FACTORS ASSOCIATED WITH ELEVATED PLASMA LEVELS OF LIPOPROTEIN (a) IN INDIGENOUS BLACK ZAMBIANS WITH DIABETES MELLITUS TYPE 2 IN THE OUTPATIENT MEDICAL CLINIC AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA

BY

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A dissertation submitted to the University of Zambia in partial fulfilment of the requirements for the degree of Master of Science in Pathology (Chemical Pathology)

THE UNIVERSITY OF ZAMBIA, LUSAKA, ZAMBIA

September, 2014

Declaration

I, **SINYANI ANGELA** this 19th day of September, 2014, declare that this dissertation represents my own work. This work has not been done in Zambia before and neither has it been published for any qualification at the University of Zambia or any other University. Various sources to which I am indebted are clearly indicated in the text and in the references

Angela Sinyani

Signed…… Date:…….

Certificate of completion of Dissertation

The supervisors have read this dissertation and are satisfied that this is the original work of the author under whose name it is being presented. It is therefore confirmed that the work has been completed satisfactorily and is ready for presentation to the examiners.

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The University of Zambia approves this Dissertation on "Factors Associated With Elevated Plasma Levels Of Lipoprotein (a) In Indigenous Black Zambians With Diabetes Mellitus Type 2 In The Outpatient Medical Clinic At The University Teaching Hospital, Lusaka."

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Abstract

Introduction: Diabetes mellitus (DM) is an established risk factor for cardiovascular disease (CVD) and is considered to be a CVD equivalent. Lipoprotein(a) [Lp(a)] is an independent risk factor for cardiovascular diseases. Since Diabetes Mellitus Type 2 (T2DM) patient numbers continue to rise, and since patients continue to present with cardiovascular disease-related complications, it is possible that some of these patients have high plasma levels of Lp(a). The aim of the study was to investigate the plasma levels of Lp(a) in T2DM patients and also to assess the factors that may be associated with the plasma levels of Lp(a) among indigenous black Zambians with T2DM.

Materials and methods: We conducted a cross sectional study that enrolled 155 participants, 79 T2DM patients attending the outpatient medical clinic of the University Teaching Hospital and 76 community-based healthy individuals. A short questionnaire was used to record the social demographic characteristics and anthropometric measurements. 4ml of venous blood was collected from which all the analytes were measured. The factors that were assessed for association with Lp(a) included; social demographic characteristics, social economic status, duration of illness, dietary composition, physical fitness, BMI, fasting blood sugar (FBS), renal function, hepatic function, acute phase response, lipid profile, and glycaemic control. Therefore the variables that were measured were as follows; Age, sex, marital status, occupation, residence, dietary fat content, dietary carbohydrate source, dietary protein source, frequency of exercise, BMI, FBS, ALT, urea, creatinine, C-reactive protein, Triglycerides, total cholesterol, low density lipoprotein, high density lipoprotein, HbA1c and lipoprotein(a) respectively. The data were expressed as median (interquartile range). The Mann-Whitney U test was used to compare the median values between the two groups of the study participants (Diabetics and healthy individuals) for continuous variables, the Wilcoxon rank-sum test or Kruskal-Wallis test for the ordinal data whereas the Chi-squared test was used to compare the proportions for the nominal data. SPSS version 21(IBM) was used to perform a multiple linear regression analysis to identify the *9/et of variables that would best predict the plasma levels of Lp(a)

Results: The median plasma levels of Lp(a) in the diabetics (20.0 (11.8-37.4)mg/dl) was significantly higher ($p<0.001$,) than the healthy individuals (13.6 (9.4-21.5)mg/dl). 17 % of the diabetics had plasma levels of Lp(a) higher than 30 mg/dl. Of all the independent variables assessed, the results showed that glycemic control (HbA1c), FBS (glucose) triglycerides and residence were significant ($p<0.001$, $p=0.030$, $p=0.040$, $p= 0.004$ respectively) predictors of plasma levels of Lp(a). The linear relationships showed that the plasma levels of Lp(a) had a positive relationship with HbA1c ($r=5.220$) and FBS ($r=0.660$) whereas the relationship with triglycerides ($r= -4.794$) and residence ($r= -7.165$) were inverse

Conclusion: . The plasma levels of Lp(a) in the T2DM patients were significantly higher than the non-diabetic healthy individuals. Glycemic control (HbA1c) , triglycerides (TG), fasting blood glucose (FBS) and social economic status (medium density residence) were predictors of serum levels of Lp(a).

Key Words: Diabetes mellitus type 2 (T2DM), Lipoprotein(a) [Lp(a)], Cardiovascular disease (CVD).

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Acknowledgements

I thank the Lord almighty to whom I am entirely indebted through the progression and indeed the completion of this project. However, this project would not have been possible without the support of many people.

Many thanks to my supervisors/mentors Dr .T. Kaile and Dr .S. Nyirenda for their patience and dedication to this work

Thanks to the staff in the Outpatient Medical Clinic and the Clinical Chemistry Laboratory for the technical support they rendered. Many thanks to the Section head of the Clinical Chemistry Laboratory, Mr. Kabalanyana Mubita and the chief laboratory scientist Mr Katenga for the technical guidance and support.

I would like to thank the University of Zambia (SDF office) and the Southern African Research Support Consortium (SACORE) for all of the funding they provided to make this work possible.

Many thanks to my family and friends for their prayers, motivation, and love throughout this process.

Dedication

This paper is dedicated to my mother, Cecilia Munansangu Sinyani, who had always been a source of inspiration, a true example of hard work and perseverance. Without whom, I would not have been who I am today. MHSRIP.

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List of abbreviations

ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CRP	C-reactive Protein
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
EDTA	Ethylenediamine Tetra-acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FBS	Fasting Blood Sugar (glucose)
$HbA1_c$	Glycated Haemoglobin
HDL-C	How Density Lipoprotein Cholesterol
LDL-C	Low Density Lipoprotein Cholesterol
Lp(a)	Lipoprotein(a)
NCD	Non Communicable Disease
TC	Total Cholesterol
TG	Triglycerides
UTH	University Teaching Hospital
WHO	World Health Organisation

Glossary of terms

Cardiovascular disease: is the class of diseases that involves the heart or blood vessels: arteries and veins.

Cardiovascular risk: the risk of developing a cardiovascular disease

Diabetes Mellitus: is defined as a "metabolic disorder caused by different factors characterized by a chronic high level of blood glucose with disturbances in the carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both.

Diabetes Mellitus Type I: is characterized by defects in insulin secretion by the pancreatic beta cells.

Diabetes Mellitus Type II: is characterized by the combination of defects in insulin secretion and insulin action.

Insulin: Insulin is a hormone produced by the beta cells of the pancreas that permits glucose to enter cells and helps the body use glucose for energy. Insulin controls the amount of glucose in the blood.

Lipid profile: The lipid profile is a group of tests that are often ordered together to determine risk of coronary heart disease. Blood levels examined in a lipid profile include those for total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C), and triglycerides (TG).

Lipoprotein (a): is a *lipoprotein* subclass. The Lp(a) test is used to identify the presence of Lp(a) as a possible risk factor in the development of cardiovascular disease (CVD

Lipoprotein: is a combination of a fat and protein molecule. The protein helps to transport fat to where it is needed in the body.

