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HEPATITIS B AND HEPATITIS C VIRAL INFECTIONS AND THEIR ASSOCIATED FACTORS AMONG DIABETES PATIENTS ATTENDING UNIVERSITY OF GONDAR REFERRAL TEACHING HOSPITAL, NORTHWEST ETHIOPIA

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LIST OF ABBREVIATIONS AND ACRONYMS

Ab: ………………………………………..…Antibody

Ag: ……………………………………… Antigen

ALP: ……………………………………….Alkaline Phosphatase

AST: ……………………………………….Aspartate Transaminase

CDC: ……………………………………….Center for Disease Control

CHB: ……………………………………….Chronic Hepatitis B Infection

CLD: ……………………………………… Chronic Liver Disease

DM: ……………………………………… Diabetic Mellitus

DNA: ……………………………………….Deoxy Ribose nucleic acid

EIA: ………………………………………...Enzyme Immunoassay

ELISA: ……………………………………..Enzyme Linked Immune Sorbent assay

HAV: ……………………………………….Hepatitis A virus

HBIG: ……………………………………....Hepatitis B immune Globulin

HBsAg: ……………………………………..Hepatitis B surface Antigen

HBV: ………………………………………..Hepatitis B Virus

HCC: ……………………………………… Hepatocellular Carcinoma

HCV: ………………………………………..Hepatitis C Virus

OPD: ……………………………………… Out Patient Department

RNA: ………………………………………..Ribose Nucleic Acid

SOPS: ……………………………………….Standard Operating Procedures

T1DM: ………………………………………Type 1 Diabetic Mellitus

T2DM: ………………………………………Type 2 Diabetic Mellitus

TMB: ………………………………………..3, 3’, 5, 5’-Tetramethylbenzidine

ABSTRACT

Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic viruses whose primary replication occurs in the liver. A range of extrahepatic manifestations such as arthralgias, thyroiditis and diabetes are linked with HCV infections. Patients with diabetes mellitus are predisposed to develop a spectrum of liver diseases.

Objective: To determine the burden of Hepatitis B and Hepatitis C virus and their associated Factors among diabetes patients attending University of Gondar Referral Teaching Hospital, Northwest Ethiopia.

Methods: A comparative cross sectional study design was employed on 305 diabetes and 305 diabetes free individuals from October 2016 to February 2017. Pre-tested structured interviewer based questionnaire was used to collect data. Blood specimen was collected and serum was separated to determine hepatitis B surface antigen (HBsAg) and anti-HCV by Enzyme linked immunosorbent Assay (ELISA) assay. Data were entered with epidata; then exported to, cleared and analyzed by the statistical software SPSS version 20. Univariable and multivariable logistic regression analyses were performed to identify factors that contribute to the occurrence of Hepatitis B and Hepatitis C viral infections. Statistical significance was considered whenever p-value was less than 0.05.

Result: Out of the total, 10.7% was found to be positive for Hepatitis infections, of which 14.43 % and 6.89% study participants were sero-positive for at least one of the viruses in diabetes and control groups respectively. The sero-positivity of Hepatitis B was 8.5% and 4.6% (95% CI, 0.96 - 4.02); and Hepatitis C viral infections 7.5% and 2.3% (95%CI, 1.46 - 8.68) in diabetes and control groups respectively. History of blood transfusion (95% CI, 1.36 - 12.71) and unprotected sex (95% CI, 1.25 - 10.15) were found to be significantly associated with HBsAg sero-positivity and type of diabetes (95% CI, 1.25 - 10.89) was independently associated with anti-HCV seropositivity.

Conclusion and recommendation: Even though no statistically significant difference detected in HBV infection, the sero-positivity against anti- HCV antibody in diabetes and controls groups was found to be 7.5% and 2.3%, respectively and the difference was statistically significant (p-value< 0.001). Prospective studies are needed to confirm temporal association and elucidate the reasons of association between DM and Hepatitis B and C viruses.

Key words: HBV, HCV, DM, Coinfection, University of Gondar Referral Teaching Hospital

1. INTRODUCTION

1.1. Background

Hepatitis B virus and hepatitis C virus (HBV and HCV) infections are common diseases of the world, infecting an estimated 2 billion and 3.9 million people. HBV and HCV are hepatotropic virus whose primary replication occurs in the liver (1). These infections have a high rate of development of liver cirrhosis and can cause serious mortality, raising a major concern for global health (2). HBV and HCV are transmitted via similar routes that is through blood or blood products, including sexual contact, exposure to infected blood, body secretions, and breast feeding (3).

HBV is a partially double stranded, enveloped DNA virus that belongs to the Hepadnaviridae family and Orthohepadna virus genus. Its size ranges from 40 to 42 nm, replicates in the liver and causes hepatic abnormalities(4) (5). An infection with HBV may spontaneously resolve and lead to protective immunity, chronic infection and, in rare cases, acute liver failure with a high risk of death(6). In contrast to HBV, an infection with HCV becomes chronic in most cases. People with chronic HBV and/or HCV infection remain infectious to others and are at risk of serious liver disease such as liver cirrhosis or hepatic cell carcinoma (HCC) later in life (7, 8).

HCV is an enveloped, single-stranded, positive sense RNA virus belonging to the Hepacivirus genus within the Flaviviridae family. The genome is 9.6 kb with a single open reading frame (ORF) that encodes a polyprotein which is further cleaved to yield 10 mature viral proteins namely the structural proteins (C, E1, E2) and non-structural proteins (p7,NS2, NS3, NS4A, NS4B, NS5A, NS5B) (9, 10). Hepatitis C virus exhibits high genetic diversity, characterized by regional variations in genotype prevalence. This poses a challenge to the improved development of vaccines and pan-genotypic treatments (11, 12).

Like HBV, HCV replicates in the liver, causes liver abnormalities also leads to many serious complications including cirrhosis, liver injury and hepatocellular carcinoma (13). Infection with HCV has been shown to produce both hepatic and extrahepatic manifestations, the latter including insulin resistance, essential mixed cryoglobulinemia, and glomerulonephritis (14).The world health organization (WHO) estimates that there are 350 million people with chronic HBV infection and 170 million people with chronic HCV infection worldwide. The estimated hepatitis B surface

antigen (HBsAg) seroprevalence ranges between 0.1% – 20 % in different parts of the world. The prevalence of chronic HBV infection is about 5% worldwide, but this figure varies substantially between regions. Infection rates are low (2.0%) in the United States and Western Europe, intermediate (2.8%) in Mediterranean countries and Japan, and high (8.0-20.0%) in South-East Asia and the sub-Saharan regions. HCV infection is of epidemic proportions worldwide. Countries with high rates of chronic infection are Egypt (22%), Pakistan (4.8%), and China (3.2%) (15). In Ethiopia, the prevalence of HBsAg in chronic liver disease was 43 (35.8%) and 27 (22.5%) for anti-HCV-Ab (16-18).

Diabetes is an enormous and growing clinical and public health problem. In 2015, the International Diabetes Federation (IDF) estimated that 415 million adults had diabetes and that, by 2040, the number will increase to 642 million (19), hence Diabetes has become an important public health problem (20). Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (21). Patients with diabetes mellitus are predisposed to develop a spectrum of liver diseases which includes fatty liver, steato-hepatitis, and fibrosis to cirrhosis and hepatocellular carcinoma (22). Liver regeneration capacity is impaired in animals and humans with fatty liver after partial hepatic resection. It, therefore possible that diabetic having non-alcoholic fatty liver disease (NAFLD) may have poor regenerating capacity leading to prolonged and complicated course of acute hepatitis (23).

1.2. Statement of the problem

Both diabetes and hepatitis C virus (HCV) infection are severe health problems worldwide, especially in the developing countries. The development of diabetes has been observed to be strictly associated with aging, obesity and lack of physical activity. Conversely, study has also revealed that hepatitis virus infections could be a potential risk factor for diabetes. Previous studies have already shown that hepatitis C virus (HCV) infection is a risk factor for developing type 2 diabetes and chronic hepatitis B virus (HBV) infection (CHB) is also related to an increased risk of diabetes (24, 25). Adults with diabetes mellitus are at an increased risk of acquiring HBV infection because they are subjected to more frequent medical interventions (26).

Moreover, a range of extrahepatic (EH) manifestations such as arthralgias, thyroiditis and diabetes are linked with HCV infections. Studies have shown that patients infected with hepatitis C virus (HCV) have more glucose intolerance than the general population. It is well recognized that the levels of reactive oxygen species (ROS) are higher in both diabetic and HCV infected patients. This potential synergism of HCV and diabetes is credited to the complex interactions between HCV and glucose metabolism (23).

There is a strong relationship between diabetes (DM) and infection. Infections can elicit diabetes or make glycaemic control difficult. On the other hand; experimental models showed, viruses' appear capable of both accelerating as well as decelerating the immunological processes leading to type 1 diabetes (T1 DM) (27).

Hepatitis C virus core protein associates with the mitochondria, alters electron transport and leads to increase ROS. This results in decreased glutathione (GSH) and hinders the cell's antioxidant response. Elevated ROS levels lead Kupffer cell to burst and release TNF-α, TGF-β, and ROS (28). TNF--α alters insulin receptors IRS-1 and 2 and down-regulates the glucose transporter (GLUT4 or GLUT2) in adipocytes and hepatocytes. This promotes collagen synthesis, and has been shown to increase the risks for insulin resistance (29-31).

HCV infection leads to a post receptor defect in IRS-1 association with the IR and insulin signaling defects in hepatic IRS-1 tyrosine phosphorylation and PI3-kinase association/activation may contribute to insulin resistance, which leads to the development of type 2 diabetes mellitus in patients with HCV infection (32-34).

1.3. Literature review

1.3.1. Hepatitis B viruses

A study aimed to assess the prevalence of hepatitis B and C viruses and the risk factors among Turkish patients with type 1 and type 2 diabetes mellitus showed that 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 2 groups with respect to either marker. Shared risk factors for both hepatitis infections were increased aminotransferase levels and history of hospital admission (35).

A study in Turkey, using a third-generation commercial chemiluminescence assay showed that the seropositivity rate for the HBsAg was (3.8% vs 3.0%, P>0.43) among diabetic and non-diabetic groups respectively. History of blood transfusions and surgical procedures were positively associated with HBsAg seropositivity (36).

