PREVALENCE AND PATTERN OF HEPATITIS C AMONG TYPE 2 DIABETIC PATIENTS ON FOLLOW UP AT JIMMA MEDICAL CENTER, JIMMA: A FACILITY-BASED CROSS-SECTIONAL STUDY JIMMA, SOUTHWESTERN ETHIOPIA

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JIMMA UNIVERSITY INSTITUTE OF HEALTH, FACULTY OF MEDICINE,

DEPARTMENT OF INTERNAL MEDICINE

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ABSTRACT

Background: Hepatitis C virus infection is an important risk factor for the development of diabetes mellitus. A number of studies have shown prevalence of HCV infection is higher in patients with type 2 diabetes. Although there are increasing numbers of literatures on the seroprevalence of hepatitis C virus on type 2 diabetes mellitus patients, the studies are limited in Ethiopia including Jimma medical center (JMC)

Objective: To assess the prevalence and pattern of HCV infection in type2 DM at JMC

Method: Institutional based cross sectional study was conducted in JMC from 15/3 /2014 to 15/5/2014 E.C. A total 220 patients having type 2 were included. Data were collected by 3 trained nurses using structured questionnaire prepared for interview. Blood specimen was collected and serum serum was separated to determine 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 26. Based on the result, conclusions and recommendations were made.

Result: Hundred- forty- eight (148) type 2 diabetic patients were males while seventy-two (72) were females. Their mean age was 53.65 ± 10.13 years and median duration of diabetes was 5years. One subject tested positive for HCV infection.

Conclusion: There is a low prevalence of anti- HCV antibodies among type 2 diabetic patients.

Key words: hepatitis C virus, prevalence type 2 diabetes, Jimma, Ethiopia

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ABBREVIATIONS

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

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

JMC…….. Jimma Medical Center

ADA----------American Diabetes Association

BMI----------Body Mass Index

DM----------Diabetes Mellitus

DCCT----------Diabetes control and complication trail

HbA1C----------Glycated Adult hemoglobin

HTN----------Hypertension

OHA----------Oral Hypoglycemic Agent

SPSS----------Statistical Package for Social Science

CHAPTER 1: INTRODUCTION

1.1.1 Background

Hepatitis C virus (HCV) infections is common diseases of the of the world, infecting an estimated 185 million people, and three to four million are newly infected each year(1)

HCV are hepatotropic virus whose primary replication occurs in the liver. This infections has a high rate of development of liver cirrhosis and can cause serious mortality, raising a major concern for global health

HCV is an enveloped, single-stranded, positive sense RNA virus belonging to the Hepacivirus genus within the Flaviviridae family. Its genome is a positive, single strand RNA molecule which includes two untranslated regions(2)

History of blood transfusion, tattooing, intravenous drug abuse, hemodia lysis, abortion, non-disposable needle exposure, and frequent dental procedures are all common routes for contracting HCV infection

Infection with HCV has been shown to produce both hepatic and extrahepatic manifestations, the latter including insulin resistance, essential mixed cryoglobulinemia, and glomerulonephritis. The mechanisms through which these develop include those which are immunological, in which the chronic persistence of virus leads to the circulation of immune complexes (mixed cryoglobulinemia) and other autoimmune phenomena, and those which are virological and related to the extrahepatic tropism of the virus to other tissues.(3)

HCV infection is of epidemic proportions worldwide. Countries with high rates of chronic infection are Egypt (22%), Pakistan (4.8%), and China (3.2%)(4). . In Ethiopia, the prevalence of ant-HCV-Ab in chronic 27 (22.5%) (5)

Chronic hepatitis C is a systemic disease inducing metabolic alterations leading to extrahepatic con-sequences. In particular, hepatitis C virus (HCV) infection seems to increase the risk of incident type 2 diabetes mellitus in predisposed individuals, independently of liver disease stage.(6)

Diabetes mellitus type 2 (DM2) patients have higher risk to be infected with parenterally

Transmitted viruses like hepatitis C virus and high prevalence HCV was found in DM2 patients.