Non- Communicable Disease: is a disease which is not contagious. A medical condition or disease which is non-infectious and non-transmissible between people

CHAPTER 1

1.0 Background

According to the World Health Organisation (WHO) estimates, 347 million people have Diabetes Mellitus (DM). In 2004 about 3.4 million people died from DM related disorders with 80% of these deaths occurring in low and middle income countries[1].

In the African region WHO estimates that Non-Communicable Diseases (NCDs), (Diabetes included) will cause around 3.9 million deaths by the year 2020. Behavior-related risk factors including, sedentary life and unhealthy diets are responsible for the majority of these deaths through cardiovascular complications[2].

In Zambia, particularly at the University Teaching Hospital (UTH), there has been a marked increase in numbers of diabetes patients owing to the increase in the newly diagnosed cases at the Medical admission ward (filter clinic) and increasing number of patients attending the Out-Patient Medical Clinic. At UTH, diabetes is the second most common non-infectious cause of admissions and death[3].

Diabetes is a chronic disease related to a defect in insulin production and/ or utilisation. In Type 1(Insulin Dependent or Juvenile Onset) there is deficient insulin production while in Type 2 (non-insulin dependent or adult onset) it is ineffective use of insulin. Up to 90% of diabetics around the world are type 2, and it is largely due to excessive weight and sedentarism[4].

Symptoms of Type 2 diabetes mellitus (T2DM) are less marked than Type 1 and hence it takes several years to diagnose it. By the time the diagnosis is made, complications would have already occurred. Most patients who are seen at UTH, present for the first time with complications of diabetes or are known diabetics who are being followed up as out-patients in the Medical Clinic (Clinic 5)[5]. Complications of T2DM as seen at the UTH are mostly cardiovascular and neurological. Medical complications (cardiovascular and neurological, nephropathy) are by far the commonest. Other complications are surgical (gangrene, ophthalmic) and gynaecological[3].

Diabetes mellitus complications arise due to poor control of plasma glucose. Studies have showed an association between plasma Lipoprotein (a) (Lp[a]) and cardiovascular

complications in T2DM. Lp(a) is a unique lipoprotein which recently has been linked to the development of vascular diseases. Previously classified as low density lipoprotein variant, it has an additional protein (apo (a)) which is associated with cardiovascular disease risk. Plasma levels greater than 300mg/l are associated with increased risk of cardiovascular disease. Levels of Lp(a) among DM patients at UTH remain unknown. Lp(a) is metabolised in the liver and kidneys. Thus dysfunction of these organs may raise levels of serum Lp(a).

Plasma levels of Lp(a) have not been studied in Zambia among T2DM patients who are asymptomatic or those with cardiovascular complications, neither have determinants (risk factors) of raised serum Lp(a). This study investigated plasma levels of Lp(a) and its determinants among T2DM adult patients who are routinely followed up as out-patients.

1.2 Statement of the problem

The University Teaching Hospital attends to most of the diabetic patients in the Lusaka province. At least every diabetic patient in Lusaka has been to UTH in their lifetime. The patient may have been brought in as an emergency, may have come for routines checkup or with a DM-related complication. The mainstay of therapy is either non-pharmaceutical (Diet control, exercises) or pharmaceutical (Insulin, Glibeclamide, and Metformin). Different Clinicians with different experiences have different approaches to the management of these patients. Most of the patients are obese. Strokes, heart diseases, amputations and diabetic emergencies (Diabetic ketoacidosis, comas, hypoglycaemias) continue to be reasons for admission and referral to UTH. There is no specialised diabetes clinic at the UTH. This may suggest that the glycemic control and overall comprehensive care of these patients is inadequate.

Since T2DM patient numbers continue to rise, and since patients continue to present with cardiovascular disease-related complications, it is possible that some of these patients have high plasma levels of Lp(a).

There are numerous large clinical trials that showed that individuals with high plasma levels of Lp(a) are at risk of developing cardiovascular disease[5]. This has not been studied in Zambia. Determinants of raised plasma levels of Lp(a) in adult indigenous

black Zambians in T2DM patients and the cardiovascular diseases that are associated with raised plasma Lp (a) have also not been studied at UTH or in Zambia.

Diabetes causes complications in virtually all systems in the body including the brain, liver, kidneys, and the immune system. A significant number of patients do present with these complications although others still remain largely undiagnosed.

To investigate plasma levels of Lp(a) and what might influence its levels among T2DM at UTH, an analytical prospective cross-sectional study was done.

1.3 Justification

This study would provide evidence-based information on the association between T2DM and plasma Lp(a) levels among indigenous black Zambians. The baseline data that were obtained from this study enabled us to know the levels of Lp(a) among the diabetic patients and also in future ascertain the categorical levels (i.e. desirable levels, high risk and very high risk) of Lp(a) in relation to cardiovascular risk, specific for our population.

This provided prognostic information for those who are diabetic and have either low or high levels of Lp(a). It also provide further prognostic information for diabetic patients who had no cardiovascular disease and would therefore be at risk.

The results may also enable the clinicians to informatively improve the management of the disease besides being able to advise the patients concerning lifestyle modification and suitable treatment for the patients at risk.

It is also suggested that these findings could help clinicians determine whether plasma Lp(a) test could be done routinely. This would improve the management of diabetes and possibly minimize complications leading to an improved quality of life.

CHAPTER 2

2.0 Literature review

2.1.1 Diabetes Mellitus

Diabetes mellitus is now one of the most common NCDs globally. It is the fourth leading cause of death in most high-income countries. There is substantial evidence that it is endemic in many low and middle-income countries. Complications from DM, such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure and blindness are resulting in increasing disability, reduced life expectancy and enormous health costs for virtually every society. It is certain to be one of the most challenging health problems in the 21st century. The number of studies describing the epidemiology of diabetes over the last 20 years has been extraordinary[6].

In Zambia, the prevalence of diabetes was 2.1% among males and 3.0% among females of 25-34 years old, according to a study that was done in Lusaka urban district, in 2011[7].

Diabetes mellitus type 2 is characterized by the combination of peripheral insulin resistance (reduced insulin utilisation) and inadequate insulin secretion by pancreatic beta cells [77]. Insulin resistance, which has been attributed to elevated levels of free fatty acids in plasma[8], leads to decreased glucose transport into muscle cells, elevated hepatic glucose production, and increased breakdown of fat.

However, cardiovascular risk in people with DM is related in part to insulin resistance, with the following concomitant lipid abnormalities: elevated levels of small dense low-density lipoprotein cholesterol [LDL-C], low levels of high-density lipoprotein cholesterol [HDL-C], elevated levels of triglyceride-rich remnant lipoproteins and thrombotic abnormalities (i.e., elevated type-1 plasminogen activator inhibitor [PAI-1], elevated fibrinogen)[9, 10].

2.1.2 Lipoprotein (a) structure and metabolism

Lipoprotein(a) (Lp (a)) was discovered in human serum in 1963 by Kåre Berg during a study of variation in LDL antigenicity. It consists of a LDL covalently bound to a unique protein called apo(a). Apo(a) is a homologue of plasminogen, containing

multiple copies of plasminogen kringle 4, a single copy of plasminogen kringle 5, and an inactive protease domain. The number of kringle 4 (KIV) domains can vary from 12 to 51 giving rise to 34 different-sized apo(a) isoforms. Furthermore, within the repeated KIV domain exists 10 distinct types (KIV types 1–10) each present in a single copy except for KIV type 2 which exists in varying numbers[11].