A study in USA from 1999-2010, using the enzyme-linked immunoassay HBc and chemiluminescent immunoassay HBc indicated that a prevalence of 8.2% (95% CI = 6.8, 9.8) HBc seropositivity for those with diabetes which was 1.6 (95% CI = 1.3, 1.9) times as great among persons than without diabetes (37).

A study from china, patients with type 2 diabetes had higher prevalence of CHB than the controls in the overall population (13.5% vs.10.0%, P=0.004) and among patients with normal hepatic function (13.3% vs.8.8%, P=0.002) (38).

A simple randomized cross-sectional study conducted from December 2012 to April 2013, at the Diabetic Clinic of the Central Regional Hospital, Cape Coast, Central Region, Ghana, indicated that the prevalence of HBV infection in the type 2 diabetes mellitus (T2DM) participants was higher (5.5% [6/110]) than that of HCV (0/110) (2).

A comparative cross sectional study conducted at Woldiya General Hospital, Ethiopia, using 108 study populations from Diabetes and 108 non diabetes control groups reported that the prevalence rate of HBsAg was equal, 3.7%, indicating that there was no difference between the two groups. Only history of invasive procedures and chronic liver disease showed association with HBsAg seropositivity (39).

1.3.2. Hepatitis C viruses

A study conducted at a tertiary care hospital, India showed that out of 300 diabetics, 33(11%) were found to be anti-HCV positive and all of them had type 2 diabetes mellitus and concluded as there was a significant association between HCV and T2DM in the region. Serum alanine aminotransferase level was raised in positive cases as compared to the seronegative patients (40).

According to a descriptive study conducted at DHQ Teaching Hospital D.I. Khan from January 2013 to December 2013, among the 84 diabetic, 23(27.38%) patients were positive for HCV infection. Thus HCV infection was observed in almost one third patients with type 2 diabetes (41).

A case control study was conducted among 510 study participants at DHQ hospital Gujranwala, Pakistan. The burden of HCV in type II diabetes and nondiabetic controls was 10.5% and 4.3%, respectively indicating that diabetics are more likely to be seropositive for hepatitis C (42).

A study done on a sample of 3000 individuals with T2DM visiting Diabetes Clinic of Nishtar Medical College Hospital, Pakistan and 10,000 volunteer blood donors showed a Prevalence rate of 13.7% and 4.9% for HCV infection respectively. The patients with T2DM were more likely to have HCV infection as compared to the control group (OR = 3.03, 95%CI = 2.64-3.48, p = 0.001). Those with duration of diabetes 11 years and above had higher seroprevalence rates of 18.2%. There was no statistically significant difference among subjects when the distribution of HCV was studied on the basis of marital status, locality, or family history of diabetes (43).

Another study was carried out on twenty patients suffering from type 2 Diabetic mellitus in Baghdad hospital. The percentage of HCV Abs was 15% and there was highly significant ($P \leq 0.01$) differences between studied group (44).

A study in Turkey, using a third-generation commercial chemiluminescence assay showed that the seropositivity rate for anti-HCV was (3.3% vs 1.8%, $P<0.03$) among diabetic and non-diabetic groups respectively. History of blood transfusions was positively correlated with HCV infection (36).

Another study aimed to examine the frequency and risk factors of HCV among Saudi patients with diabetes. The frequency of HCV among patients with diabetes was 1.9%. Predictors of HCV among DM patients were sharing articles, elevated transaminases, occupational exposure to blood

or its products, disease duration more than 5 years, tattooing, blood transfusion, and hospitalization more than two times (45).

A cross-sectional study conducted on 303 consecutive patients with type 2 DM attending the outpatient clinic of de Clínicas de Porto Hospital, Alegre reported that anti-HCV testing was positive in 73 of 489 inpatients (15%), 39 of 303 (12.9%) outpatients and 34 of 186 (18.27%) in dialysis patients. The main risk factor for HCV infection was blood transfusion (46).

In a study where Three hundred (300) confirmed type 2 diabetic patients screened for hepatitis C virus antibodies at the Plateau state specialist hospital, Nigeria, the burden rate of HCV infection was 33(11%). Those aged 47–57 recorded the highest seroprevalence 10(30.3%) to the Hepatitis C Virus infection. The age range of 1–10 years of diabetic status recorded the highest prevalence rate 25(75.8%) (47).

A study from Mansoura Specialized Medical University Hospital, Egypt; among diabetic patients rate of HCV infection was found to be 32%. Prevalence of microvascular complication, nephropathy, retinopathy and neuropathy were found to be relatively higher in diabetics with HCV infection (48).

According to a study from Sudan, the prevalence rate of 1.7% for HCV infection was recorded among T2DM patients and no seropositivity was detected among the control group. The patients with T2DM were more likely to have HCV infection as compared to the control group (1.7%, 0.005% = p = 0.00) (49).

A case control study design was conducted at Jimma University Specialized Hospital and a total of 604 study subjects were included in the study. The prevalence of HCV in type II diabetes and non-diabetic controls was 9.9% and 3.3%, respectively. With regard to HCV risk factors which included ear piercing, body piercing, tattoo, tooth extraction, hospital admission, history of transfusion and contact with jaundiced person; none of these risk factors were significantly associated with HCV seropositivity in both diabetic and non-diabetic controls (50).

1.4. Significance of the study

Hepatitis B virus and hepatitis C virus (HBV and HCV) infections are common diseases of the world particularly developing country. In addition, HBV and HCV is a highly contagious virus, causes chronic liver disease and lead to death (2, 3), accordingly proper screening, diagnosis, treatment and prevention are very essential. Therefore, the results of this study will, if further explored, benefit the diabetes patients for screening, diagnosis, treatment and prevention purpose.

The association between DM and HCV infection has only lately been posed in the international literature and remains unexplained. There is a growing body of literature on the relationship of HCV infection and DM however, has not been fully understood and remains to be determined. Therefore, elucidate the presence of any possible relationship between HCV and HBV with diabetes will enable better management for diabetes patients, especially to prevent such added infections and its morbid consequences. This study could also be useful for health managers and planners to develop appropriate preventive services, allocate resources, decide on priorities and target certain populations.

Since the prevalence of diabetes is on the rise and is complicated by co-infection with HCV and HBV, the determination of relationship becomes even more important in this scenario. Still this study used as information for further studies.

2. OBJECTIVE OF THE STUDY

2.1. General Objective

To determine the magnitude of Hepatitis B and Hepatitis C viral infections and their associated factors among diabetes patients and non-diabetes attending University of Gondar Referral Teaching Hospital, Northwest Ethiopia.

2.2. Specific Objectives

To determine the burden of Hepatitis B viral infection among diabetic patients

To determine the burden of Hepatitis C viral infection among diabetic patients

To compare the burden of HBV and HCV infections between diabetic patients and control groups

HYPOTHESIS

This study hypothesizes that the burden of HBV and HCV in diabetes patient and non-diabetes groups is similar.

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted at Gondar Referral Teaching Hospital, Northwest Ethiopia. Grew out of the Gondar public Health College and Training Center (PHC and TC) established in 1954. A population of 2,929,628 (2007 census) located in the geographic center of Gondar which lies at an altitude of 6,998 feet (2,133 meters). The city holds the remains of several royal castles, including those in Fasil Ghebbi (the Royal Enclosure), for which Gondar has been called the "Camelot of Africa". The hospital has 400 beds, and G+5 maternal and child hospital, best standard TB ward and laboratory which acts as the referral center for four district hospitals in the area. It has a range of specialties including Paediatrics, Surgery, HIV care, Psychiatry, Outpatient department, Diagnostic laboratory and Gynaecology. Within it's over 400 staffs it employs 50 doctors, 150 nursing staff, 90 care staff and 75 laboratory professionals. The referral hospital provides referral services to about five million people in the Amhara region of the country. It also provides hands-on teaching experience for new generations of Ethiopian health care workers and continuing medical education. Diabetic clinic in university of Gondar teaching and referral hospital was established in 1985 and proper documentation and registration was started 20 years ago. The clinic is giving service to 3029 registered DM patients and among these 1497 are type II (36)(51).

3.2. Study Design and period

A comparative cross sectional study design was employed from October 2016- February 2017.

4. POPULATION

4.1. Source population for diabetes groups

All known diabetes patients who visited University of Gondar Referral Teaching Hospital during the data collection period.

4.2. Source population for control groups

All individuals who visited University of Gondar Referral Teaching Hospital for either blood donation or medical checkup during the data collection period.

4.3. Study population for diabetes groups

All known adult diabetic patients who visited University of Gondar Referral Teaching Hospital during the data collection period and were volunteer to participate and fulfill the inclusion criteria.

4.4. Study population for control groups

All adult individuals who visited University of Gondar Referral Teaching Hospital for either blood donation or medical checkup and confirmed as non-diabetes during the data collection period.

4.5. Study variables

4.5.1. Dependent variables

Sero-status of HBsAg

Sero-status of anti-HCV antibody

4.5.2. Independent variables

Socio-demographic factors of participants

Multiple sexual partners

Blood transfusion history

Abortion

History of surgical procedure

Uvuloctomy

Ear/nose piercing

Circumcision

Contact with jaundiced patients

Duration of the disease

Dental extraction

Tattooing

Shaving by barbers

DM status

4.6. Inclusion and exclusion criteria

4.6.1. Inclusion criteria

Diabetic groups

All adults who visited Outpatient Department (OPD) proved as diabetic patients during the study period.

Control groups

All adults who visited the hospital for medical checkup or blood donation and/or proved of non DM.

4.6.2. Exclusion criteria

Diabetic groups

Individuals who proved as diabetic patients during the study period but who refused to give informed consent and were vaccinated against HBV.

Control groups

Individuals who refused to give informed consent and were vaccinated against HBV.

4.7. Sample size estimation and Sampling technique

4.7.1. Sample size estimation

The sample size was determined by using double population proportion formula by considering 80% power, 95% confidence interval assuming association of hepatitis C virus infection with type II diabetes in Ethiopia the prevalence was 9.9% and 3.3% among diabetes and non-diabetic control groups respectively and bearing equal sized groups, the minimum sample size was estimated as follows (50).

Sample size is derived using the following formula:

$$n1 = \frac{(r+1)}{r}\frac{(\bar{p})(1-\bar{p})(Z_{\beta}+Z_{\frac{\alpha}{2}})^2}{(p_1-p_2)^2} = 2(0.066)\,(0.934)\,(7.84)/(0.066)^2 = 221.89$$

Where:

n_1= sample size in each group

r = ratio of larger group to smaller group

$Z\alpha$: Standard normal distribution abscissa corresponding to 95% confidence interval (1.96)

Z_β: the desired power (0.84 for 80%)

P_1= Proportion in the diabetes population to have anti HCV.

