ranging from asymptomatic infection to symptomatic. The long-term danger is that some infected persons develop persistent infection, of whom a fraction develop chronic liver disease and associated complications such as cirrhosis, portal hypertension, hepatocellular carcinoma, and hepatocellular failure (Mathaw, 2008).

Chronic HBV infection is dangerous because there are often no symptoms (even liver blood tests may be normal). As many as 2 out of 3 chronically infected persons are not aware they have been infected. People chronically infected with HBV have a 200-fold greater risk of developing liver cancer than those who are not infected. Treatment options for liver cancer are limited. Currently, there is no effective systemic chemotherapy for liver cancer. However, early detection by regular screening can lead to successful surgical removal and long-term survival (Physicians' Guide, 2007).

The relative lifetime risk for HBsAg-positive males was found to be about 20 compared to uninfected individuals. In human patients; liver cancers usually develop after several decades of infection and are often associated with cirrhosis of the liver. Consistent with the distribution of chronic HBV infections around the globe, HCC is one of the two or three most common malignant neoplasm in people living in China, Taiwan, and Southeast Asia, as well as in sub-Saharan Africa (David and Peter, 2007).

Many chronically infected persons show no outward signs of HBV infection, therefore screening for hepatitis B is necessary to: Identify individuals who have chronic HBV infection so they can receive appropriate medical management; Identify those who are unprotected so they can be vaccinated (Physicians' Guide, 2007). Individuals born in areas of high or intermediate prevalence rates for HBV (like Africa and Asia) including immigrants and adopted children should be screened (Lok and McMahon, 2009; WHO, 2002).

## 2. Objective of the Study

### 2.1 General Objective

To estimate the seroprevalence of HBV in patients with DM visiting the diabetic clinic of Woldiya Hospital and to compare them with the non diabetic controls.

### 2.2 Specific Objectives

- ❖ To assess the proportion HBsAg between diabetic and non diabetic controls.
- ❖ To compare Liver Function Test (LFT) value between diabetic and non diabetic; and between HBV sero positives and Negatives.
- ❖ To assess the association of HBV infection with Sociodemographic variables and clinical history of participants.

### 2.3 Hypotheses

∅ **H<sub>0</sub>**: Prevalence of HBV among diabetes mellitus patient is not different from that of non diabetes

$$H_0: p_1 = p_2$$

∅ **H<sub>A</sub>**: Prevalence of HBV among diabetes mellitus patient is different from that of non diabetic

$$H_1: P_1 \neq p_2$$

P<sub>1</sub>=proportion of HBV in Diabetes Mellitus patients

P<sub>2</sub>=proportion of HBV among non Diabetic Controls

## **3. Methods and Materials**

### **3.1 Study Area**

The study was conducted at Woldiya Zonal Hospital which is found in north Wollo zone in Amhara region .The zone has 1,503,283 population according to the 2007 preliminary census report (Federal Democratic Republic of Ethiopia, 2008) and 521km away from Addis Ababa. Woldiya is a hillside market town, capital of the Semen Wollo Zone, Located north of Dessie and southeast of Lalibela. The town has altitude and longitude of 11°50'N 39°41'E and an elevation of 2112 meters above sea level (The free Encyclopedia, 2009).

Currently this Hospital has 235 staffs, about 200 in patient beds, about 200 registered diabetic patients and 7177 HIV positive (4486 active ART and 2691 pre ART) clients as of March, 2011 report.

### **3.2 Study Design and Period**

The study design was a cross sectional comparative study, that the exposure and outcome variables were DM and HBV infection respectively. The study started by grouping the study subject in two groups based on their exposure status i.e diabetes, and data was collected at one point in time from November, 2010 to January, 2011. The study planned to identify the possible associated factors; compare mean LFT values between HBV positive and negative; and between diabetic and non diabetic controls.

### **3.3 Source and Study Population**

The source (sample) population comprised of those diabetic patients who followed their case at Woldiya Hospital. The study populations were those diabetic patients with age between 18 and 60 years, who came to the Hospital during data collection period and those who participated in the study after fully informed about the aim of the study.

The control population included 108 subjects who were comparable to the diabetic group for SES. These populations included blood donors and VCT service clients.

The aim of the study was explained to the patients and the control population, those who gave informed consent were interviewed for demographics, diabetic, and the potential associated clinical characteristics data.

## **3.4 Eligibility Criteria**

### **3.4.1 Inclusion Criteria**

For diabetic patients, those known diabetes mellitus patients who came to the Hospital diabetic clinic for check up and age between 18 and 60 were included in the study. The control group comprised 108 participants whose random blood glucose level  $< 126$  mg/dl as measured by senso card rapid test kit and comparable to diabetes with regard to SES.

### **3.4.2 Exclusion Criteria**

Diabetes mellitus patients with age  $< 18$  and greater than 60 years excluded to be matched with age to the controls as most of VCT clients and blood donors are in age range of 18-60 years. Control subjects having diabetes or random blood glucose level  $\geq 126$  mg/dl was specifically excluded from the analysis.

## **3.5 Description of Variables**

The aim of this study was to assess whether DM is associated with HBV infection by comparing the proportion of HBsAg among diabetic and non diabetic population providing that other confounding variables were assumed to be randomly (non differentially) distributed like hemodialysis, chronic liver disease, cancer, blood transfusion or controlled like SES.

### **3.5.1 Dependant Variables**

- Presence of HBV

### **3.5.2 Independent Variable**

- Diabetes Mellitus (types, years of living with diabetes and treatment regimens)

- Socio demographic variables (age, sex, level of education, occupation, smoking, alcohol consumption)
- Clinical history(previous experiences of jaundice, hospital admission, surgical operation, blood transfusions, intravenous drug abuse, tattooing, hemodialysis, tooth extraction, and abortion [for females only])
- LFT values (ALT and AST)

### 3.6 Sample Size Estimation

The sample size was calculated with prevalence (p1) assumed to be 50% for HBsAg among DM patients as there was no preliminary study in our country. The prevalence for the controls (p2) was taken as 70% which is for over all marker prevalence based on different studies in Ethiopia and sub Sahara Africa (Edmunds *et al*, 1996; Abebe *et al*, 2003; Pasquini *et al*, 1988 and Kefene *et al*, 1989). This sample size calculation also considers the limitation of resources. Based on this consideration, sample size was calculated using the following double proportion formula.