Circulating apo(a) is mainly synthesized by the liver as a precursor with lower molecular mass which is processed into the mature form and then secreted into the blood stream, where free apo(a) binds to circulating LDLs to generate complete Lp(a) particles. The assembly of Lp(a) occurs almost exclusively extracellularly[12] Lp(a) has a low affinity for the LDL receptor responsible for internalizing and thus clearing LDL, hence it does not contribute to the clearance of Lp(a)[13]. However, recently, an asialoglycoprotein receptor (ASGPR) was identified. It is highly expressed in the liver which binds and internalises Lp(a). Fragments of apo(a) are found in human urine suggesting that the kidney also plays a role in Lp(a) clearance although it is not a major route for Lp(a) catabolism[14].

About 90% of Lp(a) concentration is under genetic regulation. The greatest part of the variability in Lp(a) levels (over 40%) is accounted for by quantitative polymorphism in the internal sequence of the apo(a) gene. Qualitative polymorphisms in the sequence of the promoter play only a minor role (from 10 to 14%)[15]. Despite this genetic regulation, some metabolic abnormalities may have effects on Lp(a) levels in plasma. Among these: the acute-phase response, hormonal homeostasis, diabetes, liver and renal failure and defects in the ASGPR gene have all been shown to influence the still enigmatic metabolism of this lipoprotein.

Lp(a) levels are generally very resistant to changes in diet and are generally unresponsive to lipid-lowering drugs, such as the Statins or Fibrates. One exception is Nacin, which has routinely been shown to effectively lower Lp(a) levels, when given in high doses[16].

The physiological role of Lp(a) remains unknown, although a number of possible functions have been proposed such as: Lp(a) promotion of tissue repair and the inhibition of fibrinolysis and cancer growth[18].

2.1.3 Lipoprotein(a) role in the pathogenesis of cardiovascular complications

Lp(a)'s pathogenicity is linked to its atherogenic and thrombogenic activities such that shortly after its discovery, raised levels of Lp(a) were repeatedly associated with an increased incidence of a variety of cardiovascular diseases. These include silent coronary artery disease (CAD), acute myocardial infarction (AMI), asymptomatic carotid atherosclerosis, stroke, and peripheral artery occlusive disease (PAOD). Raised Lp(a) concentrations have also been observed in patients with several thrombotic occlusive disorders such as pulmonary embolism, central retinal vein occlusion and interference in placental circulation causing fetal growth retardation. It has also been proposed that Lp(a) levels are strong predictors of not only occlusive events following vascular and endovascular surgical procedures but also development of thrombotic episodes in patients with severe rheumatological diseases[17].

Lp(a) concentrations vary over one thousand fold between individuals, from < 0.2 to > 200 mg/dL. This has been observed in all populations studied. The mean and median concentrations between different world populations show distinct differences with the main being a two to threefold higher Lp(a) plasma concentration in populations of African descent compared to other populations.

The risk of developing cardiovascular disease is related to plasma levels of Lp(a). The desirable levels are those < 14 mg/dL, while borderline risk are 14 - 30 mg/dL. High risk are 31 - 50 mg/dL and very high risk being > 50 mg/dL.

2.1.4 Lipoprotein (a) and Diabetes Mellitus Type 2

There are contrasting views concerning the relationship between DM and Lp(a). In a recent prospective study it was concluded that Lp(a) was inversely associated with the risk of T2DM in which Lp(a) levels <10 mg/dl had the highest relative risk for developing T2DM as compared to higher levels[18]. In another study subjects with insulin-requiring T2DM had a higher serum Lp(a) levels and a higher prevalence of serum Lp(a) levels > 30 mg/dl than other groups of DM patients and non-DM control subjects[19]. This is in agreement with other workers who reported that T2DM is strongly associated with increased Lp(a) levels[20]. Similarly, it was observed that DM patients

have elevated levels of serum Lp(a) with a higher frequency of high risk levels as compared to healthy subjects[21].

2.1.5 Lipoprotein (a) and modulatory factors

A study showed that glycaemic control had a modulatory role on Lp(a) levels and also that Lp(a) levels positively correlate with Body Mass Index (BMI)[22]. A study done at West China Hospital showed that there was a positive correlation in the Lp(a) levels between T2DM patients and their offspring, suggesting a potential genetic control for Lp(a) levels in T2DM families[23].

For Kraft *et al*, their data clearly demonstrated that, average Lp(a) levels in Familial Hypercholesterolemia (FH) homozygotes are in a range above the 90th percentile of Lp(a) levels in healthy white populations and twice as high as in FH heterozygotes. This leaves little doubt that the LDL-Receptor mutation that results in FH also results in hyperlipoproteinemia (a)[24].

An Indian study by Bhavani *et al* showed that majority of hypertensive patients had Lp(a) levels >30 mg/dl which in general was taken as high-risk level for atherogenesis. The pathogenicity and atherogenic role of Lp (a) was greatly influenced by the concentration of other serum lipids and lipoproteins. A significant correlation was observed between HDL and LDL cholesterol levels and Lp(a). They confirmed that raised Lp(a), hypertension and dyslipidaemia were often associated[25].

Nevertheless, patients on insulin treatment have a better lipid profile (TC, HDL-C, LDL-C, TG) compared to patients on oral hypoglycaemic agents. Meanwhile, Lp(a) levels were raised in all DM patients and seemed not to be affected either by Insulin or oral hypoglycaemic treatment[26].

2.1.6 Lipoprotein (a) association with ethnicity and genetic predisposition

About 80% of the adult Caucasian white population regardless of age and gender had normal levels of Lp(a) as reflected by the frequency distribution of plasma Lp(a). Adult American blacks had higher Lp(a) levels than whites and this was also the case for black children[27]. However, in a study conducted by University of Pennsylvania affiliates, who were investigating the association between Lp(a) and coronary artery

calcification in T2DM women, found that blacks had 2–3 fold higher Lp(a) levels than whites, in both DM and non-DM samples[28]

2.1.7 Lipoprotein (a) assay and measurement

Lp(a) is currently measured by a range of commercially available immunoassays including Enzyme-Linked-Immunoabsorbant Assay (ELISA), Immunoturbidimetric and immunonephelometric assays that use antibodies specific to the apo(a) moiety of Lp(a). A dilemma exists with most of these methods with respect to the affinity of the antibodies to different apo(a) size isoforms.

There are a lot of studies that have used Immunoturbidimetry for analysing Lp(a) and others that have compared the different analytical methods, i.e. ELISA, immunonephelometric [29, 30, 31, 32] assay. It is a simple and rapid method for Lp(a) quantification which is not biased by different apo(a) isoforms.[32] It provides rapid, accurate, and precise screening of lipoprotein(a) in serum or plasma.[29]

Immunoturbidimetry measures the turbidity of a sample to determine the level of an analyte. Upon addition of the assay reagent, antibodies and antigen cluster to form an immune complex that precipitates, increasing the turbidity of the sample. The level of analyte is determined by comparison with a calibrator of known concentration. In this case specific anti Lp(a) antibody agglutinates with Lp(a) in the test sample. The agglutination is measured as an absorbance change at 700nm proportional to the change in Lp(a) concentration in the sample. This assay is not affected by Apo(a) size related bias.

The current study used the immunoturbidimetic assay on the Olympus AU400 chemistry analyser to measure Lp(a) in plasma.

2.2 Research questions

1. Is there a difference in the plasma levels of Lp(a) between the T2DM patients and healthy population in the adult indigenous black Zambians.