P_2= Proportion in the non-diabetes control population to have anti HCV.

$\overline{P}$= $(p_1+p_2) \div 2$= poled Proportion in the target population to have anti HCV.

Therefore, a total of 488 including non-response of 10% were considered for both diabetes and non-diabetic control group.

Even though initially the minimum sample size was determined to be 488 by double population formula, the actual sample size taken was increased to 610 as the detection of HBsAg and anti-HCV was low to further determine the associated factors contributing to viral hepatitis. Moreover, increased sample size improves the power of study.

4.7.2. Sampling technique

Systematic random sampling was employed as diabetes patients were coming for follow-up, either glucose monitoring or treatments. Any adult who came for blood donation or any clients who have a medical check-up or diagnosed other than DM were recruited as a control groups.

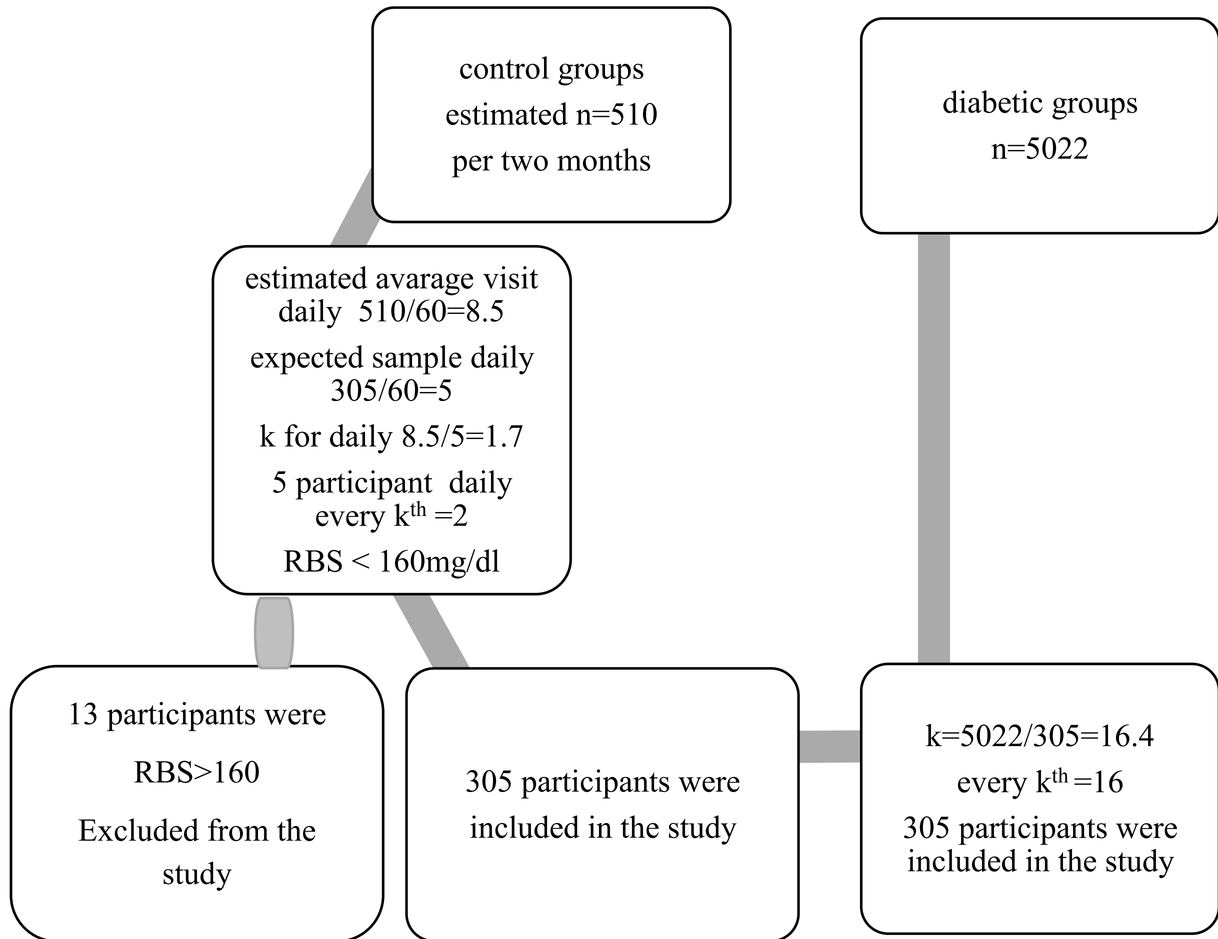

Figure 1: Flowchart of participants enrolled in diabetes and non-diabetes groups for investigation of the burden of HBV and HCV in University of Gondar Referral Teaching Hospital (October 2016- February 2017).

4.8. Data Collection and Processing

Laboratory confirmed DM patients and non DM (control groups) were consented for the study and to give blood. All eligible DM patients and control groups who were volunteered were contacted for both blood collection and interview. Socio-demographic factors such as age, sex, religion, occupation, marital status, ethnicity, and educational Status were collected. Other associated risk factors such as multiple sexual partners, blood transfusion, abortion (for female participants only), dental extraction at health facility, circumcision, hospital admission, surgical procedure, venous or body piercing for treatment delivery by traditional birth attendant, ear piercing, nose piercing, uvuloctomy, tattooing on body, tattooing on gum, shaving, contact with jaundiced patient, and frequent alcohol consumption status, were also collected with a structured interviewer-based pretested questionnaire by trained nurse professionals. Blood sample collection was undertaken under aseptic condition by licensed medical laboratory professionals.

5.7. Laboratory methods

5.7.1. Sample collection and processing

Five ml of venous blood was collected aseptically by vacuntainer needle in serum separating tube; samples were left at room temperature for 1 hour to facilitate clotting and were centrifuged at 3000 rpm for 5 minutes to get clear serum for serological analyses. Serum sample was kept in Nunc tubes and stored at -20 degree centigrade until it was processed.

HBsAg EIA Test

A) Specimen containing HBsAg: (Daillab.at, Neudrof Australia)

The HBsAg EIA is a solid-phase simultaneous sandwich immunoassay, which employs monoclonal antibodies and polyclonal antibodies specific for HBsAg. Microtiter well are coated with monoclonal antibodies specific for HBsAg. A serum specimen is added to the antibody coated Microtiter wells together with enzyme conjugated polyclonal antibodies. HBsAg, if present, will form an antibody-HBsAg-antibody-enzyme complex. The plate is then washed to remove unbound material. Finally, a solution of substrate is added to the wells and incubated. A blue color will develop in proportion to the amount of HBsAg present in the specimen. The enzyme-substrate reaction can be stopped and the result is visualized by naked eye or read by EIA plate reader for absorbance at the wavelength of 450 nm.

B) Specimen without HBsAg:

A serum specimen is added to the antibody coated Microtiter wells together with enzyme conjugated polyclonal antibodies. HBsAg, if absent, will not form an antibody-HBsAg-antibody-enzyme complex. The plate is then washed to remove unbound material. Finally, a solution of substrate is added to the wells and incubated. A Colorless to light blue color will develop. The enzyme-substrate reaction can be stopped and the result is visualized by naked eye or read by EIA plate reader for absorbance at the wavelength of 450 nm. Positive sample generates a medium to dark blue color. No color or very pale blue color indicates a negative reaction. The intensity of the reaction is photometrically quantitated.

5.7.2. HCV Antibody EIA

Multiple epitopes of HCV proteins (Core, NS3, NS4 and NS5) are bound to the micro-titer wells. When antibodies to HCV are present in the test sample, they react with recombinant proteins and

attach to the solid-phase. Non-reactive antibodies are removed with the wash buffer. Human IgGs bound to the antigen are reacted with goat-anti-human IgG peroxidase conjugate and visualized by subsequent reactions with a chromogenic substrate. Positive sample generates a medium to dark blue color. No color or very pale blue color indicates a negative reaction. The intensity of the reaction is photometrically quantitated (52).

5.7.3. Blood glucose test

Principle of the method

Glucose present in the plasma is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD). 4 aminophenazone, an oxygen acceptor, takes up the oxygen and together with phenol forms a pink coloured chromogen which can be measured at 515nm.

75 g oral glucose is given to pregnant women and then measure plasma glucose level with the same principle of blood glucose test at three different time, zero hour, an hour and two hours after oral glucose administration.

If a plasma glucose level of 70 – 110 mg/dl, 80 – 140 mg/d and 140 – l60 mg/dl for fasting, post-prandial and random sample respectively and oral glucose tolerance test (OGTT) <92 mg/dl <180 mg/dl or <153 mg/dl after fasting, an hour and two hours respectively were included under control groups (53).

5.8. Quality control

The standard operational procedures were strictly followed for the quality control issues. Both hepatitis B and C kits were checked by using known HBsAg and anti-HCV antibody positive and negative control samples. All data quality control tools were considered. The data were cheeked for completeness and representativeness prior to entry. The reliability of the study findings were guaranteed by implementing Quality control (QC) measures throughout the whole processes of the laboratory works. All materials, equipment and procedures were adequately controlled. Pre-analytical, analytical and post-analytical stages of quality assurance were strictly followed. All laboratory analyses were carried out using standard operating procedures.

5.9. Data Processing and Analysis

Data were entered using epidata and analyzed by the statistical software SPSS version 20.The burden for HBC and HCV were expressed in percentages for the respective study group. Frequencies and percentages were used to descriptively summarize characteristics of study. Comparisons between groups were made by using Student's t test for continuous variables and Pearson Chi-square was used to test the difference in proportions of hepatitis infections across categorical variables. A multivariable logistic regression model was used to determine the independent effect of various factors that will be potentially associated with the factors of hepatitis in both groups. Odds ratios (OR) and 95% confidence intervals (CI) were used as a measure of the strength of association.

5.10. Ethical Considerations

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Laboratory Sciences. Then, a letter informing to University of Gondar Referral Teaching Hospital from Ethical Committee of Department of Medical Laboratory and permission was obtained from University of Gondar Referral Teaching Hospital to access data from study population. All eligible participants were informed as the participation were voluntary and as the aim of this study was only to collect necessary information which is helpful to assess the burden of HBV and HCV with possible factors. Blood sample collection was carry out under aseptic condition by licensed medical laboratory professionals. All personal information obtained from the study participants were coded to maintain confidentiality. When the participants (from control groups) were found to be higher blood glucose level, they were informed by the health worker and received proper treatment and excluded from the study. Positive cases for either HBV or HCV were linked to the hospital's medical OPD with an internal referral form of the hospital.