Formula for calculating the sample size is

$$n1 = n2 = \frac{\left( z_{\alpha/2} \sqrt{\left(1 + \frac{1}{r}\right) \bar{p}\bar{q}} + z_{\beta} \sqrt{p1q1 + \frac{p2q2}{r}} \right)^2}{(p1 - p2)^2}$$

Where  $\bar{p} = \frac{p1+p2}{2} = \text{average proportion}$  ,  $\bar{q} = 1 - \bar{p}$

r =ratio of diabetic to controls= n1/n2=1 for equal sample size

P1=prevalence of HBsAg among diabetes mellitus patients

P2=prevalence of HBsAg among the control population

P1-p2= effect size

The difference in this prevalence was 20%.level of significance  $\alpha=0.05$ , power  $1-\beta=80\%$ ,  $\beta=0.2$

$Z_{\alpha/2}$  = the z-score corresponding to the probability with which it is desirable to be able to conclude that an observed difference of size (p1-p2) of variables between diabetes and normal population will not occur by chance, =1.96

$Z_{\beta}$  = the score corresponding to the degree of confidence with which it is desired to certain of detecting a difference size (p1-p2) between variables of that actively present, =0.84

$$\bar{p} = \frac{p1 + p2}{2} = \frac{0.5 + 0.7}{2} = 0.6$$

$$\bar{q} = 1 - \bar{p} = 1 - 0.6 = 0.4$$

$$p1 = q1 = 0.5$$

$$P2=0.7 \text{ and } q2=0.3$$

Substituting these values

$$n1 = n2 = \frac{\left( 1.96 \sqrt{\left(1 + \frac{1}{1}\right) (0.6 \times 0.4)} + 0.84 \sqrt{(0.5 \times 0.5) + \left(\frac{0.7 \times 0.3}{1}\right)} \right)^2}{(0.7 - 0.5)^2}$$

$$n1 = n2 = 94$$

If we add 15% contingency,  $94 \times \frac{15}{100} = 14$ ,  $14+94=108$

There fore at least 108 people required in each group to conduct this research. This sample size can also be calculated using Epi info statistical software version 3.3.2.

### 3.7 Sampling Methods

Due to small number of source population and homogeneity of people in the area, we did not use probability sampling method. Consecutive convenient sampling method was used and participants were asked to participate in the study after the aim of the study explained. Only

those willing to participate asked for questionnaire and to give venous blood. This research was done only by voluntary participation of our study subject.

### **3.8 Method of Data Collection**

After informed consent was gained from the participant, information for socio demographic data, history of exposure for the possible associated factors, type of diabetic and years of follow up (only for diabetic patient) and other relevant information was collected using structured questionnaire .The participant serologic status for HBsAg, SGOT and SGPT levels was done using the package insert lab testing guideline.

### **3.9 Laboratory Procedures**

#### **3.9.1 HBsAg Test Procedure**

1. Bring the sealed pouch kit to room temperature if refrigerated.
2. Open the pouch and remove the device, once opened the device must be used immediately.
3. Dispense 50µl of serum/plasma into the sample well ‘S’ using micropipette.
4. At the end of 15minute read the result macroscopically.

#### **3.9.2 SGPT/ALT and SGOT/AST Test Procedures**

1. Check the computer is connected to the clinical chemistry analyzer.
2. Switches on the clinical chemistry analyzer.
3. Start the software by double clicking the icon HumaStar 80.
4. Check wash solution container is full ,waste container is empty ,working reagents have been prepared ,standards and controls are ready for use, the reaction cells on the analytical plate are clean and sample cups &reagent racks are in place.
5. Add labeled samples in the sample well.
6. Click UTILITY button & the instrument will initialize it self.
7. Click PATIENT DATA button &enter the patient information (name, age sex, address, Department& physician) next click LINK &check all the data will appear in the upper table finally click EXIT.

8. Choose GPT and GOT tests you are going to perform, repeat for the second and the third samples...click PROCESS button.
9. Record results
10. Wash and shut down the analyzer

### **3.9.3 Random Blood Glucose/RBS/ Test Procedures**

1. Clean finger with alcohol and dry
2. Prick the dried finger with lancet
3. Take one drop of blood with inserted glucose strip
4. Read result and discard the strip in to safety box

## **3.10 Data Quality Assurance**

Besides the investigator the VCT counselor for data collection trained how to get consent and fill the questionnaire, measure weight of participants and then took sample .To avoid information and measurement bias, the specific detail of the study was not told to the counselor. To avoid the measurement bias that encountered during testing of the serum (plasma) sample, Internal Quality control were done. Special emphasis was given during coding the data sheet as well as the sample. **VISITECT HBsAg**, (Omega, UK), 100% sensitive and specific, rapid two site sandwich immunoassay kit was used. For LFT test, closed system computerized huma star 80 chemistry analyzer was used and for screening of rapid blood glucose, senso card machine was used.

## **3.11 Data Analysis**

After sociodemographic, clinical history and laboratory data was collected using questionnaire and lab report format; it entered and analyzed using SPSS version16. Variables descriptively expressed as mean  $\pm$  SD or number and percent. Comparisons between groups made using Student's *t* test for continuous variables and Chi-square or fisher's exact test for categorical data. A multivariate logistic regression model used to determine the independent effect of various factors that were potentially associated with the risk of hepatitis in both groups. All tests were two-sided with  $\alpha$ -level of 0.05 and 80% power.

### 3.12 Operational Definitions

- ✓ **Acute Hepatitis:** A patient is said to have acute infection of HBV if both HBsAg and anti-HBc antibody IgM is detected in patients' blood. Usually a self-limiting disease marked by inflammation in the liver in association with a transient HBV infection
- ✓ **Chronic HBV Infection:** An individual is considered chronically infected if HBsAg is present for more than six months. Three markers are used to determine the stage of chronic infection: HBsAg, HBeAg, and anti-HBc total. persistent HBV infection accompanied by ongoing liver injury and resulting risk of cirrhosis and HCC
- ✓ **Diabetes participant:** Those known diabetes who follow their cases at the hospital and came to the hospital diabetic clinic for check up and age between 18 and 60 years.
- ✓ **Inactive HBsAg carrier state:** Persistent HBV infection of the liver without significant, ongoing necroinflammatory disease and persistently normal ALT/AST levels.
- ✓ **The control groups:** Normal health individuals whose random blood glucose level < 126 mg/dl those come to VCT for marriage purpose, general health examination or for blood donation and age between 18 and 60.
- ✓ **Type I DM:** patients who use insulin as initial therapy and continuing using it.
- ✓ **Type II DM:** patients who used oral ant diabetic as initial therapy and continuing using it or switch to insulin.

### 3.13 Ethical Considerations

Ethical clearance and permission was obtained from the Department ethics committee of Microbiology, Immunology and Parasitology, college of Health science, Addis Ababa University; and a letter of support was written to Woldiya general Hospital.

The study participant informed about the purpose of the study and the importance of their participation in the study. And also they informed as they could not participate; stop at any time in between data collection or jump (decline) to answer some of the questions if they feel uncomfortable with out losing the benefit that would got in the institution .Their participation were purely on voluntary.

After they gave their informed consent, they asked to sign on the Amharic written consent and patient information sheet for this particular study. About 5 ml venous blood was taken by a trained counselor and lab personnel .Anonymous testing was undertaken, that was sample was coded and positive individuals were not identified by their name. For Participants whose lab result was clinically significant like positive for HBsAg; the investigators consulted a physician and communicated for further diagnosis and treatment.