2. What factors are associated with the serum levels of Lp(a) in the T2DM patients

2.3 Objectives

2.3.1 General Objective

To investigate plasma levels of Lipoprotein(a) and its associated factors among adult T2DM patients in indigenous black Zambians who routinely attend the medical outpatient clinic at the University Teaching Hospital.

2.3.2 Specific objectives

1. To determine plasma levels of Lp(a) among adult T2DM patients.

2. To Investigate factors that may influence plasma levels of Lp(a) in adult T2DM patients.

CHAPTER 3

3.O Methodology

3.1 Study Design and Study Site

This was an analytical prospective cross-sectional study involving adult patients with T2DM attending the outpatient medical clinic, at the University Teaching Hospital.

The study was conducted at the outpatient medical department from June to August 2013. The clinic consisted largely of diabetic and cardiovascular patients who had been referred from urban, periurban clinics of Lusaka, a few from district hospitals and health centres in various provinces, for further management.

3.2 Target population and study population

The target population was DM Patients attending the outpatient medical clinic between ages of 18 to 75 years who were from urban and periurban areas of Lusaka. Some also came from other provinces.

The study population however consisted of the T2DM patients who met the inclusion criteria.

The control group was community based non-diabetic adults. They were matched according to the social-demographic properties i.e age, sex, economic status.

3.3 Sample Size and Sampling method

The sample size was initially 226 participants for both arms (study and controls) calculated by the *sample size for frequency in a population* formula. Sample Size for Frequency of DM in the population (for the finite population correction factor) (*N*) was 351. The hypothesized percentage frequency of outcome factor in the population (*p*) was set at 50% with confidence limits as % of 100 (absolute +/- %) (*d*) at 5%. The design effect (for cluster surveys-*DEFF*) was 1. Using the equation**: Sample size n = [DEFF*Np(1-p)]/ [(d2/Z21-α/2*(N-1)+p*(1-p)].** The sample size at different confidence levels (%) were 230(99%), 202(97%), 184 (95%), 154(90%) and 113(80%). However, the sample size at 80% confidence level was selected.

Therefore, after data clean up the final sample size was 155, 79 diabetics and 76 healthy individuals. The missing data files were left out. The sampling method used was systematic purposive sampling.

3.4 Case definition

Type 1 diabetic patients were considered to be patients whose onset of diabetes was in childhood or those who had entirely been on insulin treatment from the onset of disease.

Type 2 diabetics on the other hand were considered to be those whose onset of disease was in adulthood *or* those who were and had been taking oral hypoglycemic drugs, and also those who were currently on insulin but had previously taken oral hypoglycemic drugs *or* those who usually and were controlling their glycaemia through diet control.

However, the limitation of this case definition is that firstly, Type 1's who might have been started on oral hypoglycemic drugs and also the rare adult onset might have been missed. And secondly, the type 2's who might have been started on insulin at the time of diagnosis and also the rare childhood onset might have been missed.

3.5 Inclusion criteria and Exclusion criteria

Patients with T2DM of either gender and provided an informed written consent were included. Those who were excluded were T2DM patients who had kidney failure, liver dysfunction and major surgery within the past one month, non Negroid Zambians and the pregnant women. This information was obtained from the patient's files and interviews. (See appendices 7.1 for more details).

3.6 Data Collection

3.6.1 Clinical Data

Recruitment was done during normal working hours at outpatient medical clinic, starting from 07:00 hours to 11 hrs from Monday to Friday Study participants were recruited by the research team after they explained the study to them. Participants were given a study information sheet (see appendices**: 7.2** for full details). After they read and were agreeable, they signed the consent forms (see appendices **7.3)**. Participants were then assigned a serial number. It was after this that blood samples were obtained from them. Information obtained was collected systematically by use of a short

questionnaire (see appendices: **7.4**). Patient files were reviewed to verify some of the information obtained during interviews.

Fasting Blood Sugar (FBS) was measured on the spot using a glucometer. Weights and height were done and recorded.

3.6.2 Laboratory sample analysis

Four (4) millilitres (ml) of blood was collected by venepuncture of the antecubital vein. Half of this was put an EDTA tube while the other 2 ml in a Lithium-heparin tube. These tubes were labelled with the patient's newly assigned serial number and immediately transported to the clinical chemistry laboratory within UTH.

The samples were analysed using an Olympus AU400 chemistry analyser available at UTH in the clinical chemistry laboratory (see appendices: **7.5** for more details). The reagents were stored within the required conditions (see appendices: **7.7**). Calibration and Quality control of all the tests was performed on the analyser before running the samples to ensure precision, accuracy and validity of results. The lithium-heparin tube samples were used to measure Urea, Creatinine, AST, ALT and albumin, lipid profile (TG, LDL-C, HDL-C, TC), Lp (a) and CRP.

Immunoturbidimetry (IT) was the analytical method for Lipoprotein(a) and CRP. Photometric colour test, enzymatic colour test, UV kinetic test were used to analyse AST, ALT, Albumin, Urea, Creatinine, Lipid profile, and $HbA1_c$.(see appendices : **7.8**).

3.7 Ethical considerations

Permission to conduct the study was sought from the: UTH medical superintendent, the consultants at the Department of Medicine, and the Department of Research and Graduate studies (DRGS) through the Assistant Dean, Postgraduate. Approval to conduct the study granted by the University of Zambia Biomedical Research Ethics Committee (UNZA-BREC).

Study participants were provided with an information sheet for them to understand the study after which an informed consent was obtained prior to recruitment. Information collected from patients and laboratory results were confidential. Access to this information was restricted to the research team and clinicians only.

3.8 Data processing and statistical analysis

3.8.1 Variables

Variables measured in the study included the outcome variable Lipoprotein (a) [Lp(a)] and independent variables; Age, FBS, HBA1c, Total Cholesterol, Triglycerides, LDL, HDL, Urea, Creatinine, ALT, CRP, Duration of illness, Exercise, BMI, Residence, Sex, Occupation, Dietary protein source, Dietary carbohydrate type and Dietary fat content. Then nature of the variables are listed in table below.

Table 2.8.1a: List of all the variables that were analysed in the study.

Variable	**Type of variable**	**Units**
Outcome variable		
Lipoprotein (a) [Lp(a)]	Continuous	Mg/dl
Independent variables		
Age	Continuous	years
FBS	Continuous	Mmol/l
HBA1c	Continuous	%
Total Cholesterol	Continuous	Mmol/l
Triglycerides	Continuous	Mmol/l
LDL	Continuous	Mmol/l
HDL	Continuous	Mmol/l
Urea	Continuous	Mmol/l
Creatinine	Continuous	Mmol/l
ALT	Continuous	IU
CRP	Continuous	Mmol/l
Duration of illness	Continuous	years
Exercise	Ordinal	
BMI	Ordinal	Kg/m^2
Residence	Ordinal	
Sex	Nominal	M/F
Occupation	Nominal	
Dietary protein source	Nominal	

Dietary carbohydrate type	Nominal	
Dietary fat content	Ordinal	

The factors that were used to assess the predictors of Lp(a) levels among the T2DM patients included; renal function measured by urea and creatinine, liver function measured by ALT, lipid profile measured by LDL, HDL, triglycerides and total cholesterol. Acute phase response was measured by CRP. Dietary characteristics were assessed by dietary fat content, dietary carbohydrate type and protein source in diet. On the other hand the social economic status was assessed by occupation and residence whereas glycemic control was measured by HBA1c and physical fitness by frequency of exercise. Finally, social demographic characteristics were assessed by age, sex, and BMI as summarized in the table below.