5.11. Dissemination of results

After conducting the research, the results of the study will be submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences, so that it can serve as a reference in the library. In addition, a copy of this material will be given to Gondar Referral Teaching Hospital, be presented on annual conferences of professional societies and other concerned bodies. The finding of the study will also be presented to the medical scientific community and manuscript will be submitted to peer-reviewed journals for publication.

6. RESULTS

6.1. Socio-demographic characteristics

A total of 610 participants (305 cases and 305 controls) were included in this study. The mean age was 37.74 among diabetic and 36.33 years among controls. The majority of participants were male among diabetic (56.4%) and control groups (59.7%). In individuals who were tested with HBV and HCV serology tests , there were no significant differences in age sex wise among cases and controls (p=0.056 and p=0.412, respectively). The mean duration of diabetes was 6.38 ± 5.29 (range 1-22) years and the mean random blood sugar for control subjects was 89.43 ± 9.05 mg/dl, (Table 1).

Table 1: Socio-demographic characteristics of participants with diabetes and non-diabetes investigated for burden of HBV and HCV in University of Gondar Referral Teaching Hospital (October 2016- February 2017).

Socio-demographic characteristics		**No. (%)**	
		Diabeties	Non-Diabeties
Age (years)	≤35	110(36.1)	128(42.0)
	36-45	123(40.3)	133(43.6)
	46-55	72(23.6)	44(14.4)
Sex	Male	172(56.4)	182(59.7)
	Female	133(43.6)	123(40.3)
Residence	Rural	74(24.3)	38(12.5)
	Urban	231(75.7)	267(87.5)
Occupation	Self-employed	85(27.9)	75(24.6)
	Driver	23(7.5)	20(6.6)
	House Wife	87(28.5)	18(5.9)
	Student	19(6.2)	20(6.6)
	Farmer	48(15.7)	6(2.0)
	Civil servant	43(14.1)	166(54.4)
Religion	Orthodox	281(92.1)	261(85.6)
	Muslim	20(6.6)	44(14.4)
	Others	4(1.3)	0(0.0)
Ethnicity	Amhara	284(93.1)	294(96.4)
	Others*	21(6.9)	11(3.6)
Educational Status	Illiterate	99(32.5)	10(3.3)
	Read and write	24(7.9)	18(5.9)
	Primary and High school	147(48)	135(44.2)
	College/university	35(11.5)	142(46.6)
	Total	**305(100)**	**305(100)**

*Oromo and Tigray

6.1. Burden of HBV and HCV infection

Out of the total, 10.7 %(65/610) was found to be positive for hepatitis infections, of which 14.43 % (44/305) and 6.89% (21/305) study participants were sero-positive for at least one of the viruses in diabetes and control groups respectively. The burden of HBV infection in diabetes and controls was found to be 8.5% (26/305) and 4.6% (14/305), respectively and the difference was not statistically significant (COR=1.94; 95%CI = 0.99, 3.79 P-value = 0.053). The burden of sero-positivity against anti- HCV antibody in diabetes and controls groups was found to be 7.5% (23/305) and 2.3% (7/305), respectively and the difference was statistically significant (COR= 3.47; 95%CI = 1.47, 8.23; P-value = 0.005**)**. About 1.64% (5/305) of the study participants had HBV and HCV co-infection among diabetes groups, while none were detected among control groups, (Table 2).

Table 2: Burden of Hepatitis B and Hepatitis C virus infection among diabetes and control groups at University of Gondar Referral Teaching Hospital (October 2016- February 2017) (n=305).

Variables		**Diabetic patients**	**Control subjects**	**COR(95%CI)**	**AOR(95%CI)**	**p-value**
HBsAg	Positive	26(8.5%)	14(4.6%)	1.94(0.99,3.79)	1.96(0.96, 4.02)	0.06
	Negative	279(91.5%)	291(95.4%)			
Anti-HCV	Positive	23(7.5%)	7(2.3%)	3.47(1.47,8.23)	3.56(1.46,8.68)	**0.00***
	Negative	282(93.8%)	298(97.7%)			

*statistically significant association (p-value less than 0.05), p-value is for AOR

6.2. Associated factors for HBV and HCV infections

Hospital admission (P-value < 0.001), surgical procedures (P-value < 0.001) and Venous or body piercing (P-value = 0.02) had statistically significant differences between diabetic and non-diabetic groups. In bi-variable analysis; only history of blood transfusion (COR= 3.05, 95%CI; 1.12: 8.29) and unprotected sex (COR= 2.75 95%CI; 1.15:6.56) were significantly associated with HBsAg sero-positivity, (table 3). The odds of detecting of HBsAg in participants having history of blood transfusion, unprotected sex and circumcision were 4.15, 3.56 and 4.58 times greater than in those who had no history blood transfusion, unprotected sex and circumcision respectively, (Table 4).

Table 3: Bi-variable analysis of factors for HBsAg sero-positivity among diabetes patients at University of Gondar Referral Teaching Hospital (October 2016- February 2017).

Clinical and diabetic characteristics		**HBsAg (-) N (%)**	**HBsAg(+) N (%)**	**COR(95%CI)**	**P-value**
History of STD/STI	Yes	13(4.7)	2(7.7)	1.70(0.36,8.00)	0.49
	No	266(95.3)	24(92.3)	1.00	
Multiple sexual partner	Yes	23(8.2)	5(19.2)	2.65(0.91,7.68)	0.07
	No	256(91.8)	21(80.8)	1.00	
History of blood transfusion	Yes	25(9.0)	6(23.1)	3.05(1.12,8.29)	**0.03***
	No	254(91.0)	20(76.9)	1.00	
Abortion**	Yes	25(20.5)	3(27.3)	1.45(0.36,5.89)	0.60
	No	97(79.5)	8(72.7)	1.00	
Dental extraction at health facility	Yes	59(21.1)	7(26.9)	1.37(0.55,3.42)	0.49
	No	220(78.9)	19(73.1)	1.00	
Circumcision	Yes	191(68.5)	22(84.6)	2.53(0.85,7.57)	0.09
	No	88(31.5)	4(15.4)	1.00	
Hospital admission	Yes	206(73.8)	18(69.2)	0.80(0.33,1.91)	0.61
	No	73(26.2)	8(30.8)	1.00	
Surgical procedure	Yes	55(19.7)	7(26.9)	1.50(0.60,3.74)	0.38
	No	224(80.3)	19(73.1)	1.00	
Venous or body piercing	Yes	107(38.4)	10(38.5)	1.00(0.44,2.30)	0.99
	No	172(61.6)	16(61.5)	1.00	
Delivery by TBA**	Yes	35(28.7)	4(36.4)	1.42(0.39,5.16)	0.59
	No	87(71.3)	7(63.6)	1.00	
Ear piercing	Yes	83(29.7)	11(42.3)	1.73(0.76,3.93)	0.18
	No	196(70.3)	15(57.7)	1.00	
Uvuloctomy	Yes	143(51.3)	11(42.3)	0.70(0.31,1.57)	0.38
	No	136(48.7)	15(57.7)	1.00	
Tattooing on body	Yes	46(16.5)	7(26.9)	1.87(0.74,4.70)	0.19

	No	233(83.5)	19(73.1)	1.00	
Tattooing on gum	Yes	25(9.0)	4(15.4)	1.85(0.59,5.79)	0.29
	No	254(91.0)	22(84.6)	1.00	
Shaving	Yes	119(42.7)	12(46.2)	1.15(0.51,2.58)	0.73
	No	160(57.3)	14(53.8)	1.00	
Contact with jaundiced patient	Yes	36(12.9)	6(23.1)	2.02(0.76,5.38)	0.15
	No	243(87.1)	20(76.9)	1.00	
Frequent alcohol consumption	Yes	100(35.8)	12(46.2)	1.534(0.68,3.44)	0.30
	No	179(64.2)	14(53.8)	1.00	
Unprotected sex	Yes	45(16.1)	9(34.6)	2.75(1.15,6.56)	**0.02***
	No	234(83.9)	17(65.4)	1.00	
Type of diabetes	Type I	150(53.8)	15(57.7)	1.17(0.52,2.64)	0.70
	Type II	129(46.2)	11(42.3)	1.00	
Any confirmed diseases other than diabetes	Yes	59(21.1)	3(11.5)	0.49(0.14,1.68)	0.25
	No	220(78.9)	23(88.5)	1.00	
Duration of disease(year)	<5	151(54.1)	11(42.3)	1.52(0.54,4.32)	0.42
	6-10	74(26.5)	9(34.6)	0.91(0.31,2.72)	0.87
	>11	54(19.4)	6(23.1)	1.00	
Treatment type	Oral	94(33.7)	4(15.4)	1.00	
	Injection	185(66.3)	22(84.6)	2.80(0.94,8.34)	0.66
	Total	**279(91.5%)**	**26(8.5%)**		

COR=crude odds ratios, CI= Confidence Interval, *statistically significant association (p-value less than 0.05), **= concerning female

Table 4: Multi-variable analysis of factors for HBsAg sero-positivity among diabetes patients at University of Gondar Referral Teaching Hospital (October 2016- February 2017).

Factors	**Category**	**COR(95%CI)**	**AOR(95%CI)**	**P-value**
History of blood transfusion	Yes	2.800(1.035,7.573)	4.15(1.36,12.71)	**0.01***
Circumcision	Yes	2.534(0.848,7.574)	4.58(1.26,16.66)	**0.02***
Unprotected sex	Yes	2.753(1.155,6.562)	3.56(1.25,10.15)	**0.01***

COR=crude odds ratios, AOR=adjusted odds ratios, CI= Confidence Interval, *statistically significant association (p-value less than 0.05)

Only type of diabetes was significantly associated with anti-HCV sero (AOR=3.69, 95%CI; 1.25:10.89). The odds of detecting of anti-HCV antibody was 3.69 times greater in T2DM than T1DM, (Table 6).

Table 5: Bi-variable analysis of factors for anti-HCV sero-positivity among diabetes patients at University of Gondar Referral Teaching Hospital (October 2016- February 2017).