## 4. Results

A total of 216 participants were included in this study, of whom 108(50%) participants were diabetes and the rest 108(50%) were non diabetes. Sex, weight, level of education, smoking, alcohol consumption and most of clinical characteristics were comparable between diabetes and non diabetes control. The descriptive statics for age, weight and LFT values is summarized in table1.

Table1. Age, weight and liver function test results of participants, Woldiya, Ethiopia, 2011

<b>Variables</b>	<b>Min</b>	<b>Max</b>	<b>Mean</b>	<b>SD</b>
<b>Age</b>	18	60	33.4	12.5
<b>Weight</b>	38	65	54.3	4.9
<b>GOT/ALT/</b>	8	108	29.1	14.6
<b>GPT/AST/</b>	2	60	22.9	11.6

In the study, 96 (44.4%) female and 120 (55.6%) male were included. Mean age and weight of participants were 33.4 years and 54.3 kg respectively. Of a total of 216 study participants, 90(41.7%) married, 80(37%) single, 36(16.7%) divorced and 10(4.6%) widowed were included. Majority of participants 80(37%) were illiterate, 89(41.2%) were farmer.

With regard to diabetic participants, most of them, 75 (69.4%) were type I diabetic and 33 (30.6%) were type II diabetic indicating that type I diabetic is highly prevalent in the study area. The results for comparison of sociodemographic variables and laboratory results between DM patients and non diabetic control were summarized in table2 below.

Among the 216 participants, 69(31.9%) had abnormal ALT (>40IU/l) and 85(39.4%) had abnormal AST (>40IU/l).

Table2. Comparison of Sociodemographic rate of HBsAg positivity and LFT values between DM patients and control subjects, Woldiya, Ethiopia, 2011.

	Diabetic patients	Control subjects	p-value
<b>Age (mean)</b>	37.6±13	29.2±10.4	0.001
<b>Weight (mean)</b>	54.6±4.8	54.1±5	0.824
<b>Sex (n)</b>			
<i>Male</i>	57 (52.8%)	63 (58.3%)	0.494
<i>female</i>	51 (47.2%)	45 (41.7%)	
ALT (Iu/l)	25.2±1	20.5±11.7	0.001
AST (Iu/l)	30.4±13	27.8±15.7	0.001
<b>HBsAg (n)</b>			
<i>positive</i>	4 (3.7%)	4 (3.7%)	1.000
<i>negative</i>	104 (96.3%)	104 (96.3%)	
<b>Active smoking (n)</b>			0.614
<b>Yes</b>	1 (1%)	3 (2.8%)	
<b>no</b>	107 (99%)	105 (97.2%)	
<b>Alcohol consumption (n)</b>			
<i>Yes</i>	9 (9.3%)	32 (29.6%)	0.00*
<i>no</i>	99 (91.7%)	76 (70.4%)	
<b>Total</b>	108 (100%)	108 (100%)	

\*=Even though p-value shows significant association, the 95%CI for crude odds ratio less than unity indicating that non significant.

Of a total of 216 study participants, 8 (3.7%) subjects were positive for HBsAg, four from each of diabetic (3.7%) and non diabetic (3.7%) indicating that no significant difference between the

prevalence of HBsAg in diabetic and that in non diabetic (Odds Ratio [OR] =1.00; 95% CI: 0.244-4.1; p=1.00) . Among positive participants, four were females and the rest four were males showing no difference between female and male participants. Comparison of mean age, ALT, AST, were done between diabetes and non diabetes and it was found to (37.6±13 Vs 29.2±10.4, 25.2±1 Vs 20.5±11.7, 30.4±13 Vs 27.8±15.7) respectively, indicating that there is significance difference between these two groups (p=0.001).

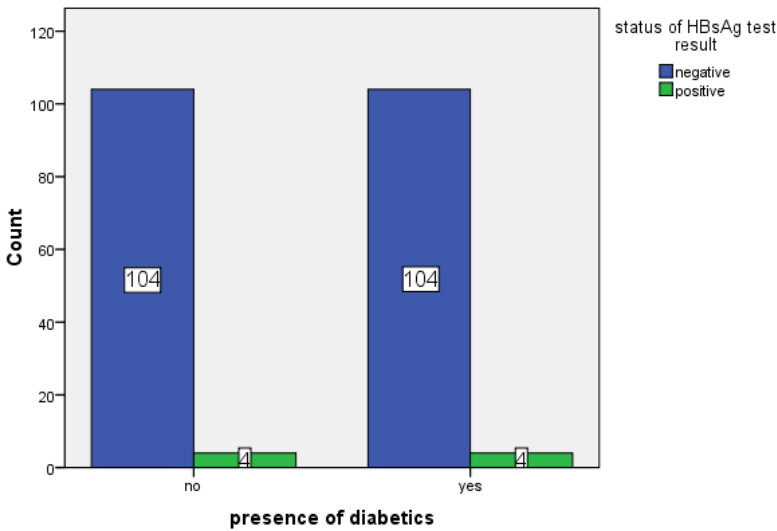


Figure 1.Bar chart showing the number of HBsAg positive and negative among diabetes and non diabetes.

Among HBsAg positive participants; four were married, two were single and the remaining two were divorced; four were illiterate and the rest four were whose level of education greater than or equal to grade seventh; three were farmer, two private employ, two government employ and the remaining one participant with no job.

Table 3.comparison of the mean value of continuous variables between HBsAg positive and negative participants, Woldiya, Ethiopia, 2011.

HBsAg Test Result		GPT/ALT/	GOT/AST/	age (year)	weight (kg)
Negative	Mean	22.4	28.3	33.4	54.3
	SD	11.3	13.5	12.5	4.9
Positive	Mean	34.8	49.8	34.1	54.5
	SD	13.8	25.2	14.5	5.9

Note: ALT and AST are measured in IU/l

This study also tried to observe the association of the presence of HBsAg with sociodemographic variables; and clinical characteristics of the study participant using multivariate logistics regression method. Except history of liver disease, no clinical and sociodemographic characteristic were associated with HBV infection in our study. In any HBsAg positive participants, no probable source of infection could be identified. Among 73 participants with history of Hospital admission, 24 with history of surgery, 8 with history of blood transfusion ,46 people with history of tattooing/body piercing, 19 participants with history of CDK and 30 with history of tooth extraction ,2(2.7%), 2(8.3%), 1(12.8%) ,3(6.5%),1(5.2%) and 2(6.7%) were sero positive for HBsAg respectively (table 5).

On the other hand active smoking, alcohol consumption, history of abortion and history of Household contacts with viral hepatitis could not be detected in any of the positive participants (Table 4 and 5).

Table 4. Sociodemographic variables and status of diabetes types related to the risk of HBV, Woldiya, Ethiopia, 2011.