Patients with diabetes mellitus are predisposed to develop a spectrum of liver diseases which includes fatty liver, steatohepatitis, and fibrosis to cirrhosis and hepatocellular carcinoma(7)

A range of extrahepatic (EH) manifestations such as arthralgia's, thyroiditis and die- bets are linked with HCV infections. Studies have shown that patients infected with hepatitis C virus (HCV) have more glucose intolerance than the general population.(8)

Retrospective and cohort study done in China, Hospital of Sichuan University from January 2008 to January 2017(n=1099) showed patients with HCV has higher prevalence of insulin resistant(9)

Cross- sectional and clinical studies shows that there is a high prevalence of anti-HCV anti-body in T2DM patients

1.1.2 Statement of the problem

Both diabetes and hepatitis C virus (HCV) infection are severe health problems worldwide, especially in the developing countries.

Type 2 diabetes mellitus (DM) is a chronic metabolic disorder in which prevalence has been increasing steadily all over the world. it is fast becoming an epidemic in some countries of the world with the number of people affected expected to double in the next decade due to increase in ageing population, thereby adding to the already existing burden for healthcare providers, especially in poorly developed countries(10)

Hepatitis C virus (HCV) infection is associated with increased rates of glucose abnormalities, including diabetes mellitus and insulin resistance. The presence of glucose abnormalities in HCV infected patients, including diabetes mellitus and insulin resistance, is associated with negative liver-related outcomes(11)

Molecular mechanisms provide explanations for which HCV infection might increase the risk for development of T2DM or worsen glycemic control in patients with established T2DM(12)

Globally, the number of people with diabetes mellitus has quadrupled in the past three decades, and diabetes mellitus is the ninth major cause of death. About 1 in 11 adults worldwide now have diabetes mellitus, 90% of whom have type 2 diabetes mellitus (T2DM) .The new estimates of diabetes prevalence, deaths attributable to diabetes and healthcare expenditure due to diabetes present a large social, financial and health system burden across the world(13)

In 2017, approximately 5 million deaths worldwide were attributable to diabetes in the 20–99 years age range. The global healthcare expenditure on people with diabetes was estimated to be USD 850 billion in 2017(13)

HCV infections were associated with diabetes prevalence and risk of incident diabetes. Studies add evidence suggesting that diabetes is an additional metabolic complication of HCV infection.(13)

During hepatitis C virus (HCV) chronic infection, extrahepatic manifestations are frequent and polymorphous .Beyond the risk of developing liver complications, that is, cirrhosis and liver cancer, patients with HCV infection have an increased risk of morbidity and mortality related to non-liver diseases(14)

Eradication of HCV is associated with improved glycemic control in patients with diabetes as evidenced by decreased mean HbA1c and decreased insulin use. (15)

CHC is associated with a four-fold increased risk of insulin resistance and type 2 diabetes mellitus. Reduced insulin resistance, with improved insulin sensitivity seen at 12 weeks and 24 weeks, and at end of therapy with antiviral(3)

A high prevalence of HCV infection was detected in diabetic patients, and most of anti-HCV positive patients presented with abnormal LFTs(16)

In 2016, the viral hepatitis elimination strategy for Africa aims at 30% reduction in new cases of chronic viral hepatitis C infections and 10% reduction in viral hepatitis C–related deaths by 2020 through country ownership, partnership and multisectoral cooperation, universal health coverage, integration, and a public health approach based on simplified and standardized interventions.

Many of the challenges faced are unique to sub-Saharan Africa and the development of strategies is complicated by a scarcity of good data from countries and regions within sub-Saharan Africa.(4)

The seroprevalence of hepatitis C virus is significantly high in diabetic subjects, particularly type 2 diabetics, it is suggested systematic screening for this infection in any diabetic patient.(17)

The pooled prevalence of anti-HCV in Ethiopia was high. Strengthening the scope of existing prevention and control programs and implementing novel approaches, including screen-and-treat, could significantly help to tackle this critical public health issue(18)

Various studies have shown high HCV seropositivity among patients with T2DM as compared to the control group(3,7,13) .Although there are Series of studies found that prevalence of HCV infection is higher in patients with diabetes than in those without diabetes ,there are few of literatures in Ethiopia, as far as my knowledge was concerned there was no study done particularly in study site, so this study is therefore aimed to determine the prevalence and associated factors of HCV and in type 2 diabetic patients at JUMC

1.1.3 Significance of the study

The ever-increasing global hepatitis C infection is fueling the burden of diabetes mellitus, which exaggerates various complications and may be a cause of morbidity and mortality millions particularly developing country.