Table 2.8.1b: List of features assessed to investigate the factors that influence the Lp(a) levels

Factors assessed	**Measurements**
Social demographic characteristics	Age Sex BMI
Fasting blood sugar	FBS
Glycemic control	HbA1c
Duration of DMT2 illness	Years
Lipid profile	LDL HDL TG TC
Hapatic function	ALT
Renal function	Urea Creatinine
Acute phase response	CRP
Dietary composition	Dietary fat content Dietary carbohydrate type Protein source in diet
Physical fitness	Frequency of exercise
Social economic status	Occupation Residence

3.9.2 Data analysis

Descriptive analysis: The data was expressed as median and interquartile range, for continuous variables and proportions (percentages) for categorical variables. Mann-Whitney U test was used to compare the median values between the study group and the controls (Diabetics and healthy individuals) for continuous variables, whereas the Chi-squared test was used to compare the proportions for the nominal data. Wilcoxon rank-sum test for the ordinal data.

Inferential analysis: Automatic linear modelling was used to identify the combination of independent variables that best predicted the outcome variable. Assumptions of multiple linear regression; normality, goodness of fit, homoscedasticity and outliers were met. Eight outliers were identified and trimed whereas independent variable data were transformed.

Linear modelling was "forward variable selection" method meaning variables were added one at a time and at each stage regression assumption were tested. All potential independent variables were assessed for co-linearity and also tested for interactions. A significance level of 0.05 was used to include or exclude factors in the final equation.

IBM SPSS Statistics version 19 for windows and Microsoft Excel 2013 was used to analyse the data. Results were summarised on to tables and graphs as given below. All statistical tests were performed at 5% significance level or 95% confidence interval and differences were considered significant if 2-tailed $p<0.05$.

CHAPTER 3

3.0. **Results**

3.1 Descriptive analysis

3.1.1 Social demographic characteristics

The median ages of the two groups of the study participants were significantly different (*p* <0.001, with the diabetics [56(44-63) years] being much older than the healthy individuals [31(24-38) years] (table 3.1.2a).

The proportions in sex and BMI between the diabetics and healthy individuals were similar with no significant difference (p= 0.993 and p= 0.292 respectively). However, the female proportion in both groups were more than the males (figure3.1.1a) and the majority of the BMI proportions among the healthy individuals were normal (46.1%) whereas the majority of the proportions among the diabetics were overweight (36.7%) and obese (30.4%)(figure 3.1.1b).

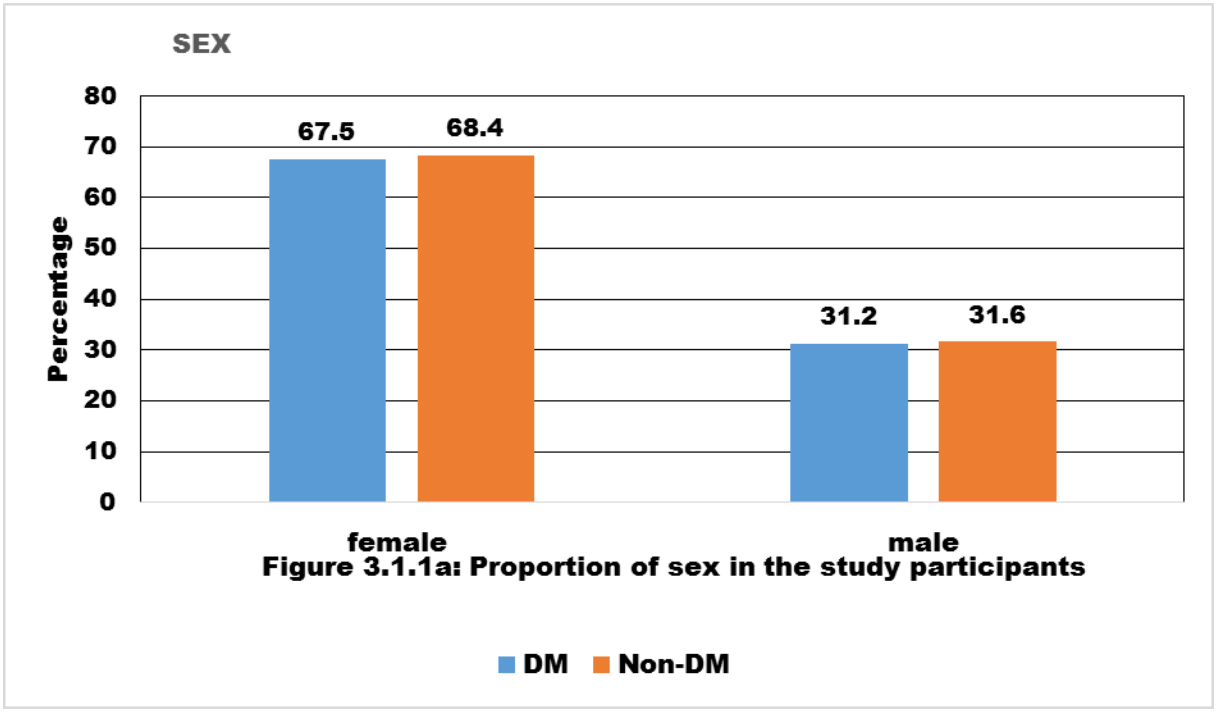

Figure 3.1.1a: Proportion of sex in the study participants

p-value= 0.993

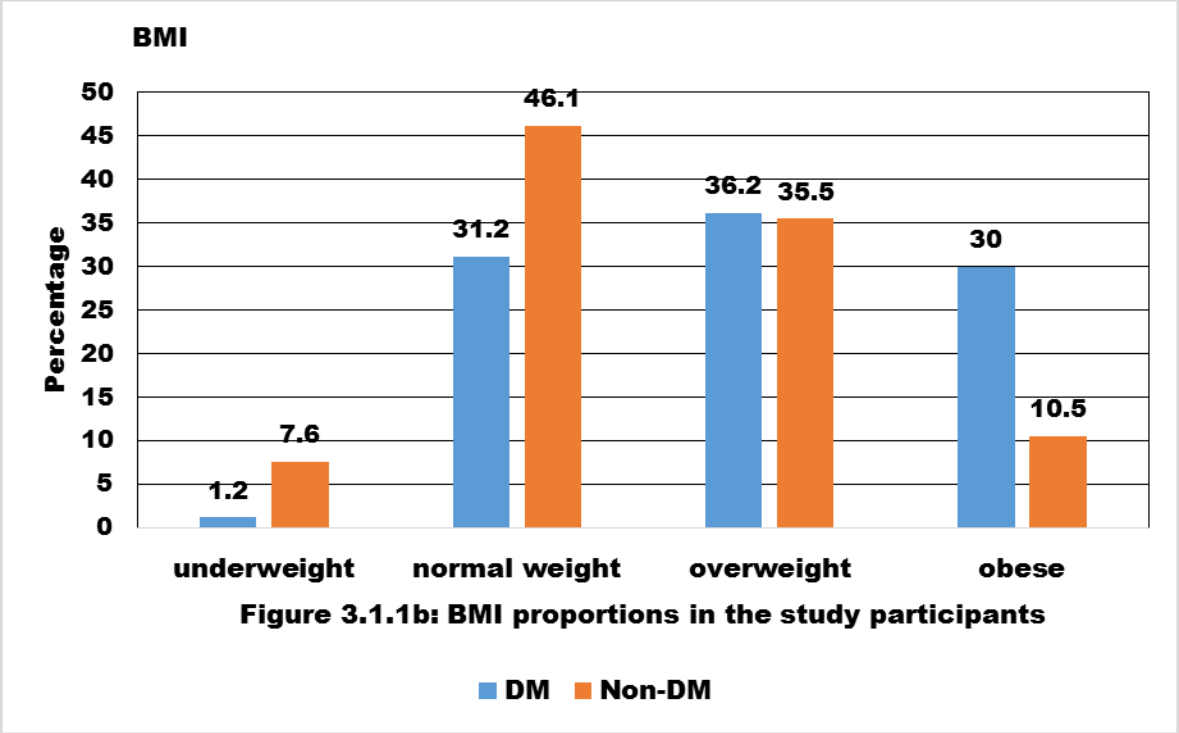

Figure 3.1.1b: BMI proportions in the study participants

P= 0.292

3.1.2 Clinical/ laboratory

Lipoprotein (a), fasting blood sugar and glycaemic control and duration of T2DM illness