Clinical and diabetic characteristics		**Anti-HCV (-) N (%)**	**Anti-HCV(+) N (%)**	**COR(95%CI)**	**P-value**
History of STD/STI	Yes	14(5.0)	1(4.3)	0.11(0.11,6.93)	0.89
	No	268(95.0)	22(95.7)	1.00	
Multiple sexual partner	Yes	25(8.9)	3(13.0)	1.54(0.43,5.55)	0.50
	No	257(91.1)	20(87.0)	1.00	
Blood transfusion	Yes	27(9.6)	4(17.4)	1.99(0.63,6.27)	0.24
	No	255(90.4)	19(82.6)	1.00	
Abortion**	Yes	26(20.3)	2(40.0)	2.62(0.42,16.47)	0.31
	No	102(79.7)	3(60.0)	1.00	
Dental extraction at health facility	Yes	60(21.3)	6(26.1)	1.31(0.49,3.46)	0.59
	No	222(78.7)	17(73.9)	1.00	
Circumcision	Yes	196(69.5)	17(73.9)	1.24(0.47,3.26)	0.65
	No	86(30.5)	6(26.1)	1.00	
Hospital admission	Yes	205(72.7)	19(82.6)	1.78(0.59,5.41)	0.30
	No	77(27.3)	4(17.4)	1.00	
Surgical procedure	Yes	59(20.9)	3(13.0)	1.76(0.51,6.14)	0.37
	No	223(79.1)	20(87.0)	1.00	
Venous or body piercing	Yes	107(37.9)	10(43.5)	1.26(0.53,2.97)	0.60
	No	175(62.1)	13(56.5)	1.00	
Delivery by TBA**	Yes	38(29.7)	1(20.0)	0.59(0.64,5.47)	0.64
	No	90(70.3)	4(80.0)	1.00	
Ear piercing	Yes	91(32.3)	3(13.0)	3.18(0.92,10.96)	0.06
	No	191(67.7)	20(87.0)	1.00	
Uvuloctomy	Yes	144(51.1)	10(43.5)	0.74(0.31,1.74)	0.48
	No	138(48.9)	13(56.5)	1.00	
Tattooing on body	Yes	48(17.0)	5(21.7)	1.35(0.479,3.83)	0.57

	No	234(83.0)	18(78.3)	1.00	
Tattooing on gum	Yes	28(9.9)	1(4.3)	0.41(0.05,3.18)	0.39
	No	254(90.1)	22(95.7)	1.00	
Shaving	Yes	117(41.5)	14(60.9)	2.19(0.92,5.24)	0.07
	No	165(58.5)	9(39.1)	1.00	
Contact with jaundiced patient	Yes	38(13.5)	4(17.4)	1.35(0.44,4.19)	0.60
	No	244(86.5)	19(82.6)	1.00	
Frequent alcohol consumption	Yes	101(35.8)	11(47.8)	1.64(0.70,3.86)	0.25
	No	181(64.2)	12(52.2)	1.00	
Unprotected sex	Yes	47(16.7)	7(30.4)	2.19(0.85,5.61)	0.10
	No	235(83.3)	16(69.6)	1.00	
Type of diabetes	Type I	160(56.7)	5(21.7)	1.00	
	Type II	122(43.3)	18(78.3)	4.72(1.70,13.07)	**0.00***
Any confirmed diseases other than diabetes?	Yes	57(20.2)	5(21.7)	1.10(0.39,3.08)	0.86
	No	225(79.8)	18(78.3)	1.00	
Duration of disease(year)	1-5	147(52.1)	15(65.2)	0.89(0.31,2.57)	0.83
	6-10	80(28.4)	3(13.0)	2.42(0.56,10.56)	0.23
	>11	55(19.5)	5(21.7)	1.00	
Treatment type	Oral	91(32.3)	7(30.4)	1.00	
	Injection	191(67.7)	16(69.6)	1.09(0.43,2.74)	0.85
Late diabetic complications	Yes	145(50.7)	16(69.6)	2.25(0.90,5.64)	0.08
	No	141(49.3)	7(30.4)	1.00	
	Total	**282(91.5%)**	**23(7.5%)**		

COR=crude odds ratios, CI= Confidence Interval, *statistically significant association (p-value less than 0.05), **= concerning female

Table 6: Multi-variable analysis of factors for anti-HCV sero-positivity among diabetes patients at University of Gondar Referral Teaching Hospital (October 2016- February 2017).

Variables	Category	COR(95%CI)	AOR(95%CI)	P-value
Type of diabetes	Type II	4.72(1.70,13.07)	3.69(1.25,10.89)	**0.02***
Ear piercing	Yes	3.18(0.92,10.96)	0.53(0.14,2.10)	0.37
Shaving	Yes	2.19(0.92,5.24)	1.66(0.61,4.54)	0.33
Unprotected sex	Yes	2.19(0.85,5.61)	1.03(0.36,2.95)	0.96
Late diabetic complications	Yes	2.25(0.90,5.64)	1.47(0.54,3.96)	0.45

COR=crude odds ratios, AOR=adjusted odds ratios, CI= Confidence Interval, *statistically significant association (p-value less than 0.05), p-value is for AOR.

7. DISCUSSION

Our study included 610 participants divided into two groups according to the presence and absence of diabetes; and 305 diabetic patients and 305 age and sex were matched control groups were included. We compared the burden of HBV and HCV infection in the studied groups to establish an association between HCV and HBV with diabetes. 10.7 % was found to be positive for hepatitis infections where as 14.43 % and 6.89% were sero-positive for at least one of the viruses in diabetes and control groups respectively. Hospital admission, surgical procedures and Venous or body piercing had statistically significant differences between diabetic and non-diabetic groups. History of blood transfusion, unprotected sex and circumcision were significantly associated with HBsAg sero-positivity. Type of diabetes was significantly associated with anti-HCV sero-positivity. Therefore, diabetes patients are at higher risk of acquiring HCV infection and may be related to either the disease itself or frequent parenteral exposure or patients who have been infected with HCV might have acquired diabetes due to damage to β-cells of the pancreas so as to induce diabetes.

A study in USA from 1999-2010, using the enzyme-linked immunoassay and chemiluminescent immunoassay for detection of HBc, indicated that a prevalence of 8.2% (95% CI = 6.8, 9.8) HBc seropositivity for those with diabetes (37), which is in line with the present study. On the other hand, a study from china reported that a higher prevalence of HBV infection 13.5% among diabetes patients (38), as compared to 8.5% prevalence in this study. This might be due to a variation in socio-demographic and epidemiological factors.

The magnitude of HBV infection in the present study (8.5%) is higher than a study from Ghana, a simple randomized cross-sectional study conducted from December 2012 to April 2013 (2), from Turkey, a study using a third-generation commercial chemiluminescence assay (36), and from Ethiopia, a comparative cross sectional study using rapid diagnostic test in 2014 (39), indicated a prevalence rate of 5.5%, 3.8% and 3.7% respectively. The higher magnitude of HBV infection in the present study compared to a study in Woldiya General Hospital, Ethiopia might be due to the small sample size and a less sensitive diagnostic technique (rapid test) was employed by the researchers.

The burden of HCV infection in this study (7.5%) was close to a case control study from Jimma Referral Teaching Hospital, Ethiopia (9.9%) screened by rapid antibody test (50). The present

study was slightly lower than 11% Nigeria, 2009 (47), 11% India, 2016 (40), 12.9% Alger, 2012 (46), 13.7% Pakistan, 2010 (43), whereas it was much lower than reports of 15% Iraq, 2015 (44), and 27.38% DHQ Teaching Hospital, 2014 (41) and 32% Egypt, 2007 (48). On the other hand a higher (7.5%) burden of HCV infection in patients with diabetes was observed in the present study as compared to a study from Sudan, 2014 (1.7%) (49), Saudi, 2016 (1.9%) (45), and Turkey, 2015 (3.8%) (36).

In this study the odds of detection of anti-HCV antibody was 3.69 times greater in T2DM than T1DM (AOR= 3.69, 95%CI; 1.25:10.89), which was in line with a study from tertiary care hospital, India, showed all of them had type 2 diabetes mellitus and concluded as there was a significant association between HCV and T2DM in the region (40). Also, HCV infection was observed in almost one third patients with type 2 diabetes in DHQ Teaching Hospital, Pakistan (41). Thus a higher burden of HCV infection in T2DM can explain the fact that HCV infection leads to a post receptor defect in IRS-1 association with the insulin receptor and insulin signaling defects in hepatic IRS-1 tyrosine phosphorylation and PI3-kinase association/activation may contribute to insulin resistance leading to T2DM (32, 33).

Even though this study was not detected any significant epidemiological factors to HCV infection other studies showed predictors of HCV among DM patients were sharing materials, elevated transaminases, occupational exposure to blood or its products, disease duration more than 5 years, tattooing, blood transfusion, and hospitalization more than two times in Dammam, KSA (45) The main risk factor for HCV was blood transfusion Alger, 2012 (46). Having multiple sexual partners was identified as the only significant risk factor for hepatitis C (OR = 9.148) (41). Also other study reports that DM duration of >5 years increased the probability of HCV risk to 3.7 fold while insulin users were 3.2 times more likely to have HCV infection. Increased hospital admission (3–4 times) also increased HCV risk by 11.5 times and 13.6 times among patients with ≥5 admissions. Similarly, having 3–4 surgical procedures increased HCV risk by 8.6 times and 39.3 times with≥5 procedures. HCV transmission is 4 times more likely by blood transfusion. Those who shared personal items were 8.5 times more likely to have HCV, tattooing increased HCV risk by 6.7 times (28). Blood transfusions and surgical procedures were found to associate with anti-HCV seropositivity (35). Similarly with this study none of these risk factors were significantly associated with HCV seropositivity in both diabetic and non-diabetic controls (38).

In the present study, history of blood transfusion (AOR=3.94, 95%CI; 1.29:12.00), unprotected sex (AOR=3.79, 95%CI; 1.33:10.79) and circumcision (AOR=4.84, 95%CI; 1.30:17.97) were HBsAg sero-positivity predictors. Only history of invasive procedures and chronic liver disease showed association with HBsAg seropositivity Ethiopia, 2014 (39). Duration of diabetes mellitus, poor diabetic regulation, and insulin treatment usage were found to relate to HBsAg (35).