Several studies have reported that hepatitis C virus infection is an important risk factor for the development of diabetes mellitus

Chronic Hepatitis C infection type 2 diabetic patient worsen glycemic control

Therefore, determining the prevalence of HCV and associated factors
diabetes will enable better management for diabetes patients, especially to prevent such added infections and its morbid consequences as treatment of HCV in diabetic patient showed good glycemic control , hence it has become very necessary for a screening exercise to determine the prevalence rate of HCV among diabetic patients at our location of study, so as to increase awareness of the population and health practitioners on the dangers of the co-infectious status of this virus among diabetes

CHAPTER 2: LITERATURE REVIEW

2.1 prevalence of HCV among type 2 diabetic patients

A study conducted at tertiary care hospital, India , out of 300 type 2 diabetic patient33(11%) were found to be anti-HCV positive , which showed there was a higher prevalence of HCV sero-positivity among patients with type 2 diabetes compared to the general population(19)

Another study done on 3000 individuals with T2DM visiting Diabetes Clinic of Pakistan, showed Prevalence rate of 13.7% for HCV infection was recorded among subjects having T2DM with seropositivity rate of 4.9% among the control group of volunteer blood donors without diabetes(20)

A cross-sectional study on DM2 patients recruited at two geo- graphical regions in Brazil. A total of 537, (36.12%) males and (63.87%) female concluded high prevalence of HCV in DM2 patients.(21)

Another study aimed to determine prevalence of HCV infection among Saudi Arabia, study revealed that Among T2DM and non-diabetics, a seroprevalence of 8.0% and 2.0% respectively was found(1)

According to study conducted at tertiary health-care facility South-west, Nigeria a total of 180 diabetic patients were recruited for this study of 71(39.4%) males and 109(60.56%) females study showed a slightly higher prevalence of hepatitis C infection in type 2 diabetics. Overall

prevalence of HCV infection among diabetes patients assayed was 13.3% out of which 8(11.3%) Risk factors considered showed that, 7 (18.9%) seropositive subject were alcoholic consumers(P value = 0.2621;P > 0.05) while 5 (8.9%) recorded history of sharing sharp objects P = 0.2427;P > 0.05)(22)

Study in diabetic subjects attending Kisangani University Clinics and General Hospitals of Kisangani City Democratic Republic of Congo, showed seroprevalence of hepatitis C virus in diabetics was 24.8% compared to 1.9% in volunteer blood donors (p = 0.0000) . Hepatitis C virus infection was more common in type 2 diabetics (p = 0.006) and significantly associated with age of diabetic patients (p = 0.002).(17)

As the study conducted at outpatients' clinics of Suez Canal University Hospital, (n=226), from June 2015 to June 2016; Occurrence of HCV infection in diabetic patients was 1.99 times higher than its occurrence in non-diabetic (OR=1.99); (P< 0.05)(15)

Healthy facility based case control study was done North West Tigray, Ethiopia (n=460 , males 265 (57.6%) with a mean age of 45.8 ± 11.8 ;highest percentage 64 (28%) of HCV) was detected in diabetic study subjects as compared to non-diabetic (23)

Another institutional based cross-sectional study was conducted at Felege Hiwot Regional Referral Hospital Bahir-Dar, Northwest Ethiopia, {n=385 (58.7%) were male} showed the seroprevalence rate of hepatitis C virus infection was 6.5%. Male diabetic's patients have higher prevalence (4.4%).) (24)

2.2 Patterns of HCV infection among type 2 diabetic patients

According to study conducted in 2011-2013,seroprevalence of hepatitis c infection in type 2 diabetic patient, India showed HCV infection was higher male age group of 40–59 years Transaminases levels, were significantly elevated in the sero-positive group (P = 0.000)(19)

Study done at in Peshawar. Pakistan comprised of 100 Diabetic patients, 36% were found to be anti HCV positive. Serum Transaminase level was raised in 75% of the positive cases and the seropositive cases had a comparatively higher blood sugar level(25).