<b>Sociodemographic Characteristics</b>	<b>HBV positive, N (%)</b>	<b>HBV negative, N (%)</b>	<b>AOR</b>	<b>P-value</b>
<b>Age:</b>				
<i>18-45</i>	6 (75)	170 (81.7)	1	0.228
<i>46-60</i>	2 (25)	38 (18.3)	4.83 (0.37-62.5)	
<b>Sex:</b>				
<i>female</i>	4 (50)	92 (44.3)	1	0.884
<i>Male</i>	4 (50)	116 (55.7)	0.84 (0.08-8.82)	
<b>Marital status:</b>				
<i>Marriage</i>	4 (50)	86 (43.4)	1	0.972
<i>Single</i>	2 (25)	78 (37.5)	0.96 (0.08-11.3)	
<i>Divorced</i>	2 (25)	34 (16.3)	2.02(0.24-8.2)	
<i>widowed</i>	0 (0)	10 (4.8)	NA	
<b>Educational status:</b>				
<i>Illiterate</i>	4 (50)	76 (36.5)	1	0.997
<i>Read and write only</i>	0 (0)	33 (15.9)	NA	
<i>Priesthood education</i>	0 (0)	5 (2.4)	NA	
<i>1-6 Grade</i>	0 (0)	32 (15.4)	NA	
<i>7-10 grade</i>	1 (12.5)	37 (17.8)	0.17(0.01-5.08)	
<i>11-12 grade</i>	1 (12.5)	7 (3.4)	4.36	
<i>&gt;12+</i>	2 (25)	18 (8.6)	2.2(0.025-208)	

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**Occupation:**

<i>Has no job</i>	1 (12.5)	19 (9.1)	1	
<i>Government employee</i>	2 (25)	18 (8.7)	1.01(0.02-52.8)	0.998
<i>Farmer</i>	3 (37.5)	86 (41.3)	0.24(0.01-5.24)	0.361
<i>House wife</i>	0 (0)	26 (12.5)	NA	0.996
<i>private employee</i>	2 (25)	26 (12.5)	2.86(0.13-64.5)	0.508
<i>student</i>	0 (0)	33 (15.9)	NA	0.995

**Alcohol consumption** 0.998

<i>no</i>	8 (100)	167 (80.3)	NA
<i>Yes</i>	0 (0)	41 (19.7)	

**Active smoking** 1.00

<i>no</i>	8 (100)	204 (98.1)	NA
<i>Yes</i>	0 (0)	4 (1.9)	

**Types of diabetic** 1.00

<i>Type one</i>	3 (75)	72 (69.2)	1
<i>Type two</i>	1 (25)	32 (30.8)	0.75(0.03-8.62)

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OHA = oral hypoglycemic agents; AOR =Adjusted odds ratio; NA = not available.

Table 5. Clinical characteristics and LFT value related to the risk of HBV between HBsAg positive and negative subjects, Woldiya, Ethiopia, 2011.

Clinical Characteristics	HBsAg positive, N (%)	HBsAg negative, N (%)	AOR	P-value
<b>History of jaundice(chronic liver disease)</b>				
<i>no</i>	7 (87.5)	206 (99.04)	1	0.033
<i>Yes</i>	1 (12.5)	2 (.96)	338(1.6-7.07)	
<b>History of hospital admission</b>				
<i>no</i>	6 (75)	137 (65.9)	1	0.052
<i>Yes</i>	2 (25)	71 (34.1)	0.01(0.0-1.033)	
<b>History of surgical operation</b>				
<i>no</i>	6 (75)	186 (89.4)	1	0.609
<i>Yes</i>	2 (25)	22 (10.6)	0.42(0.02-11.6)	
<b>History of blood transfusion</b>				
<i>no</i>	7 (87.5)	201 (96.6)	1	0.077
<i>Yes</i>	1 (12.5)	7 (3.4)	262(0.5-1.27)	
<b>History of tattooing and /or body piercing</b>				
<i>no</i>	5 (62.5)	165 (79.3)	1	0.071
<i>Yes</i>	3 (37.5)	43 (20.7)	14(0.79-250)	
<b>History of hemodialysis (CKD)</b>				
<i>no</i>	7 (87.5)	190 (91.3)	1	0.413
<i>Yes</i>	1 (12.5)	18 (8.7)	5.9(0.08-424)	
<b>Receiving corticosteroids or immunosuppressive drugs</b>				
<i>no</i>	7 (87.5)	194 (93.3)	1	0.509
<i>yes</i>	1 (12.5)	14 (6.7)	0.232(0.003-17)	
<b>History of household contacts</b>				
<i>no</i>	8 (100)	205 (98.6)		0.999
<i>Yes</i>	0 (0)	3 (1.4)	NA	

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**History of multiple sexual partner**

<i>no</i>	6 (75)	141 (67.8)	1	0.841
<i>Yes</i>	2 (25)	67 (32.2)	0.806(0.09-6.7)	

**History of tooth extraction**

<i>no</i>	6 (75)	180 (86.5)	1	0.442
<i>Yes</i>	2 (25)	28 (13.5)	2.68(0.22-33.2)	

**GPT**

<24 I $\mu$ /l	1 (12.5)	125 (60)	1	
24-40 I $\mu$ /l	5 (62.5)	64 (30.8)	7.1(0.3-165)	0.224
>40 I $\mu$ /l	2 (25)	19 (9.2)	3.32(0.09-125)	0.517

**GOT**

<24 I $\mu$ /l	1 (12.5)	87(41.8)	1	
24-40 I $\mu$ /l	1 (12.5)	84(40.4)	0.86(0.023-32)	0.934
>40 I $\mu$ /l	6 (75)	37(17.8)	26 (0.54-1.3)	0.099

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## 5. Discussion

We compared diabetes and non diabetes with some sociodemographic variables and LFT values, as we can see from table 2, except age, all the sociodemographic variables (weight, sex, smoking and alcohol consumption) did not show any difference indicating that the two groups are comparable. The LFT result show differences between diabetes and non diabetes in this study. The prevalence of high ALT levels may reach 20% in diabetes. Elevation of these enzymes is strongly related to obesity, diabetes and dyslipidemia, and their measurement may act as a surrogate marker of non alcoholic fatty liver disease (NAFLD) presence (Judi *et al*, 2010; Sheth and Chopra, 2010).

In this study, prevalence rates of HBsAg in diabetic patients and non diabetes controls were the same and estimated to be (3.7% versus 3.7%; Odds Ratio [OR] =1.00; 95% CI: 0.244-4.1; p=1.00) indicating that there was no significant difference in the prevalence rate of HBsAg seropositivity between diabetic patients and the control population. These findings showed that that the vast majority of patients with diabetes have no increased susceptibility to infection by HBV than the general population. This study also suggest the study area to be of intermediate endemicity (2-8%) with HBV and consistent with previous serologic data from most region of Ethiopia (Abebe *et al* (2003) ; Negero *et al* (2011); Tessema *et al* (2010) ; Shimelis *et al* (2007) even though there is regional differences in HBV marker prevalence.