8. STRENGTH AND LIMITATIONS OF THE STUDY

The study adds to the limited data on the subject available in this region and will help in increasing awareness regarding association of HCV and HBV with diabetes, thereby will help in reducing morbidity and cost associated with this comorbidity in the long run. The study even more important as double burden, HBV and HCV infections among diabetes and control groups, was determined. This study was, however, limited by our inability to assay RNA in those positive for HCV antibody.

9. CONCLUSION

The burden of sero-positivity against anti- HCV antibody in diabetes and controls groups was found to be 23/305(7.5%) and 7/305(2.3%), respectively and the difference was statistically significant. There is a significant association between Hepatitis C virus infection and diabetes in the region. Except the type of diabetes, the lack of any significant epidemiological factor for HCV infection in our diabetic population suggests that HCV may have a direct role in the development of diabetes. The burden of HBV infection in diabetes and control groups were 26/305(8.5%) and 14/305(4.6%), respectively and the difference was not statistically significant. History of blood transfusion and unprotected sex were significantly associated with HBV infection.

10. RECOMMENDATIONS

HBV and HCV infection continuous as a major health problem in patients with diabetes. Therefore, the following recommendations were forwarded:

- All adult patients diagnosed of diabetes would be considered for screening for HBV and HCV virus with indication of potential associated factors.
- Vaccination against HBV is helpful in the prevention of HBV infection, and is recommended for all adults who are at increased risk for infection particularly diabetes patients as they are immuno-compromised and have frequent medical interventions.
- Awareness of patients with diabetes on modes of transmission of the hepatitis B and hepatitis C viruses is central to prevent viral infection.
- It remains to be seen whether diabetes was a risk factor for the HCV infection or vice versa. Therefore prospective, multicenter studies are needed to establish temporal association, elucidate the reasons of association as well as the mechanism and determination of other aspects of the relationships.

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ANNEX I: PARTICIPANT INFORMATION SHEET

My name is Yihenew Million. I am a medical laboratory technologist and postgraduate student at Addis Ababa University. Now I am conducting a study entitled burden of HBV and HCV infections among diabetic patients at Gondar referral teaching hospital, North Gondar Ethiopia. You are invited to participate in this study. Please read the following statements and ask any unclear points before you agree to participate. If you agree to be included in this study, I would like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical specimen.

The topic of this study is burden of HBV and HCV infections among diabetic patients at Gondar referral teaching hospital, North Gondar Ethiopia. Since HBC and HCV is one of the major health problems in our country, the result of the study can be helpful in planning and intervention of the diseases. Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw at any time, there will be no consequences and you will get all the services provided in the hospital with no problem. If you decide to participate, you have to sign on the permission template form and you may obtain a copy of this information sheet.

Expected from participants

As a participant of this study, you are expected to give blood. Being asked to give sample does not necessarily mean that you have the disease. When you are found to be positive for the micro-organism, you will be informed by the health worker and receive proper treatment. You need to know that your results might be discussed with other appropriate individual out of this hospital. But your name and address will not be disclosed rather an identification code will be used in such conditions.

Time required for participating

You will spend 10-15 minutes until the specimen is collected and permission form is signed.

Risks of participant

Specimen collection will have no effect and you will not get any risk as the sample will be collected by well trained professionals. But you may fill minor temporary pain during sample collection.

Confidentiality

The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional will have access the information. The information will be encoded in a computer and saved with password protection.

Benefits of participation

By participating, you will get no financial benefits. Even though there is no direct benefit due to participation in this study, the findings of the study is useful for better understanding of the problems of HBV and HCV among DM patients. You will also obtain all the results of the analysis for free and communicated to your physician for the appropriate management.

Rights of participants

Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Communication

In case if you have any questions, unclear ideas and doubt about the project, contact addresses are:
Investigator: Yihenew Million (BSc), +251918224345

Email- millionbeza@gmail.com

Advisor: Kassu Desta (BSc, MSc, PhD fellow), +251911107099

For additional information, please contact Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences at: Telephone +251112755170

Your signature below indicates that you have read /or listened, and understand the information provided for you about the study. Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have question.   I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Name of the participant: ------------------------------Signature: ----------- Date: ----------------------

ለጥናቱ መረጃና ተሳታፊነት መግለጫ ቅጽ

የጥናቱ ዓላማ

ሄፓታይተስ “ቢ” እና ሄፓታይተስ “ሲ”ቫይረሶች በስኳር ህሙማን መካከል ያለዉን ስርጭት ለማጥናት የታቀደነዉ፡፡

በጥናቱ ስለመሳተፍ

በዚህ ጥናት መሳተፍ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ ስለሆነም የስኳር ህሙማን በጥናቱ እንዲሳተፉ ፈቃደኛነትዎን እንጠይቃለን፡፡ለመሳተፍ ከፈቀዱ፤5ሚሊሊትር የደም ናሙና ከክንድዎ ተወስዶ የላቦራቶሪ ምርመራ ይደረግለዎታል፡፡ የላቦራቶሪ ምርመራውም ሄፓታይተስ “ቢ ”እና “ሲ ” ቫይረስን በደምዎ ዉስጥ መኖርና አለመኖሩን ማረጋገጥ ይሆናል፡፡ደም ከመወሰዱ በፊት እና ከውጤት በሁዋላ በሰለጠነ ባለሙያ የምክር አገልግሎት ያገኛሉ፡፡የደም ናሙናዉም የሚወሰደዉ ንጽህናዉ በተጠበቀ አዲስ እና በታሸገ መርፌ እና ስሪንጅ ነዉ፡፡

በጥናቱ ሊከሰቱ የሚችሉ ተያያዥ ችግሮች

አምስት ሚሊ ሊትር የደም ናሙናዉን ለመዉሰድ መርፌ ሲገባ ከሚፈጥረዉ የቅጽበት ህመም ስሜት በስተቀር የጎላ ችግር አያመጣም ነገር ግን ምቾት ካልተሰማዎት ሀኪም እንዲያይልዎት ይደረጋል፡፡

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

የደም ናሙና የላብራቶሪ ዉጤት ምንም አይነት ችግር ካሳየ የመድሃኒት ትእዛዝና የባለሙያ ምክር ይሰጠዎታል፡፡

የጥናቱ መረጃዎች ሚስጥራዊነት

በጥናቱ ውስጥ የተሰበሰቡ ማናቸዉም ግላዊ መረጃዎች ሚስጥራዊነታቸዉ የተጠበቀ ይሆናል፡፡ከማንነትዎ ጋር በቀጥታ ተያያዥነት ያላቸዉ መረጃዎች በሙሉ በዋና ተመራማሪዉ ሚስጥራዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኃላ ለምርምር ሂደቱ ብቻ የሚዉሉ ይሆናሉ፡፡

የጥናቱን ዉጤት ስለማሳወቅ

የዚህ ጥናት ዉጤት በተለያዩ የህትመት ውጤቶች የሚቀርብ ሲሆን ይህ ከማንነትዎ ጋር የተያያዘ ምንም አይነት መረጃን አያካትትም፡፡ስለዚህም የጥናቱን ዉጤት በሪፖርት እናቀርበዉ ዘንድ ፈቃዶን እነጠይቃለን፡፡

ከጥናቱ ስለመዉጣትና ስለማቋረጥ

ይህ ጥናት በፈቀደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማንኛዉም ወቅት በፈቃድዎ ከጥናቱ መውጣት ይችላሉ፡፡ከጥናቱ ቢወጡም እንኳን የተለመደዉን የህክምና እርዳታ በጤና ተቋሙ ዉስጥ በማንኛዉም ጊዜ የማግኘት መብት አለዎት፡፡

ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ፡፡ ይኸነዉ ሚሊዮን ሞባይል፤ +251918224345 ኢ-ሜይል፤ millionbez@gmail.com

ለተጨማሪ መረጃ፡ አዲስ አበባ ዩኒቨርሲቲ ፤የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ፡፡ ስልክ+251112755170

ANNEX II: CONSENT FORM

Consent form

Burden of HBV and HCV infections among diabetic patients at Gondar referral teaching hospital, Northwest Ethiopia.

I, ____ request and give consent to involve in the research study "Burden of HBV and HCV among diabetic patients" at Gondar referral teaching hospital, Northwest Ethiopia. I acknowledge that the benefits and risks of the research project and alternatives to participation, especially as far as they affect

--- Have been fully explained to me by ----------------- -------------------------------------- and my consent is given voluntarily.

I have understood and I am satisfied with the explanations that I have been given.

I have been provided with a written information sheet.

I understand that my involvement in this research study and/or the procedure(s) may not be of any direct benefit to me and that I may withdraw my consent at any stage without affecting my rights to future medical treatment.

Name of study participant: ---

Signature: --- Date ---------------------------------------

ስለ ስምምነቱ ማረጋገጫ ፊርማ

እኔ ስሜ ከታች የተገለፀዉ የጥናቱ ተሳታፊ ለመሆን ስወስን የጥናቱን አላማዎች አሰራሮችና ቅድመ ሁኔታዎች በግልጽ በመረዳትና ከጥናቱ ተሳታፊነት ፈቃደኛነቴን በማንኛዉም ደረጃ የማንሳት መብቴን በማረጋጥ ነዉ፡፡

እኔ-- በጥናቱ ተሳታፊ መሆኔን በፊርማዬ እያረጋገጥሁ ይህንን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎች በሚገባ የተረዳሁና ከጥናቱ በማንኛዉም ደረጃ ለመሰረዝ ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እነደማይነፈጉኝ በማመን ነዉ፡፡ እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑንን በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊው ሙሉ ስም---ፊርማ-----------------

የተመራማሪዉ ሙሉ ስም --- ፊርማ-----------------

የምስክር ሙሉ ስም --- ፊርማ----------------

ANNEX III: QUESTIONNAIRE

Addis Ababa University

Faculty of Medicine

Department of Medical laboratory Sciences

For data collectors: For each question please encircle the answer.

If you make a mistake; simply cross out the mistake and encircle the correct choice.