In this cross-sectional study done , 1054 Saudi diabetic patients, 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 in those who had history of; hospital admission (11.5 times) , having surgical procedures(13.6 times) , blood transfusion(4 times), shared personal items (8.5 times) tattooing (6.7 times). The likelihood of HCV infection was also higher among DM patients with liver diseases and elevated liver enzymes(26)

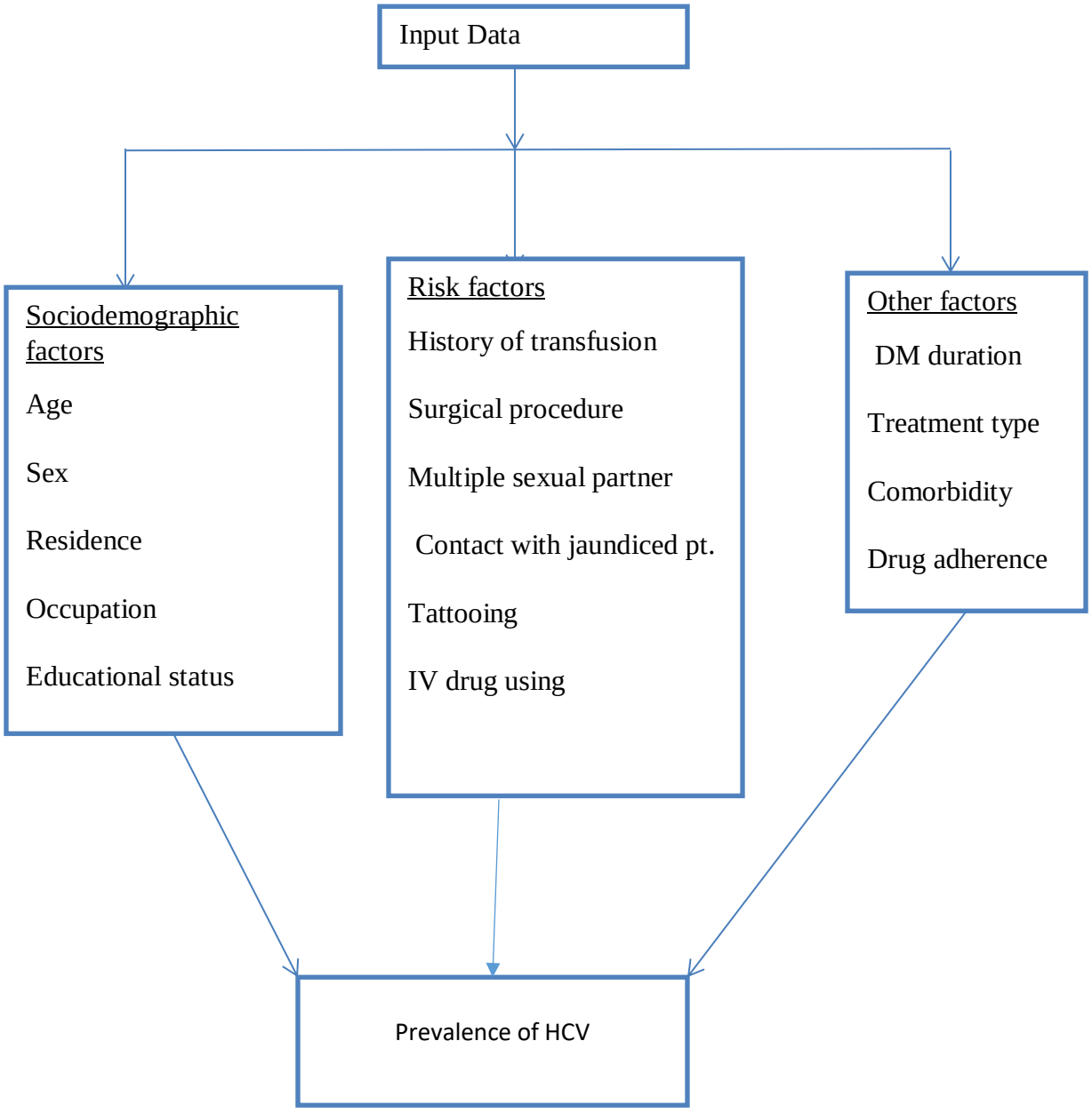
A descriptive cross-sectional study in diabetic subjects in Democratic Rebuplique Congo, Hepatitis C virus infection was significantly associated with age of diabetic patients (p = 0.002).(17)

A study done on the prevalence of Hepatitis C virus infection in diabetic patients attending a tertiary health-care facility South-west Nigeria (n: 180), 71(39.4%) males and 109(60.56%) females prevalence of HCV infections (13.3%). Risk factors considered showed that, 7 (18.9%) showed seropositive subject were alcoholic consumers(P value = 0.2621;P > 0.05) while 5 (8.9%) recorded history of sharing sharp objects P = 0.2427;P > 0.05) (22)