A well structured questionnaire was used to assess the association of HBsAg prevalence with sociodemographic variables, laboratory results and clinical history of participants. Except history of liver disease, demographic variable and clinical characteristics were not associated with HBV as we observed the p-value and adjusted odds ratio along with 95% confidence interval from table 4 and 5). Therefore, in any HBsAg positive participants, no probable source of infection could be identified. Many patients, perhaps up to one third, will not know how they acquired the infection (British liver trust, 2009). The source of infection cannot be identified in about 35% cases (WHO, 2002). Unable to identify, source of infection with these clinical history, increased the probability of acquiring infection during neonatal and childhood period.

History of liver disease was associated with HBsAg. The main cellular target of HBV is the hepatocyte, and in humans, these are the only cells convincingly shown to replicate the virus. HBV was responsible for a chronic hepatitis, leading to cirrhosis and liver cancer in many parts of the world (David and peter, 2007). HBV is causally associated with primary hepatocellular carcinoma (PHC) (Murray et al, 2005).

Normal serum alanine aminotransferase (ALT=34.8 IU/l  $\pm$ 13.8 SD) levels in patients with HBsAg in our result may indicate the inactive HBsAg carrier state (After spontaneous HBeAg seroconversion, 67% to 80% of carriers have low or undetectable HBV DNA and normal ALT levels with minimal or no necroinflammation on liver biopsy) (Lok and McMahon, 2009; Sharma *et al*, 2005) or may reflect mild chronic hepatitis B or hepatitis B with fluctuating disease activity (Fauci *et al*, 2008) . Serum testing for hepatitis B e antigen and hepatitis B virus DNA can help resolve these different patterns (Fauci *et al*, 2008). HBV infected patients with ALT values close to the upper limit of normal may have abnormal histology and can be at increased risk of mortality from liver disease especially those above ages 40 (Lok and McMahon, 2009). Young carriers with high levels of hepatitis B may have normal ALT levels (British liver trust, 2009).

AST was higher than the normal value (AST=49.8 IU/l  $\pm$ 25.2 SD) but statistically not different from the sero negative group [AOR=26; 95% CI: 0.54-1.3; P=0.099], one of the reason for this situation might be the effect of diabetes, The presence of diabetes remained an independent risk factor for chronic liver diseases and HCC after adjustment for alcohol use or viral hepatitis in the studies that evaluated these factors (Blonski *et al*, 2010). An elevated level of AST only suggests damage to heart muscle (myocardial infarction), skeletal muscle or kidney. Even though additional seromarker tests are required to differentiate between acute and chronic HBV infection in these study group, the likely hood of being chronic is higher based on LFT value where the ratio of AST: ALT (49.8:34.8=1.43) where the normal range is from 0.7-1.4 and the value of ALT only which will be higher if the infection is acute hepatitis.

History of transfusion, tattooing and history of alcohol consumption were not associated with HBsAg in our study. All this results are consistent with Shimelis *et al*, 2007 study. Transfusion with blood or blood products is no longer an important risk factor for acute viral hepatitis (Fauci

*et al*, 2008; Lok and McMahon 2009); Tattooing and body piercing (for hepatitis B and C) and are frequently mentioned but are actually quite rare types of exposure for acquiring hepatitis (Fauci *et al*, 2008) and our finding agreed with this situation. No specific dietary measures have been shown to have any effect on the progression of chronic hepatitis B. However, heavy use of alcohol (>20 g/d in women and >30 g/d in men) may be a risk factor for the development of cirrhosis (Lok and McMahon, 2009).

History of abortion and multiple sexual partner did not associate with HBsAg positivity in our study hemodialysis also did not associate with hepatitis seropositivity in contrast to (El-Ottol *et al*, 2010) study. As we can see from table 4 and 5; the confidence interval is so wide indicating that the sample size is small and the point estimate is imprecise. Because of these reason, we could not strongly conclude that the mentioned clinical characteristics, laboratory and sociodemographic variables did not associated with HBV infection.

History of house hold contact is not associated with HBsAg positivity in this study and this also agree with other most recent study (Shimelis *et al*, 2007). Non-sexual interfamilial spread is not commonplace but may occur. It becomes common when the first family member to be infected is an infant or child and is seen most clearly amongst adoptive parents of chronic HBsAg carrier children (British liver trust, 2009).

Since there was no research that used questionnaire to assess the association of sociodemographic variables with HBsAg prevalence on diabetes patients and non diabetes controls; these finding could not compared rather will be used as base line information for the scientific community for further studies. No association was observed with HBsAg positivity and sociodemographic variables like, age, sex, marital status, occupation, and educational status. Even though direct comparison is difficult due to differences in study population, geography and ethnic variability, this finding agrees with other most recent study done in Shashemene General Hospital VCT center by (Negero *et al*, 2011). Except with age and sex, it also agree with (Shimelis *et al*, 2007) study.

Many previous studies, conducted in various nations, including Taiwan by Chen *et al* (2003) (13.5%Vs12.4%), South Africa by Kew *et al* (2001) (4.6%Vs 4.3%), Turkey by Kilic *et al*

(2007 )(3.4%Vs 2.2%), Nigeria by Onyekwere *et al* (2002) (20% Vs17.3%) and Turkey by Gulcan *et al* (2008 )(5.1%Vs 3.8%) reported a higher prevalence rate of hepatitis B in diabetic patients than non diabetic, but no significant difference was found. Our finding which was (3.7%Vs3.7%) apparently consistent with the above mentioned studies as there was no statistical difference between diabetes and non diabetes. Unlike other studies our result showed equal prevalence between diabetic and non diabetic. This might be due to small sample size that we used as indicated by wide range of confidence interval; (AOR=1.00; 95% CI: 0.244-4.1) and chance as indicated by p-value (p=1.00).

Another study from Turkey by Demir *et al* in (2002) and by Khuri *et al* in (1987) using 100 participants from diabetic and non diabetic found (11% Vs 3%) and (51% Vs 25%) respectively which were significant in both finding. The reason behind this finding that made it different from our study is that, in case of Demir *et als'* study, it might be because of difference in the type of prevalence marker which they used. They were assessing prevalence of occult HBV infection which is relatively frequent among patients with immune suppression .The impairment of the immune system is well demonstrated in DM. Our finding was also different from kuri et al study; the possible reason that this result being different from ours is due to the difference in the type of HBV markers they studied which was mainly HB surface antibody. Another possible reason for this difference is that the sample size between their diabetic and control groups and also the type of control groups. They used 100 diabetic and 395 healthy health care workers as control where selection bias might be high due to knowledge of their previous HBV status.