Identification number: ------------------------ Date of data collection-----------------------

1. Socio-demographic information			
Code	Question	Answer	remark
101	Sex	1. Male 2. Female	
102	Age	--------------------------years	
103	Residence	1. Urban 2. Rural	
104	Current occupational status	1 Self-employed 4. Student 2. Driver 5. Farmer 3. House Wife 6. Other specify _________	
105	Religion	1. Christian 3. doesn't have 2. Muslim 4. others specify _________	
106	Marital status	1. Married 3. divorced 2. single 4, widowed	
107	Ethnicity	1. Oromo 4. Gurage 2. Amhara 5. Wolayta 3. Tigray 6. others specify ____________	
108	Educational Status	1. Illiterate 4. Grade 9-10 2. Read and write 5. Grade 11-12 3. Grade 1-8 6. College/university	

3. Hepatitis B and C related risk factors Have you ever practiced the following?			
code	Question	Answer	
200	History of STD/STI	1. Yes 2. No	

201	Multiple sexual partner	1. Yes 2. No	
202	Blood transfusion	1. Yes 2. No	
203	Abortion	1. Yes 2. No	
204	Dental extraction at health facility	1. Yes 2. No	
205	Circumcision	1. Yes 2. No	
206	Hospital admission	1. Yes 2. No	
207	Surgical procedure	1. Yes 2. No	
208	Venous or body piercing for treatment	1. Yes 2. No	
209	Delivery by TBA	1. Yes 2. No	
210	Ear piercing	1. Yes 2. No	
211	Nose piercing	1. Yes 2. No	
212	Uvuloctomy	1. Yes 2. No	
213	Tattooing on body	1. Yes 2. No	
214	Tattooing on gum	1. Yes 2. No	
215	Shaving	1. Yes 2. No	
216	Contact with jaundiced patient	1. Yes 2. No	
217	Frequent alcohol consumption	1. Yes 2. No	
218	Unprotected sex	1. Yes 2. No	
2.	Diabetic variables		
300	Type of diabetes	1. T1DM 2 T2DM.	
301	Any confirmed diseases other than diabetes?	1. Yes 2. No	
302	Duration of disease	specify in year --------	
303	Treatment type	1. Oral 2. Injection	
304	Late diabetic complications	1. Yes 2. No	

305	Do you have glucometer (point of care)	1. Yes 2. No	
306	If Q 305 is Yes how often you use it	specify in number ------	
307	If Q 305 is Yes do you use a lancet for more than one	1. Yes 2. No	
307	Follow-up for glucose monitoring in health center/clinic/hospital	1. Yes 2. No	
308	if Q 307 is Yes how often	specify in number -------	
309	History of ulcers/blisters	1. Yes 2. No	
310	if Q 309 is Yes how long it lasts	specify in number -------	

THANK YOU!!!

ID.no ----------------------	Laboratory result
HBsAg	1. Positive 2. negative
Anti-HCV	1. positive 2. Negative

አዲስ አበባ ዩኒቨርስቲ

የህክምና ፋኩልቲ

የላቦራቶትሪ ትምህርት ክፍል

ለመረጃ ሰብሳቢዎች፤ጥያቄዉን ከጠየቃችሁ በኋላ መልሱን ከተሰጡት አማራጮች ያክብቡ፡፡

1. መለያ ቁጥር________________መረጃው የተሰበሰበበት ቀን__________________________

1. የማህበራዊና ስነ-ህዝብ ሁኔታ የሚዳስሱ ጥያቄዎች			
ኮድ	ጥያቄ	መልስ	ምርምራ
101	ጾታ	1. ወንድ 2. ሴት	
102	እድሜ	---------------------------ዓመት	
103	መኖሪያ አካባቢ	1. ገጠር 2. ከተማ	
104	ስራ	1 የግል 4. ተማሪ 2. ሹፌር 5. ገበሬተማሪ 3. የቤትእመቤት 6. ሌላ ይገለጽ _______________________	
105	ሃይማኖት	1. ክርስቲያን 3. የለም 2. ሙስሊም 4. ሌላ ይገለጽ_________	
106	የጋብቻ ሁኔታ	1. ያገባ /ች 3. የፈታ/ች 2. ያላገባች/ች 4, የሞተበት/ባት	
107	ብሔር	1. ኦሮሞ 4. ጉራጌ 2. አማራ 5. ወላይታ 3. ትግሬ 6. ሌላ (ይገለጽ) ---------	
108	የትምህርት ሁኔታ	1. ማንበብ እና መፃፍ የማይችል 4. ከ9ኛ-10ኛ ክፍል . 2. ማንበብና መፃፍ ብቻ የሚችል 5. 11ኛ-12ኛ ክፍል 3. ከ1ኛ-8ኛ ክፍል 6. ኮሌጅ/ዩኒቨርሲቲ	

2.ሄፓታይተስ "ቢ " እና "ሲ "ን በተመለከተ ጥያቄዎች ከዚህ በታች ያሉት በህይወትዎ አድርገው/ ተከስቶብዎ ያውቃሉ?			
ኮድ	ጥያቄ	መልስ	
200	ያባለዘር በሽታ	1. አዎ 2. አይደልም	
201	ከአንድ በላይ የትዳር ጓደኛ	1. አዎ 2. አይደልም	
202	ደም መቀበል	1. አዎ 2. አይደልም	
203	ማስወረድ	1. አዎ 2. አይደልም	

204	ጤና ድርጅት ጥርስ ማስነቀል	1. አዎ 2. አይደልም	
205	ግርዛት	1. አዎ 2. አይደልም	
206	ሆስፒታል መተኛት	1. አዎ 2. አይደልም	
207	ማንኛዉም አይነት ቀዶ ጥገና	1. አዎ 2. አይደልም	
208	ሞኝባገኝ መቆረጥ/መወጋት	1. አዎ 2. አይደልም	
209	በልምድ አዋላጅ መዉለድ	1. አዎ 2. አይደልም	
210	ጆሮ መበሳት	1. አዎ 2. አይደልም	
211	አፍንጫ መበሳት	1. አዎ 2. አይደልም	
212	እንጥል ማስቆረጥ	1. አዎ 2. አይደልም	
213	ሰዉነት መነቀስ	1. አዎ 2. አይደልም	
214	ድድ መነቀስ	1. አዎ 2. አይደልም	
215	ፀጉር ቤት ፂም መላጨት	1. አዎ 2. አይደልም	
216	የወፍ በሽታ ከያዘዉ ሰዉ ጋር ንክኪ	1. አዎ 2. አይደልም	
217	አዘውትሮ መጠጥ መጠጣት	1. አዎ 2. አይደልም	
218	ልቅ የሆነ ግብረ ሥጋ- ግንኙነት	1. አዎ 2. አይደልም	
3. የስኳር በሽታ ህመም ሁኔታ			
300	የስኳር በሽታ አይነት?	1. T1DM 2. T2DM	
301	ከስኳር በሽታዉ በተጨማሪ ሌላ የታወቀ በሽታ አለብዎት?	1. አዎ(ይገለጽ----------------------- 2. የለም	
302	በሽታው ከያዘወት ምን ያህል ጊዜ ሆነዎት?	--------------(በቁጥር ያስቀምጡ)	
303	መድሀኒት የሚወስዱበት መንገድ	1. በመዋጥ 2. በመርፌ	
304	በበሽታው መባባስ ምክንያት ከፍተኛ የጤና እክል ተከስቷል?	1. አዎ 2. አይደልም	
305	የስኳር መጠን መለኪያ በቤት ውስጥ ተጠቅመው ያውቃሉ ?	1. አዎ 2. አይደልም	
306	ለ ጥያቄ ቁ. 305 መልስዎ አዎ ከሆነ ከመቸ ጀምሮ እና በምን ያህል ጊዜ ይጠቀማሉ?	--------------(በቁጥር ያስቀምጡ)	
307	ለ ጥያቄ ቁ. 305 መልስዎ አዎ ከሆነ ስለት(lancet) ከአንድ ጊዜ በላይ ተጠቅመዋል፡፡	1. አዎ 2. አይደልም	
307	በጤና ተቋም የስኳር መጠን ምርመራ አድርገው ያውቃሉ?	1. አዎ 2. አይደልም	
308	ለ ጥያቄ ቁ.307 መልስዎ አዎ ከሆነ ከመቸ ጀምሮ እና በምን ያህል ጊዜ ይጠቀማሉ?	--------------(በቁጥር ያስቀምጡ)	
309	በስኳር ህመም ምክንያት በእግር ጫማ ላያ ቁስለት ተከስቶ ያውቃል?	1. አዎ 2. አይደልም	
310	ለ ጥያቄ ቁ. 309 መልስዎ አዎ ከሆነ ለምን ያህል ጊዜ?	--------------(በቁጥር ያስቀምጡ)	

ANNEX IV: LABORATORY METHOD

1. HBsAg EIA TEST

INTRODUCTION

HBsAg ELISA is used for the qualitative determination of Hepatitis B surface antigen (HBsAg in human serum or plasma. This test is indicated for the screening of blood and blood products to be used for transfusion and an aid for the diagnosis of existing or previous hepatitis B infection.

HBsAg is one of the earliest markers that appear in the blood following infection with Hepatitis B virus (HBV).

Hepatitis B surface antigen (HBsAg) appears 1-7 weeks before biochemical evidence of liver disease or jaundice. Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, the HBsAg persists for long periods (6-12 months) with no sero-conversion to the corresponding antibodies. Therefore, screening for HBsAg is highly desirable for all donors, pregnant women and people in high-risk groups.

PRINCIPLE OF THE TEST

The HBsAg EIA is a solid-phase simultaneous sandwich immunoassay, which employs monoclonal antibodies and polyclonal antibodies specific for HBsAg. Microtiter well is coated with monoclonal antibodies specific for HBsAg. A serum specimen is added to the antibody coated Microtiter wells together with enzyme conjugated polyclonal antibodies. HBsAg, if present, will form an antibody-HBsAg-antibody-enzyme complex. The plate is then washed to remove unbound material. Finally, a solution of substrate is added to the wells and incubated. A blue color will develop in proportion to the amount of HBsAg present in the specimen. The enzyme-substrate reaction can be stopped and the result is visualized by naked eye or read by EIA plate reader for absorbance at the wavelength of 450 nm.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Plasma samples containing EDTA, heparin or oxalate may interfere with test procedures and should be avoided. Specimen with extensive particulate should be clarified by centrifugation prior

to use. Covered specimens may be stored for up to 48 hours at 2°-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for mix prior to testing. Avoid repeated freeze thaw. At least, two wells of negative and positive controls each should be run in every assay.

PRECAUTIONS

1. Caution: Some components of this kit contain human serum. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.

2. Wear disposable gloves while handling kit reagents and specimens and thoroughly wash hands afterwards.

3. Dispose off all specimens and materials used to perform the test as if they contained infectious agents.

4. Do not mix reagents from kits with different lot numbers.

5. Cross contamination between reagents will invalidate the test results.

6. All reagents and components except the conjugate must be equilibrated at room temperature prior to use.

STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2°-8°C upon receipt. Micro titer plate, once opened, should be kept in a sealed bag with desiccants to minimize exposure to damp air. To remove the required number of strips from the micro titer plates, bring the sealed pouches to room temperature first and then open the pouches.