According to study done seroprevalence of Hepatitis C virus among patients with Diabetes mellitus in North West Tigray, Ethiopia, showed that study subject with uvulotomy, previous

history of immunosuppressive disease, and study subjects with fast blood glucose (≥ 126 mg/dl) showed statistically significant association with HCV infection [AOR (12.4 (3.5–18.3); 0.1 (0.03–0.5); and 8.6 (1.7–13)] respectively (27)

Conceptual Framework



CHAPTER 3: OBJECTIVE OF THE STUDY

3.1General Objective

To determine the prevalence of HCV infection and its patterns among type 2 diabetes at Jimma Medical Center (JMC).

3.2 Specific Objectives

3.2.1 To determine the burden of HCV among type 2 DM patients

3.2.2 To identify factors associated HCV infection among type 2 DM patients

CHAPTER 4: MATERIALS AND METHODS

4.1 Study area and period

The study was done in Jimma university medical center (JUMC). JUMC is located in Jimma town which is 355km away from Addis Ababa in the south west, Jimma zone, Oromia regional state, Ethiopia. It is one of the oldest public hospitals in the southwest part of the country and comes under Jimma University. The hospital serves as a referral site and provides specialized care for about 20 million population.

It has one diabetic follow-up clinic. The clinic provides comprehensive care with regular follow-up for drug dose adjustments, evaluation for side effects of the drugs, detection of comorbidities and detection of acute and chronic complications for about 3500 diabetic patients of which about 2000 are type 2 diabetic

The study was conducted from November, 2021 to Juanary, 2022

4.2 Study design

Facility based cross-sectional study was used.

4.3.1 Source population

All patients with type 2 diabetes mellitus who have follow up at JUMC

4.3.2 Study population

4.4 inclusion and exclusion criteria

4.4.1 Inclusion criteria

Adults visiting Outpatient Department (OPD) who are proved as type 2 diabetic patients and > 18 years

4.4.2 Exclusion criteria

Individuals who proved as diabetic patients during the study period but who refused to give

Informed consent

4.5 Sample size determination and sampling procedure

4.5.1 Sample size determination

The sample was calculated using a formula for estimation of single population proportion taking seroprevalence of type 2 DM patients to be p=16.3% (from previous study in Ethiopia) ,margin of error 5%, and using 95% confidence level.

$n = (Z\alpha/2)^2 p (1-p) / d^2$

P = 16.3%,

$Z\alpha/2$ = standard normal variable at 95% confidence level (1.96).

d= precision (tolerable margin of error

$n = (Z\alpha/2)^2 p (1-p)/d^2 = (1.96)^2 \times 0.28(1-0.28)/(.05)^2 = 209.55$

Assuming for non-response of 5% size would give 220 patients

4.5.2 Sampling Method

Consecutive convenient sampling method was used as diabetes patients coming for follow-up, either glucose monitoring or treatments and participants were asked to participate in the study after the aim of the study explained. Only those willing to participate were asked for questionnaire and to give venous blood.

4.6 Study variables

4.6.1 Dependent variables

- ✓ HCV infection

4.6.2 Independent Variable

- ✓ years of living with diabetes
- ✓ 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, multiple sexual partner)

4.7.1 Method of Data Collection

After informed consent was gained from the participant, information for socio demographic data such as age, sex, religion, occupation, marital status, ethnicity, and educational Status was collected. History of exposure for the possible associated factors, years of follow up and other relevant information was collected with a structured interviewer-based pretested questionnaire by trained nurse professionals the participant serologic status for HCV antibody was done after questionnaire using the package insert lab testing guideline by licensed medical laboratory professional under aseptic condition.

4.7.2 Laboratory methods

Sample collection and processing

Five ml of venous blood was collected aseptically by vacutainer needle in serum separating tube; samples was left at room temperature for 1 hour to facilitate clotting and was 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.

4.8.3 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 17 attach to the solid-phase.

4.9 plan for data processing & analysis

Collected data was rechecked for completeness by principal investigator. Finally, the data was entered in to Epi data entry software and then it exported to SPSS 26 version for descriptive analysis and inferential statistics.

Descriptive statistics: percentages, means, Medians, standard deviations and ranges will be used to describe findings. A bivariate analysis will be done to sort variables candidate for multiple logistic regression having value less or equals to 0.25. Multivariate logistic regression analyses will be conducted to generate factors strongly associated with the dependent variable. Finally association will be declared with P value less than 0.05.