Three studies from China by Huo *et al* (2003), Cheng *et al* (2006) and Lao *et al* (2006); and one study from Tehran by Alavian and Tabatabae (2010) found different outcome association, but these studies could not be compared with our finding because of differences in the study design, methodology, study subjects and outcome variables.

Studies done in Ethiopian on different target population, for example a study done by Abebe *et al* (2003) in Addis Ababa, by Pasquini *et al* (1988) in Arsi , Shimelis *et al* (2007) at Saint Paul's General Specialized Hospital on VCT clients, by Negero *et al* (2011) among VCT clients at Shashemene Hospital and by Tessema *et al* (2010) at University of Gondar on blood bank showed 7%, 10% and 5.7%,5.7% and 4.7% HBsAg prevalence, respectively. When we

compared these finding with ours, the four studies showed intermediate endemicity prevalence like the current study but slightly higher. The possible reason for these differences laid on differences in method, type of lab test kit used; sample size, geographic distribution and socio demographic variables. For example Abebe *et al*(2003) used Venous blood from 4736 individuals under 50 years of age from 1262 households in Addis Ababa, selected using stratified cluster-sampling with ELISAs. Pasquini *et al* in (1988) in Arsi used 300 outpatients living in the catchment area of the Asella Hospital using ELISA. Shimelis *et al* (2007) used cross sectional study on 384 VCT attendants using ELISA kits. In our case we used VISTECT HBsAg (Omega, UK) rapid test kit which is 100% sensitive and specific.

Our low prevalence might also be attributed to the failure to identify infected patients because of the serologic window during the incubation period following infection, the presence of some rare variants escaping the serologic assay for HBsAg, particularly when concurrent testing for anti-HBc is not performed (David and Peter, 2007), and the problem of occult HBV infections (David and Peter, 2007; Lok and McMahon, 2009), in which neither HBsAg or anti-HBc are detected (David and Peter, 2007). Since the assay is serologic, there might be false negative, especially for HBV, patient antibody may be bound with viral antigen in immune complexes, thereby preventing antibody detection (Murray *et al*, 2005). Some persons may test positive for anti-HBc but not HBsAg or anti-HBs. This situation is not uncommon among persons from areas with high prevalence of HBV infection and in those with human immunodeficiency virus (HIV) or hepatitis C virus (HCV) infection (Lok and McMahon, 2009).

Generally this study and other mentioned studies in Ethiopia showed lower prevalence than WHO report which says greater than 8% for HBsAg. This is expected, because in endemic area of Africa and Asia, most infections occurs in infants and children as a result of maternal-neonatal transmission or close childhood contact (WHO, 2002; Lok and McMahon, 2009) and these studies done on adults which have lower prevalence than infants, children and special group of populations who are at special risk making the prevalence of these study lower than WHO report.

The final point that we want to discuss is that the proportion of type I and type II diabetes in his study .In this study the proportion of type I and type II diabetes were 75(69.4%) and 33(30.6%) respectively unlike many other studies that found 62.0% type II by *Worku et al (2008)*, 90% type

II diabetes by (Fauci et al, 2008; Yemane et al, 2007; Levitt, 2008). The reason that might made our result of type I higher than type II is that the unusual use of insulin as initial therapy for type II diabetes. Because we defined type I diabetes; those who use insulin as initial therapy and still being on insulin. Insulin should be considered as the initial therapy in type II DM, particularly in lean individuals or those with severe weight loss, in individuals with underlying renal or hepatic disease that precludes oral glucose-lowering agents, or in individuals who are hospitalized or acutely ill (Fauci et al, 2008). Eating cheap high carbohydrate diet ('junk-food') over a long period of time and/or continuous exposure to pollution can lead to gradual exhaustion of the  $\beta$ -cells can precipitate Diabetes Mellitus known as "*Third-world Diabetes*". The condition is similar to type I diabetes but occur late in age and without ketoacidosis.

A study done by Shitaye Alemu and Peter Watkins (2004) in Northern Ethiopia found that; in urban areas, Type 2 diabetes accounts for 71% of the people with the condition and in the rural areas who are known to have Type 2 diabetes appears to be relatively very low – 23% of the people with the condition. Our study coincides with this study since most of our study subjects were people who came from rural areas. Un published data showed that the types of diabetes in Ethiopia is different from other countries and they recommend if is seen again.

## **6. Conclusion and Recommendations**

### **6.1 Conclusion**

- In our study we have observed that prevalence of HBsAg was equal between DM and non diabetic; and it was found to be 3.7% indicating that no difference between the two groups.
- The two groups of study population show statistical significance difference in their LFT tests indicating that diabetic is associated with increased value of ALT and AST.
- LFT tests and clinical characteristics of study participants did not show association with HBsAg seropositivity calling for large sample size.

Generally our findings suggest that DM has no any association with HBV infection and similar in both DM and control group and therefore would require no special anti HBV prophylaxis than the general population.

### **6.2 Recommendations**

The finding indicates that clinical and sociodemographic characteristics did not associate with HBV infection. Therefore if one wants to observe the association of HBV with sociodemographic and clinical history of participants:

- Must use larger sample size

### **6.3 Limitation of the study**

The author would like to forward the following limitations:

- Due to resource constrains, the sample size we used was small and unable to use additional confirmatory tests especially for participants who were positive by the VISITECT rapid test kit.
- The sampling method was convenient, by which generalizing the finding to the general population is sub optimal.

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# Annexes

## Annex I: Informed Consent and Patient Information Sheet (English)

My name is Daniel Mekonnen and I am M.Sc student in microbiology at AAU. I am doing a research entitled Prevalence of HBV in patients with Diabetes and normal controls.

The objective of the study is to observe the prevalence of HBV on these two groups and to see if there is a difference between being diabetic and non diabetic. If you are agree to participate in the study, about 5 ml of blood will be collected from you or you will allow us to use the sample that you will give for your medical examination and you will be interviewed. During collection of blood, you may feel some discomfort, but this does not produce serious pain. All the data obtained will be kept strictly confidential by using only code numbers and locking the data, only study personnel will have access to the files. Anonymous testing will be undertaken, that is sample will be coded and positive result will not be identified by names. There will be no costs to you as a result of taking part in this study and you are not asked to pay for the laboratory examination. I will give you the result and if your result is clinically significant, I will contact you to the physician for further diagnosis and treatment. Your participation is purely voluntary, and you can not participate or you can with draw any time after you get involved in the study or You can also jump (decline) to answer some of the questions if you feel uncomfortable. Participation and not participation has no influence on the service you seek to get.

**Participant's response:** I am free to decline to be in this study, or to withdraw from it at any point and also to jump a question that feels me discomfort. He promised to give the result with out cost .My decision as to whether or not to participate in this study will have no influence on my present or future medical service.