The levels of Lp(a) were significantly higher (p<0.001, in the diabetics (20.0 (11.8-37.4) mg/l) than that of the healthy individuals (13.6 (9.4-21.5) mg/l) (table 3.1.2a). The distribution of the diabetics across the risk stratification range shows that 17% have Lp(a) levels higher than 30mg/dl, a cut-off point for cardiovascular risk (figure 3.1.2a). The control of glycaemia was better in the healthy individuals (4.9 (4.7-5.2) %]) than the diabetics (6.6 (5.5-7.9) %,) with a significant difference (p<0.001). In a similar manner, the fasting blood sugar of the diabetics (9.8 (6.9-13.1)mmol/l) was more than the healthy individuals (4.8 (4.5-5.3)mmol/l) (p<0.001,). The median time frame of illness among the diabetics was 2 (4-9) years (table 3.1.2a).

Table 3.1.2a: Description of all the continuous variables

Variable	Study participants	Median	Interquartile range	*p-value*
Age (yrs)	Diabetics	56	44-63	<0.001
	Healthy individuals	31	24-38	
FBS (mmol/l)	Diabetics	9.8	6.9-13.1	<0.001
	Healthy Individuals	4.8	4.5-5.3	
Lp(a) (mg/dl)	Diabetics	20.0	11.8-37.4	<0.001
	Healthy Individuals	13.6	9.4-21.5	
HBA1c (%)	Diabetics	6.6	5.5-7.9	<0.001
	Healthy Individuals	4.9	4.7-5.2	
TC (mmol/l**)**	Diabetics	3.6	0.6-1.1	0.930
	Healthy Individuals	3.7	0.6-0.9	
TG (mmol/l**)**	Diabetics	1.1	0.7-1.4	0.028
	Healthy Individuals	0.9	0.7-1.1	
LDL (mmol/l**)**	Diabetics	2.0	1.5-2.7	0.002
	Healthy Individuals	2.5	2.0-2.9	
HDL (mmol/l**)**	Diabetics	0.8	0.6-1.1	0.120
	Healthy Individuals	0.7	0.6-0.9	
Urea (mmol/l**)**	Diabetics	4.1	3.2-5.2	<0.001
	Healthy	3.2	2.8-3.9	

	individuals			
Creatinine (mmol/l)	Diabetics	76.5	66.8-92.7	0.266
	Healthy Individuals	116.3	105.5-133.7	
ALT (IU)	Diabetics	2.9	1.3-4.7	0.266
	Healthy Individuals	2.3	1.5-3.5	
CRP (mmol/l)	Diabetics	3.5	1.5-7.2	0.266
	Healthy Individuals	1.2	0.4-4.0	
Duration of illness (yrs **)**	Diabetics	2	4-9	

r= 0.1 small; 0.3 medium; 0.5 large

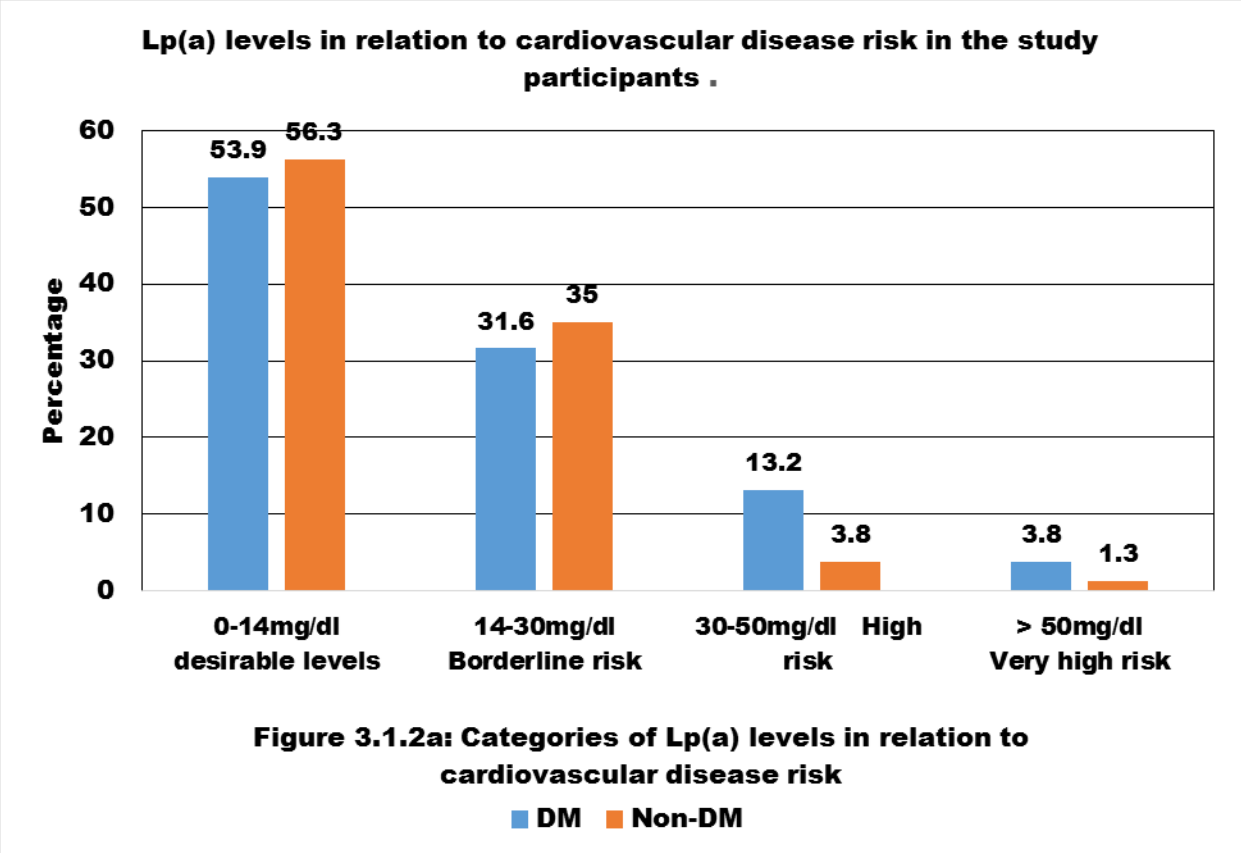

Figure 3.1.2a: Categories of Lp(a) levels in relation to cardiovascular disease risk

3.1.3 Lipid profile

The difference in the lipid profile between the two groups of the study participants was significant for triglycerides and LDL (*p= 0.028 and p=0.0025* respectively*)* having slightly higher values of triglycerides among the diabetics [1.1 (0.7-1.4)mmol/l] compared to the healthy individuals [0.9(0.7-1.1) mmol/l] whereas the LDL values among the healthy individuals [2.5(2.0-2.9)mmol/l] are higher than the diabetics [2.0(1.5-2.7)mmol/l]. The values in the HDL and total cholesterol levels are similar with no significant difference between the two groups (table 3.1.2a).