4.9.1 Data Quality Assurance

The standard operational procedures was strictly followed for the quality control issues. Hepatitis C kits was checked by using known 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 was guaranteed by implementing Quality control (QC) measures throughout the whole processes of the laboratory works. All materials, equipment and procedures was adequately controlled. Pre analytical,

analytical and post-analytical stages of quality assurance was strictly followed. All laboratory analyses was carried out using standard operating procedures.

4.10 Ethical Consideration

Ethical clearance was obtained first from Institutional Review Board (IRB) of Jimma University Institute of Health. Then, letter of cooperation from department of Internal Medicine was submitted to hospital director and then to diabetes follow-up clinic prior to data collection. Purpose & significance of the study was explained by the data collector during the interview verbally. Patient's confidentiality, equity of services and interests of patients was ensured during the study period by informing the data collectors on ethical issues. This study doesn't involve any potentially harmful intervention to the patient. The interview scripts was coded and personal identifying details were not collected

4.11 Operational Definition & Definition of Terms

Comorbidities: Co-existence of other disease with DM

Type II DM*:* patients who used oral ant diabetic as initial therapy and continuing using it or switch to insulin.

4.12 Dissemination plan

After approval by the advisors, the findings of the study will be disseminated to all relevant stakeholders through presentation and publication. Copies of the research will be given to Jimma University, College of Health Science postgraduate library, and the department of Internal Medicine, the ministry of health and other concerned institutions and stake holders for possible application and publication of the study

CHAPTER FIVE: RESULT

5.1 General Description of Subjects

A total of 220 participants were screened for antibodies to hepatitis C. In the study, 72 (32.7%) female and 148 (67.3%) male were included. Majority 64 (31.4%) of the study participants were in the age category of 55-64 years followed by 68(30.9) 45-54 years, and >64 years (18.2%).

Majorities 129 (59.3%) were from rural, 84(38.2%) were farmers, 77(35.6%) never attended formal education .They had mean disease duration of 5 years. 152(69.1%) of them were being treated with oral glucose lowering agents .Table 1 shows socio demographic characteristics

Table 1: Socio-demographic characteristics of type 2 DM patients, JMC, Jimma, Ethiopia

Variables	Category	Frequency	Percentage
Age (in years)	<25	1	.5
	25-34	6	2.7
	35-44	36	16.1
	45-54	68	30.9
	55-64	69	31.4
	>64	40	18.2
Sex	Male	148	67.3
	Female	72	32.7
Marital status	Single	1	.5
	Married	218	99.1
	Widowed	1	.5
Residence	Rural	129	59.3
	Urban	91	41.4
Occupation	Employee	56	25.5
	Merchant	4	1.8
	Farmer	84	38.2
	Housewife	58	26.4
	Others	18	8.1
Educational status	Unable to read and write	77	35.6
	Read and write only	30	13.6
	Elementary school	60	27.3
	High school	24	10.9
	Higher institution	29	13.2

5.2 Duration of Diabetes

The duration of diabetes among patients in the DM group had a wide range of between 2-30 years. The median duration of diabetes was five years.

5.3 Frequency of Probable Risk Factors of Exposure to HCV

The commonest route of exposure to a possible HCV infection was circumcision 207(94.1%) followed by ear piercing 59(26.8%), dental extraction 55(25.0%), and history of surgical procedure 39 (17.7%. Table 2 shows frequency of probable risk factors of exposure to HCV

5.4 Alcohol Consumption

More than two-third of the cases 185(84.1%) never drank alcohol. The rest of the subjects either had stopped drinking or, in any case, never drank >1 bottle of beer (about 10 g of alcohol) per day.

5.5 Prevalence of Anti-HCV

Only one subject (0.5%) was positive for hepatitis C Virus antibodies

Table 2. Frequency of probable risk factors of exposure to HCV

Serial Number	Risk Factors	Frequency	Percentage

1	Previous blood transfusion	6	2.7
2	Previous surgical procedure	39	17.7
3	Tattooing	8	3.6
4	Illicit self-injection	1	0.5
5	Multiple sexual partners	14	6.4
6	Exposure to office or household contact of jaundice	3	1.4
7	Dental extraction	55	25
8	Ear piercing	59	26.8
9	Circumcision	207	94.1
10	Uvulectomy (by native doctors)	35	15.9
11	Previous hospital admission	50	22.7

CHAPTER SIX: DISCUSSION.