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

---

Subject's signature \_\_\_\_\_ date of signature \_\_\_\_\_

---

Signature of Person Obtaining Consent \_\_\_\_\_ date of signature \_\_\_\_\_



Annex III: English Version Written Questionnaire

**ADDIS ABABA UNIVERSITY**

**SCHOOL OF GRADUATE STUDIES**

**Department of Medical Microbiology, Immunology and Parasitology.**

**Questionnaire to be filled by each participant**

**Interviewer:** -----

**Date of interview:** -----

- I. Identification**
1. Code number-----
- II. Socio demographic variables**
1. **Age** \_\_\_\_\_
- 18-45
  - 46-60
2. **Weight**-----
3. **Sex:**
- Male
  - Female
4. **Marital status:**
- Married
  - Single
  - Divorced
  - Widowed
5. **Educational status:**
- Illiterate
  - Reading and writing
  - Priesthood education
  - 1-6 Grade
  - 7-10grade
- 11-12 grade
  - >12+
6. **Occupation :**
- Have no job
  - Private employee
  - Government
  - Farmer
  - House wife
  - Sex worker/prostitute/
  - Student
7. **Active smoking:**
- Yes
  - No
8. **Alcohol consumptions:**
- Yes
  - No
- III. History of diabetes (for diabetics patient only)**
1. **Types of diabetics:**
- type1
  - type 2

**2. Duration of diabetes:**

- <1 year
- >1 year

**3. Treatment Regimens:**

- Oral Anti-diabetics
- Insulin
- Insulin+ Oral Anti-diabetics
- Diet control with/without OHA

**IV. History of exposure for potentially associated with the risk of hepatitis**

**1. Hospital Admission:**

- Yes
- No

**2. Surgical Operation:**

- Yes
- No

**3. Blood Transfusions:**

- Yes
- No

**4. Intravenous Drug Abuse:**

- Yes
- No

**5. Tattooing:**

- Yes
- No

**6. Hemodialysis (Chronic Renal Failure, Catheterization):**

- Yes
- No

**7. Abortion (For Females Only):**

- Yes

- No

**8. Previous Experiences of Jaundice (Liver Disease):**

- Yes
- No

**9. Leukemia, lymphoma, TB and cancer:**

- Yes
- no

**10. Receiving Corticosteroids or Immunosuppressive Drugs:**

- Yes
- No

**11. History of Household Contacts:**

- Yes
- No

**12. History of Multiple Sexual Exposure:**

- Yes
- No

**13. Tooth Extraction:**

- Yes
- No

Annex IV: Amharic Version Written Questionnaire

የመረጃ መሰብሰቢያ መጠይቅ ፎርም/ቅጽ/

አድስ አበባ ዩንቨርሲቲ የድህረ ምረቃ ት/ት ቤት

የማክሮባዮሎጅ ኢሚናሎጅ ዕና ፓራሲቶሎጅ ት/ት ክፍል

በጥናቱ ተሳታፊ በሆኑ ግለሰቦች የሚሞላ መጠይቅ ፎርም

የጠያቂው ስም \_\_\_\_\_ 'ቀን \_\_\_\_\_ መለያ ቁጥር \_\_\_\_\_

1. የገለሰቡ አካላዊ ማህበራዊ እና ኢኮኖሚያዊ ዝርዝር ሁኔታ

1. ዕድሜ \_\_\_\_\_

18-45

46-60

2. ክብደት \_\_\_\_\_

3. ጾታ :

ሴት

ወንድ

4. የጋብቻ ሁኔታ:

ያገባ

ያላገባ

የተፋቱ

የትዳር ጓደኛዎ የሞተበዎ

5. የትምህርት ሁኔታ:

ያልተማረ

ማንበብ እና መጻፍ ብቻ

የሀይማኖት ትምህርት

ከ1-6 ክፍል

ከ7-10ክፍል

ከ 11-12ኛ ክፍል

ከ12 ክፍል በላይ

6. ስራ:

ስራ አጥ

የመንግስት ሰራተኛ

ገበሬ

የቤት እመቤት

ሴትኛ አዳሪ

የግል ስራ

ተማሪ

7. ሲጋራ ያጨሳሉ:

አዎ አጨሳለሁ

አላጨሰም

8. መጠጥ ይጠጣሉ:

አዎ

አልጠጣም

II. የስኳር ህመምን በተመለከተ(ለስኳር ህመምን ብቻ የሚጠየቅ)

1. የስኳር ህመም አይነት:

አይነት I

አይነት II

2. የስኳር በሽታ ካመመዎት ስንት አመት ሆነዎት:

ከ1 ዓመት በታች

ከ1 ዓመት በላይ

3. የሚዎስዱት የመድኃኒት አይነት:

በአፍ የሚወሰድ ኪኒን



Annex V: HBsAg, glucose and LFT Lab. Request Form

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Department of Medical Microbiology, Immunology and Parasitology

**Laboratory request form for HBsAg ,LFT and blood glucose test**

Identification number-----Age----- Sex-----

**Result**

***HBsAg***

- Positive
- Negative

***LFT and glucose test***

- Glucose\_\_\_\_\_
- SGOT\_\_\_\_\_
- SGPT\_\_\_\_\_

Name of the technologist-----

Date of report-----

Signature-----

## Annex VI: SOP for rapid HBsAg testing

### **Introduction and Intended Use**

Acute HBV occurs by direct transmission via parenteral routes such as infected serum, blood, blood transfusion, contaminated needles or by non parenteral transmission through body fluids such as saliva, urine and semen. Tests to detect HBsAg are now widely used for the detection of infected blood products, infected patients and healthy carriers of the disease.

### **Principle of the Test**

**VISITECT HBsAg** utilizes the principles of immunochromatography, a unique two-site immunoassay on the membrane. As the test sample flows through the membrane assembly within the test device, the colored anti-HBsAg colloidal gold conjugate complexes with HBsAg in the sample. This complex moves further on the membrane to the test region where it is immobilized by the monoclonal anti-HBsAg coated on the membrane leading to formation of a pink –purple colored band which confirms a positive result. The unreacted conjugate, unbound complex, if any, and the colloidal gold conjugated rabbit IgG move further along the membrane and are subsequently immobilized by the goat anti-rabbit IgG coated on the membrane at the control region, forming a pink /purple colored band.

**VISITECT HBsAg** is capable of detecting as low as 0.8ng/ml within 15 minutes and calibrated against international standards for HBsAg 80/549.

### **Specimen Collection and Preparation**

Obtain venous blood and separate the serum or plasma. Do not use hemolysed, turbid, or contaminated samples. In case of delay put serum/plasma at 2-8°C for up to 24hrs. Devices and samples should be brought to room temperature and mixed gently prior to use.

### **Reagents and Materials Needed**

Sample, Test device, micropipette, yellow tips, glove

## **Assay Procedure**

1. Bring the sealed pouch kit to room temperature if refrigerated.
2. Open the pouch and remove the device .once opened the device must be used immediately.
3. Dispense 2 drops (50µl) of serum/plasma into the sample well 'S' using the dropper provided.
4. At the end of 15minute read the result macroscopically.