3.1.4 Renal function, hepatic function, acute phase response and physical fitness.

The renal function of the study participants was similar between the two groups with no significant difference in the creatinine clearance but a significant difference in the urea levels, however, there was no significant difference in the hepatic function and acute phase response between the diabetics and healthy individuals. (table3.1.2a).

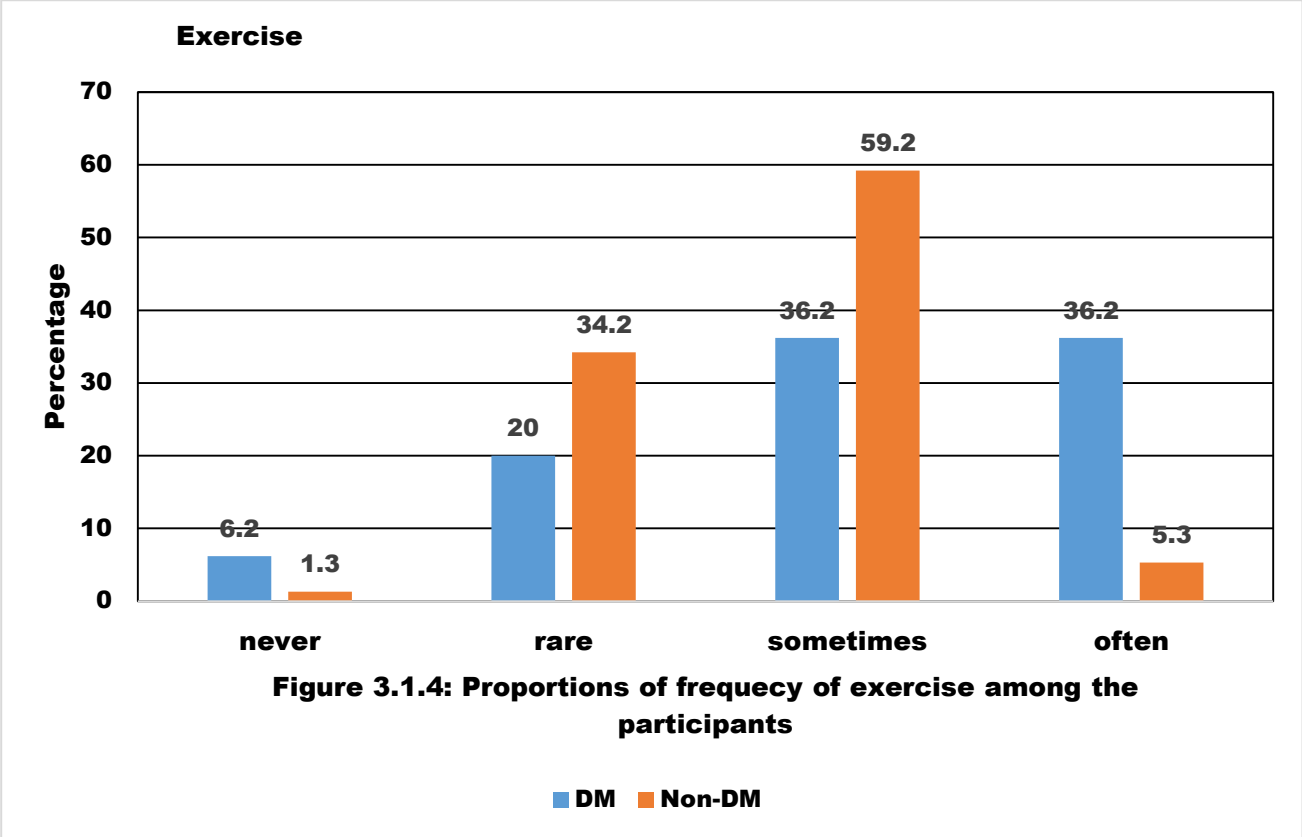

Figure 3.1.4: Proportions of frequecy of exercise among the participants

p <0.001

Figure 3.1.4 shows the proportions of the frequency of exercise among the study participants. There is a moderate significant difference (*p<0.001*) in the exercise patterns between the two groups. There was a higher proportion of diabetics (36%) who exercise more often as compared to the healthy individuals (5%).

3.1.5 Dietary composition

The fat content in diet and the major carbohydrate source in the diets of the participants showed a significant difference ($p < 0.001$ and $p<0.001$, respectively) between the two groups meanwhile the difference in the protein source in their diets was not significant as shown in the graphs below.

The results show that the major protein source in the participants was animal and plant based (figure 3.1.5a). 89% of the diabetics had a low content of fat in their diets with 80% of the healthy individuals having a higher content (figure 3.1.5b).

Refined or polished carbohydrates was the main source for both groups with the more of the diabetics (17.5%) using unpolished or complex carbohydrates compared to the healthy individuals (1.3%) (figure 3.1.5c).