Our results suggest a low HCV sero prevalence among type diabetic at JMC diabetic population

Several studies have demonstrated an increased frequency of HCV infection among patients with type 2 DM, in comparison with either the general population or blood donors

According to study done in Felege Hiwot regional referral hospital, Bahir-Dar, Northwest Ethiopia about nine years ago, the prevalence of HCV antibody was 6.5% in type 2 diabetic patient (24). Additionally, A cross sectional study was conducted among 156 prisoners from January to June 2016 Jimma, Ethiopia showed, the seroprevalence of HCV 2.6%(28). Probably, the decrease in the prevalence could be due to the attention given by the Federal Ministry of Health, health workers, and other responsible bodies for infection prevention and control. The result was, however, much lower than 3.4%, 4.8%, 6.2%, 6.5%, and 11.9% findings obtained from Egypt, Nigeria republic of Congo, Brazil ,Saudi Arabia [17-22].

However, the findings in this study are similar to Blowgun and coworkers," who reported a low prevalence rate of 0.8% among diabetic patients (29)

The finding of widespread practice of ear piercing, Dental extraction and Uvulectomy by native doctors among these predominantly illiterate subjects is not unexpected. The fact, however, that the anti-HCV prevalence is low among them in spite of this practice probably might imply that those practices are not a significant route of acquiring HCV in our environment.

Although the sample size used for this study was relatively small, it is a preliminary study, and a large multicenter study is desirable in the future to better reflect the true prevalence of anti-HCV

in DM patients. Also, it is necessary to confirm the results of anti-HCV seroanalysis by carrying out polymerase chain reaction to detect HCV-RNA. This could not be done in this study due to Financial and technical limitation

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. Conclusion

There is low prevalence of anti-HCV antibody in our study.

7.2. Recommendation

Routine screening for HCV may not be recommended in type-2 diabetic patients

Large scale study is needed to further address prevalence of HCV in type 2 diabetes.

CHAPTER EIGHT: STRENGTH AND LIMITATIONS OF THE STUDY

8.1 Strength of the study

. The study has clear and achievable objectives.

8.2 Limitations of the Study

Due to resource constrains, the sample size we used was small and unable to use additional confirmatory tests

The sampling method was convenient, by which generalizing the finding to the general population is sub optimal

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ASSURANCE OF PRINCIPAL INVESTIGATOR

The undersigned agrees to accept responsibility for the scientific ethical and technical Conduct of the research project and for provision of required progress reports as per Terms and conditions of the Faculty of Public Health in effect at the time of grant is Forwarded as the result of this application.

Name of the Resident: _____Dr.Sura oljira (Medical Resident)

_____

Date._____ Signature _____

10. Annexes

Annex I: Informed Consent and Patient Information Sheet

My name is Sura Oljira and I am Internal Medicine resident at JMC. I am doing a research entitled Prevalence and pattern of HCV among type 2 Diabetes.

The objective of the study the prevalence of HCV infection and its associated factors and among with type 2 Diabetes. 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 cannot 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

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 microorganism, 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

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 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: Dr. Sura Oljira (Resident), +251921184766

Email-suraoljira@gmail.com

Annex 1.1 Information to the Participant
Interview code no _____

Greeting self-introduction and consent

Greeting: - Good morning/afternoon.

My name is ……..We are conducting a scientific research on prevalence and pattern of HCV among type 2DM *patient at Jimma university follow up clinics during October 2021 g*. Therefore, I am happy to inform you that you are selected as one of the participants in this study. By participating in this research project, you may feel some discomfort. However, your participation is definitely important in identifying factors associated with HCV and pattern among type 2 DM patient in our hospital. The interview may take 20-25 minutes and the information gathered will be used for writing a research paper for partial fulfilment of a specialty certificate in Internal Medicine at Jimma University.

I want to assure you that any information obtained from you will remain confidential and even there is no need of writing your names or any personally identifiable information. There is no risk or direct benefit in

participating in this research project. I will proceed to ask you some information. Finally, you are kindly requested to give your genuine response in the interview.

Certificate of Consent

Do you wish to participate in the study? A. Yes B. No

If the participant agrees to participate in the study, let him/her to sign consent and proceed with interview.