## **Result Interpretation**

\* Negative =only one pink purple band appears on the control region.

\* Positive = a pink purple band on both test &control regions.

## **Limitation of the test**

When making an interpretation of the test, it is strongly advised to take all clinical data in to consideration. The presence of RF antibodies and cross reacting auto antibodies such as antibodies to HLA DR4 may give false positive results. For confirmation of results a confirmatory test must be used.

## **Evaluation Data**

Evaluation data showed that the sensitivity is from 95% to 100% and specificity from 96.78 to 100% indicating that it is highly sensitive and specific.

## Annex VII: SOP for LFT tests (SGOT and SGPT)

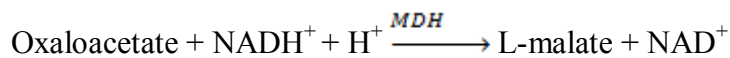
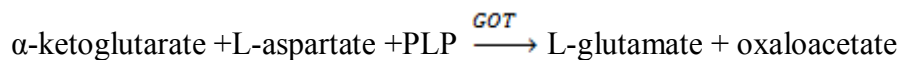
### Introduction

Elevated activities of the two serum transaminases; alanine transaminase (ALT) and aspartate transaminase (AST) maybe associated with liver disease. Elevation of the levels of any of the two enzymes has been found in 7.9% of the general population, whereas the prevalence of high ALT levels may reach 20% in diabetes. Elevation of these enzymes is strongly related to obesity, diabetes and dyslipidemia, and their measurement may act as a surrogate marker of non alcoholic fatty liver disease (NAFLD) presence. Of the two enzymes, ALT appears to have a role in gluconeogenesis, and seems to be more related to liver fat accumulation than AST. Some authors have suggested that minor elevation of this enzyme's level may be a good predictor of mortality from liver disease (Judi *et al*, 2010).

Humastar 80 is an instrument that performs clinical chemistry tests. This instrument works with external computer that has its operational software. The analyzer must be located in a dry place, free of corrosives; ambient temperature should not exceed 34°c. The analyzer should not be placed near a source of electromagnetic radiation (e.g. motors, centrifuges etc...) near a source of heat or in direct sun light. It must be located on a stable, flat surface of sufficient size, care should be taken that no objects obstruct the fan exhaust leave at least 10cm between the back of the analyzer and the nearest wall or object (Huma star 80, 2005).

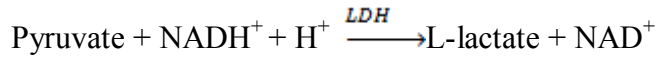
### Principles of Test

#### AST/SGOT (Serum Glutamate Oxaloacetate Transaminase)



(MDH=malate dehydrogenase, PLP= pyrodoxial phosphate)

#### ALT/SGPT (Serum Glutamate Pyruvate Transaminase)



(LDH=lactate dehydrogenase)

## Clinical Significances of the Tests

**SGOT/AST/ or SGPT/ALT/:** primarily for the diagnosis of liver disease .When there is liver cell damage the serum or plasma levels of these enzymes are raised, especially since ALT is principally found in the liver with only small amounts being present in other organs i.e. specific for detecting liver cell damage .Large amounts of AST are present in the liver, kidneys, cardiac muscle, skeletal muscles. Small amounts of the enzymes are present in the pancreas & lungs.

## Samples Needed

- 1 Specimen type : serum or heparinized plasma&K3EDTA plasma
- 2 Specimen Quality: fresh, non hemolysed, non lipemic serum or use lipid clearing factor (LCF) if used.
- 3 Amount: 3-5ml.

## Materials and Reagents Needed

Reagent racks	Wash station
Vacutainer test tube	70% alcohol
Reagents bottles of 45ml	Sample tray
Vacutainer needle	Tourniquets
Sample cups	Reagents
Marker for labeling	Micropipettes and Centrifuge

## Test Procedures

1. Check the PC is connected to the clinical chemistry analyzer.
2. Switches On the clinical chemistry analyzer.
3. Start the software by double clicking the icon HumaStar 80.
4. Check wash solution container is full ,waste container is empty ,working reagents have been prepared ,standards and controls are ready for use, the reaction cells on the analytical plate are clean and sample cups &reagent racks are in place.
5. Click UTILITY button & the instrument will initialize it self.
6. Click PRIME DILUTER button the instrument begin to prepare the hydraulic circuit.
7. In the main window click the AUTODIAGNOSIS button when the window opens click YES& the self test will run after finishing, If warnings/errors/ appear contact human distributor. Click WORK PLAN button in the session part of the main menu window.
8. Click PATIENT DATA button &enter the patient information →click LINK →click EXIT.
9. Choose the tests you are going to perform, repeat for the second and the third samples...click PROCESS button.
10. Press the SUMMARY button check the summary button is displayed next press OK.
11. Click TRAY SETUP key .check all the samples &control position &reagent trays corresponds to the work plan. Finally click OK.
12. Click STATUS ICON .It allows to see which session the instrument is performing &gives you information about wells including current temperature and time required to finish the ordered tests.
13. Click START key. Close the plexus glass cover.
14. Press RESULT key in the report part select the way you want to see: - session or report style. Finally print the results or fill on to the laboratory request paper.
15. Click UTILITY key in the utility part of main menu.
16. Place DW in washing cup then click WASH CUVETTE followed by OK button, adjust the number of washing cycle by using arrow keys.
17. Click SHUTDOWN key from the main menu and switch off the clinical chemistry analyzer next switch off the PC. Close the plexi glass cover.
18. Clean the clinical chemistry analyzer with cotton or gauze moistened with water at the end of work.

## Annex VIII: SOP for glucose test

### **Principle of the Method**

After cleaning the finger with swab and inserting the strip in to the senso-card machine prick the finger with lancet and take the drop directly by the strip, finally read the result and record immediately before discarding the used strip in to safety box.

### **Clinical Significance**

Glucose is a major source of energy for most cells of the body, insulin facilitate s glucose entry in to the cells. Diabetes is a disease manifested by hyperglycemia; patients with diabetes demonstrate an inability to produce or utilize insulin.

### **Materials and Reagents**

1. Senso-card machine
2. Glucose test strip
3. Capillary blood
4. Cotton and alcohol
5. Blood lancet
6. Glove

### **Normal Value**

Fasting= 75-115 mg/dl

Random < 126 mg/dl

### **Procedure**

1. Clean finger with alcohol and dry
2. Prick the dried finger with lancet
3. Take one drop of blood with inserted glucose strip
4. Read result and discard the strip in to safety box

## Annex IX: Assurance of the investigator

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

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Signature \_\_\_\_\_

Date \_\_\_\_\_

2. Name of examiner \_\_\_\_\_

Signature \_\_\_\_\_

Date \_\_\_\_\_