This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. Opened test kits will remain stable until the expiration date shown in 4°C, provided it is stored as described above. A micro titer plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

MATERIALS, EQUIPMENT AND KITS

Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.

Disposable pipette tips.

Distilled water.

Humidified Box capable of maintaining 37°C

Absorbent paper or paper towel.

Microtiter plate or strip-well washer

Microtiter plate reader.

Specimen Diluent: 12 ml chemically defined solution containing proteins,

Tween 20 and sodium azide in phosphate buffer

Positive Control: 1.0ml

Negative Control: 1.0ml

Enzyme Conjugate: 12 ml Goat anti-human-IgG HRP Conjugate.

Substrate Solution A: 6 ml HRP Substrate.

Substrate Solution B: 6 ml TMB.

Concentration Washing Solution (20X): 40ml phosphate buffered saline solution with Tween 20. The buffer should be diluted 20 times with distilled water before use.

Stop Solution: 6 ml 2N sulfuric Acid.

HBV

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.

6. Microtiter plate or strip-well washer

7. Microtiter plate reader.

Materials provided with the kits:

1. Microtiter Well: 8x12 or 12x8, coated with monoclonal anti-HBs antibody

2. Negative Control: 0.5ml HBsAg negative serum.

3. Positive Control: 0.5ml HBsAg positive serum.

4. Enzyme Conjugate: 6 ml, Goat anti-HBsAg-HRP

5. Wash Buffer Concentrate (20x): 25 ml, the buffer should be diluted 20 times with distilled water before use.

6. Substrate Solution A: 6 ml Urea Peroxide

7. Substrate Solution B: 6 ml TMB solution

8. Stop Solution: 6 ml 2N Sulfuric Acid

WORKING REAGENT PREPARATION, STORAGE AND STABILITY

No reagent preparation is required except for wash buffer, which is supplied as a 20 X concentrate.

WORKING WASH BUFFER

Dilute the 20X wash buffer concentrate with deionized or distilled water 1:20. For example, 5 ml of wash buffer concentrate should be diluted to a total volume of 100 mL with deionized or distilled water.

STABILITY OF OPENED KIT COMPONENTS AND DILUTED REAGENTS

The diluted wash buffer is stable for at least one week when stored at room temperature. Substrate is stable for the expiration date of the kit. The micro titer plates should be opened after they have been kept at room temperature for 20-30 minutes. After removing the required number of strips, the plates should be resealed in the foil pouch bags along with the desiccant and stored at 2°-8°C. Exposure of HBsAg plates to humidity drastically reduces the shelf life.

PROCEDURE

It is strongly advised to analyze each specimen and controls in duplicate. All the reagents should equilibrate to room temperature before use.

1. Dispense one drop (50 ul) of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control, and 50ul of serum or plasma samples into respective test wells

2. Add one drop (50 ul) of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 minute. Do not add Enzyme Conjugate to the blank well.

3. Place the microtiter plates into a humidified box, and incubate at 37°C for 30 minutes.

4. Wash each well 4 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.

5. Add one drop (50 ul) of Substrate Solution A (HRP-substrate) to each well, then add one drop (50 ul) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 15 minutes. .

6. Add 1 drop (50ul) of Stop Solution to each well to stop the color reaction.Read O.D. at 450 nm with an EIA plate reader.

INTERPRETATION OF RESULTS

A. Calculate OD ratio

Specimen OD ratio = OD Value of test sample/Average OD Value of Negative Control

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

B. Interpretations

Specimen OD ratio

Negative < 2.1

Positive ≥ 2.1

The negative result indicates that there is no detectable HBsAg in the specimen.

The specimen with a positive result should be tested duplicate again and confirmed with Western blot or other tests.

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells, The positive control OD value should be ≥ 0.8, the negative control should be ≤ 0.10):

Cut-off Calculations:

Take average OD values of Negative control and add 0.15:

1x NC + 0.15 = Cut-off.

Positive OD reading: ≥Cut-off value

Negative OD reading: < Cut-off value.

2. **HCV ANTIBODY EIA**

Hepatitis C virus is a single stranded RNA virus with some structural relations to the flavivirus family. Nucleic acid sequences of HCV cDNA clones provided the basis for the construction of recombinant peptides representing putative hepatitis C virus proteins.4,5 Antihepatitis C virus antibody screening of blood using synthetic or recombinant proteins, helped to identify apparently healthy blood donors with anti-HCV antibodies who otherwise might have transmitted the virus.

This is an enzyme linked immunosorbent assay using recombinant proteins derived from core regions of HCV virus to detect the presence of HCV antibodies in human sera.

PRINCIPLE

Multiple epitopes of HCV proteins (Core, NS3, NS4 and NS5) are bound to the microtiter wells. When antibodies to HCV are present in the test sample, they react with recombinant proteins and attach to the solid-phase. Non-reactive antibodies are removed with the wash buffer. Human IgGs bound to the antigen are reacted with goat-anti-human IgG peroxidase conjugate and visualized by subsequent reactions with a chromogenic substrate. Positive sample generates a medium to dark blue color. No color or very pale blue color indicates a negative reaction. The intensity of the reaction is photo metrically quantitated.

PRECAUTION FOR USERS

All human source material used in the preparation of this product was found to be negative for the presence of HIV-1/HIV-2 antibodies, as well as for the hepatitis B surface antigen, using a commercial licensed method. Nevertheless, because no test method can offer complete assurance of the absence of infectious agents, this product should be handled with caution.

1. Avoid contact of reagents with the eyes and skin. If that occurs, wash thoroughly with water.
2. Wear gloves.
3. Do not pipette by mouth.
4. Do not smoke.
5. Dispose all used materials in a suitable biohazardous waste container.

Remains of samples, controls, aspirated reagents and pipette tips should be collected in a container for this purpose and autoclaved 1-hour at 121°C or treated with 10% sodium hypochlorite (final concentration) for 30 min before disposal. (Remains containing acid must be neutralised prior addition of sodium hypochlorite).

6. Adjust washer to the plate used (flat bottom) in order to wash properly.
7. Do not mix reagents from different lots.
8. Do not use reagents after expiration date.
9. Extreme care should be taken to avoid microbial contamination and cross contamination of reagents.
10. Use a new pipette tip for each specimen and each reagent.
11. Soaps and/or oxidizing agents remaining in containers used for the substrate-TMB solution can interfere with the reaction.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should

be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

PROCEDURE

1. Dispense 100µl of specimen diluent into individual test wells.

2. Dispense 100µl positive control and negative control duplicate into individual wells.

3. Add 10µl of each test sample into duplicate test wells; vortex to mix.

4. Incubate for 30 minutes at 37°C

5. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.

6. Add 100µl of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 minute. Do not add Enzyme Conjugate to the blank well.

7. Incubate for 20 minutes at 37°C

8. Wash the plate 5 times as step 6.

9. Add one drop (50µl) of Substrate Solution A (HRP-substrate) to each well, then add one drop (50µl) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 minutes. .

10. Add one drop (50µl) of Stop Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA reader (59).

RESULT INTERPRETATION

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells, The positive control OD value should be ≥0.8, the negative control should be ≤0.10):

Cut-off Calculations:

Take average OD values of Negative control and add 0.15:

1x NC + 0.15 = Cut-off.

Positive OD reading: ≥Cut-off value

Negative OD reading :< Cut-off value (53)

LIMITATIONS

1. As the other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of entire content of wells completely before adding the washing solution.

2. As with all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by physician is needed for a final diagnosis.

3. Samples with positive or equivocal result must be reanalyzed in duplicate. If both retest values are lower than the cut-off, the final interpretation of the test is negative for HCV antibodies. If the result is repeatedly positive or equivocal, the sample should be further investigated with other methods.

4. Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.

5. A negative result does not exclude the possibility of exposure or infection With HCV

GLUCOSE – GLUCOSE OXIDASE METHOD

Introduction

Glucose is a reducing monosaccharide that serves as the principal fuel of all the tissues. It enters the cell through the influence of insulin and undergoes a series of chemical reactions to produce energy. Lack of insulin or resistance to its action at the cellular level causes diabetes. Therefore, in diabetes mellitus the blood glucose levels are very high. Some patients with very high blood glucose levels may develop metabolic acidosis and ketosis caused by the increased fat metabolism, the alternate source for energy. Hyperglycaemia is also noted in gestational diabetes of pregnancy and may be found in pancreatic disease, pituitary and adrenal disorders. A decreased level of blood glucose, hypoglycaemia is often associated with starvation, hyper-insulinaemia and in those who are taking high insulin dose for therapy.

Principle of the method

Glucose present in the plasma is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD). 4 aminophenazone, an oxygen acceptor, takes up the oxygen and together with phenol forms a pink coloured chromogen which can be measured at 515nm.

Specimen type, collection and storage

Plasma is the specimen of choice for glucose estimation. Plasma glucose levels have been checked to be quite stable for 6 hours at room temperature (25 -35$^{0}{}_{c}$). It is important that plasma should be separated from the cells soon after collection, preferably within 1 hour. About 2 ml of the patient's blood should be collected by venipuncture into a tube containing a mixture of potassium ethylene diaminetetraacetate (EDTA) sodium fluoride at a ratio 1:2 (W/W).

Reagents

Phosphate Buffer 100 mmol/L. pH 7.0, Colour Reagent (4 amino phenazone), Benzoic acid 1g/l, Stock glucose solution, 1 g/l, Working glucose standard 100 mg/dl.

ANNEX V: DECLARATION

I hereby declare that this MSc thesis is for the partial fulfillment of the requirement for master's degree in Clinical Laboratory Sciences (Diagnostic and public health microbiology specialty track) on the title of **"Hepatitis B and Hepatitis C Viral Infections and Their Associated Factors among Diabetes Patients Attending University of Teaching Hospital, Northwest Ethiopia".** This thesis is my real original work and it has not been previously formed the basis for the award of any Degree, Diploma of a University or other Institution of higher learning.

STUDENT'S NAME SIGNATURE DATE

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NAME OF ADVISOR 1 SIGNATURE DATE

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NAME OF ADVISOR 2 SIGNATURE DATE

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