I have adequate information about the research and I have decided to participate in the study.

Signature ----------

If the participant says "No, I don't want to participate in the study", thank him (her) and proceed to the next participant

Name of interviewer_____

Date_____/_____/____

Annex 1.2 Informed consent in Afaan Oromo
Odeeffannoo qoratamaaf kennamu

Ani maqaan koo ----------yoon ta'u qoarnnoo waa'ee baay'inna vaayiresii tiruu C dhukkubsatoota sukkaaraa fi wantoota walqabatan, "prevalence and pattern ofof HCV among type 2 diabetes *patient at jimma university follow up clinics during October 2021 G.C.*" jedhu irratti hirmaataa akka naaf taataniif kabajaan isin gaafadha. Qorannoon Kun Kan adeemsifamu bifa gaafii fi deebiitiin akkasumas kaardii yaalumsa keessanii irraa odeeffannoo fudhachuu fi saamuda dhiigaa kennuun ta'a. Odeeffannoon qorannoo kanarraa argamu hojii fuuldura adeemsifamuuf bu'aa guddaa Kan kennuu fi isin irratti immoo dhiibbaa Kan

hingeessifnedha. Odeeffannoon isin qorannaa kanaaf kennitanis Kan qaama biraaf dabarfamee hin kennamneedha. Qorannoo kana keessaas yeroo barbaaddanitti ba'uu Kan dandeessan yoo ta'u Kun immoo tajaajila isin argattanirratti dhiibbaa hin qaqqabsiisa

Qorannaa irratti hirmaachuuf yoo walii galtan bakka armaan gadii irratti mallattoon mirkaneessaa

Galatoomaa

Mallattoo hirmaataa…….

Maqaa qorataa……..

Guyyaa……

Yoo qo'annaa irratti qooda fudhachuu hinbarbaadne galateeffadhaa dhiisaa.

Annex 2. Questionnaire

Jimma University medical center

College of Public Health and Medical Sciences

Department of Internal Medicine

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		
Question	Answer	remark
Sex	1. Male 2. Female	
Age	----------years	
Residence	1. Urban 2. Rural	
Current occupational status	1 Self-employed 4. Student 2. Driver 5. Farmer 3. House Wife 6. Other specify _____	
Religion	1. Christian 3. Doesn't have 2. Muslim 4. others specify _____	
Marital status	1. Married 3. Divorced 2. single 4, widowed	
Ethnicity	1. Oromo 4. Gurage 2. Amhara 5. Wolayta 3. Tigray 6. others specify _____	
Educational Status	1. Illiterate 4. Grade 9-10 2. Read and write 5. Grade 11-12 3. Grade 1-8 6. College/university	

2. Hepatitis C related risk factors Have you ever practiced the following?	
Question	Answer
History of STD/STI	1. Yes 2. No
Multiple sexual partner	1. Yes 2. No
Blood transfusion	1. Yes 2. No
Abortion	1. Yes 2. No
Dental extraction at health facility	1. Yes 2. No
Hospital admission	1. Yes 2. No
Surgical procedure	1. Yes 2. N
Venous or body piercing for treatment	1. Yes 2. No
Ear piercing	1. Yes 2. No
Uvuloctomy	1. Yes 2. No

Tattooing on body	1. Yes 2. No
Tattooing on gum	1. Yes 2. No
Unprotected sex	1. Yes 2. No
Contact with jaundiced patient	1. Yes 2. No
Frequent alcohol consumption	1. Yes 2. No
Circumcision	1. Yes 2. No
3.Diabetic variables	
Any confirmed diseases other than diabetes?	1. Yes 2. No
Duration of disease	specify in year --------
Treatment type	1. Oral 2. Injection
Known diabetic complications	1. Yes 2. No
Do you have glucometer (point of care)	1. Yes 2. No
If above is Yes how often you use it	specify in number ------
f is Yes do you use a lancet for more than one	1. Yes 2. No
History of ulcers/blisters	1. Yes 2. No

THANK YOU!!!

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

Annex 3. Laboratory methods

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 Ant hepatitis 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 neutralized 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

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

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

ANNEX 4: DECLARATION

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

Name: _____

Signature: _____

Name of the institution: _**Jimma University**___

Date of submission: _____

This thesis has been submitted with my approval as university advisor

Name and Signature of the first advisor: _____ _

_____