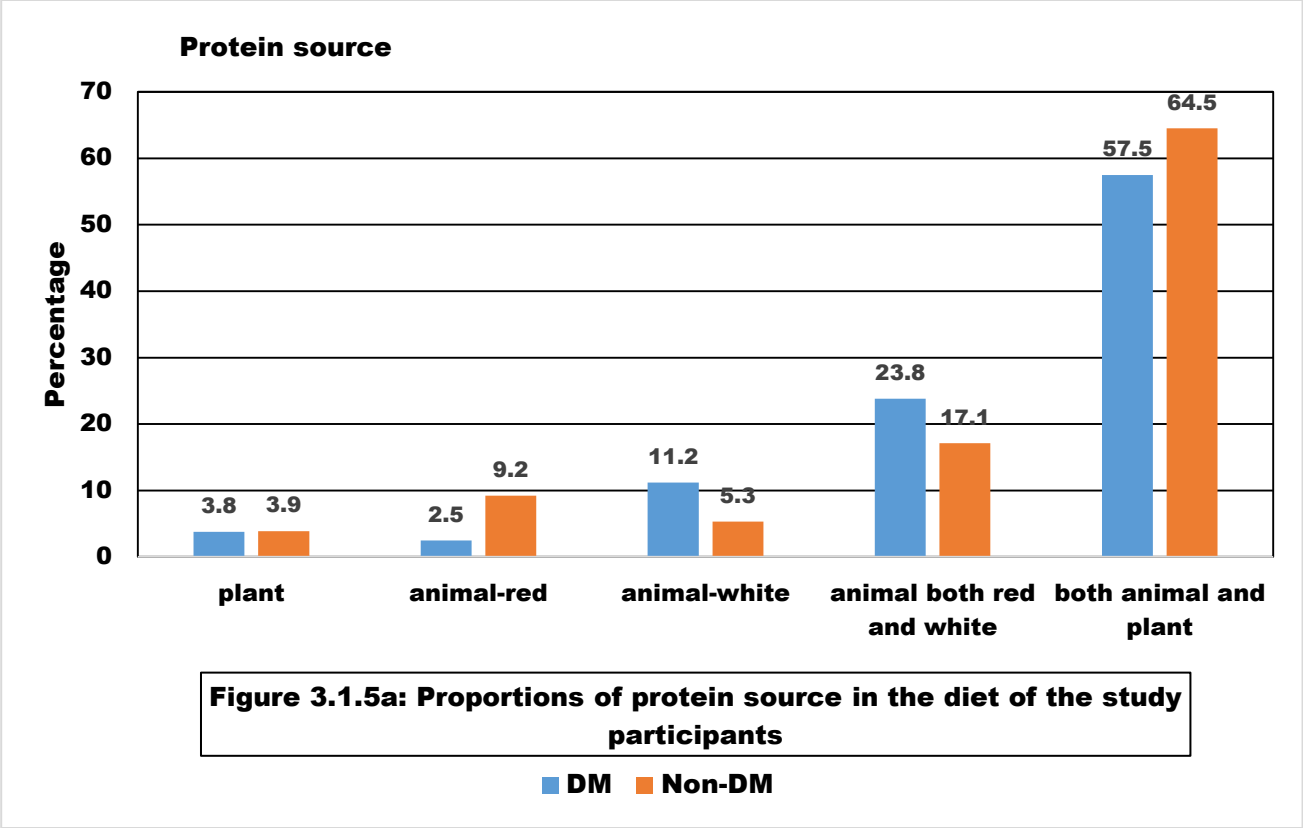

Figure 3.1.5a: Proportions of protein source in the diet of the study participants

p <0.195

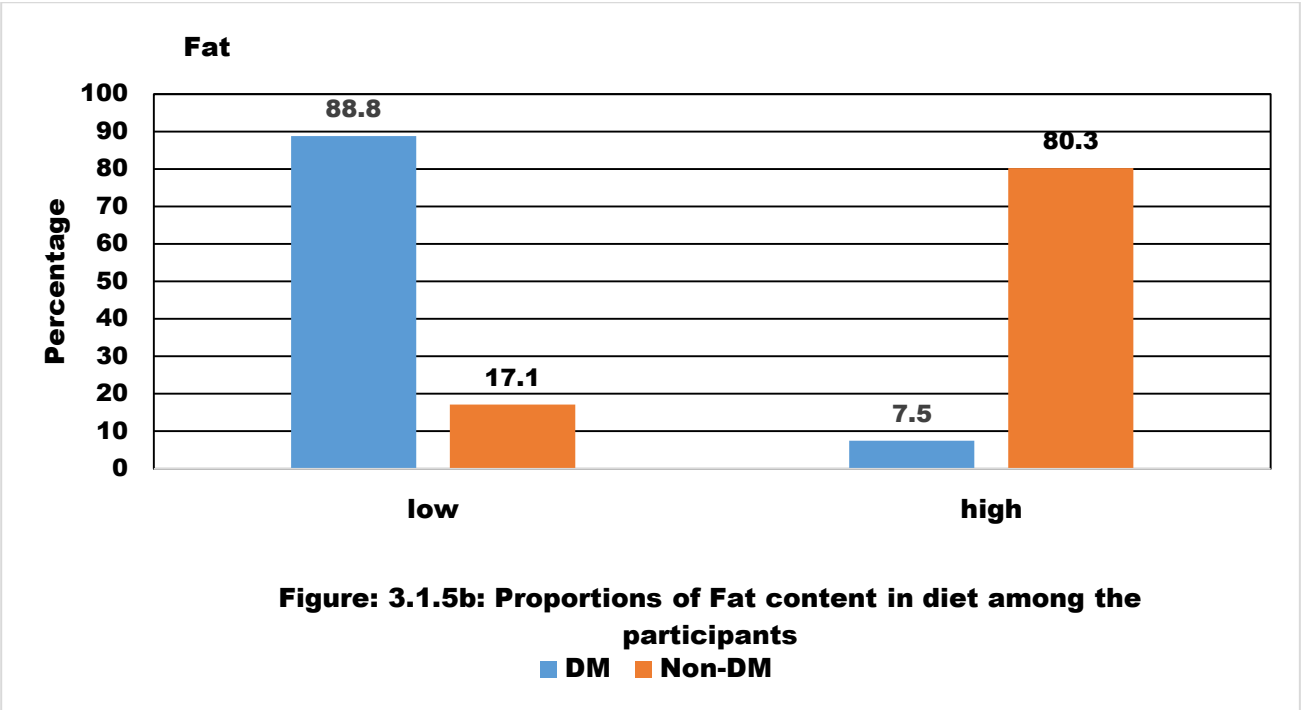

Figure: 3.1.5b: Proportions of Fat content in diet among the participants

p <0.001

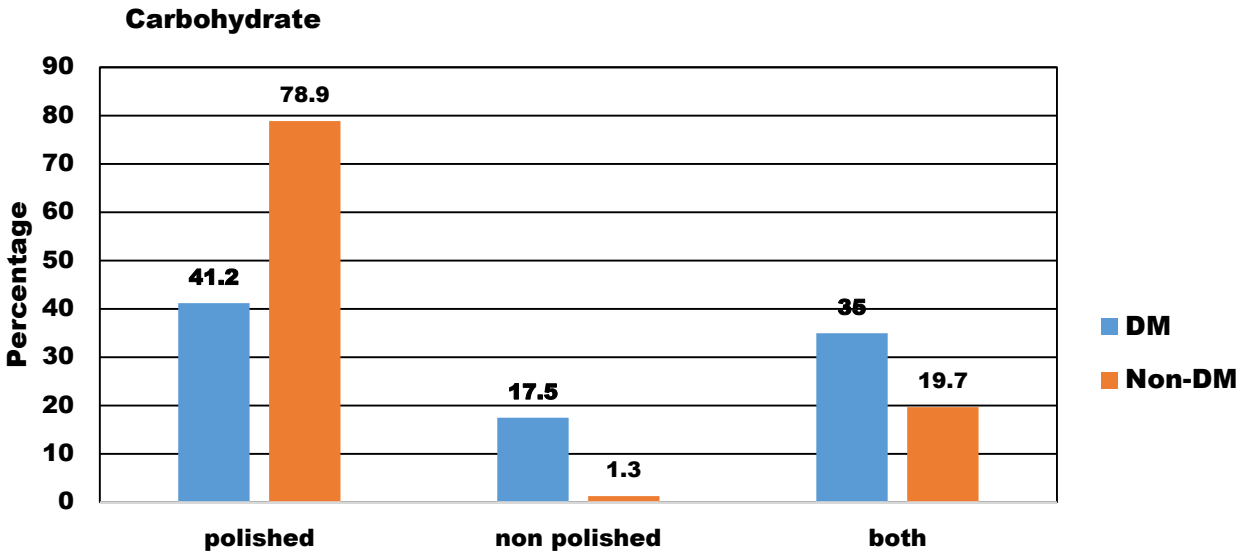

Figure 3.1.5c: Proportions of carbohydrate source in the diet of the participants

$p < 0.001$

3.1.6 Social economic status

There was a significant difference in the social economic status between the two groups of the study participants measured by occupation ($p < 0.001$) and residence(p<0.001) as seen in figure 3.1.6 and table 3.1.6 shown below.

A low income was considered to be any income below ZMR3000, moderate Income as ranging from ZMR3000 to ZMR9000 and a high income as above ZMR9000

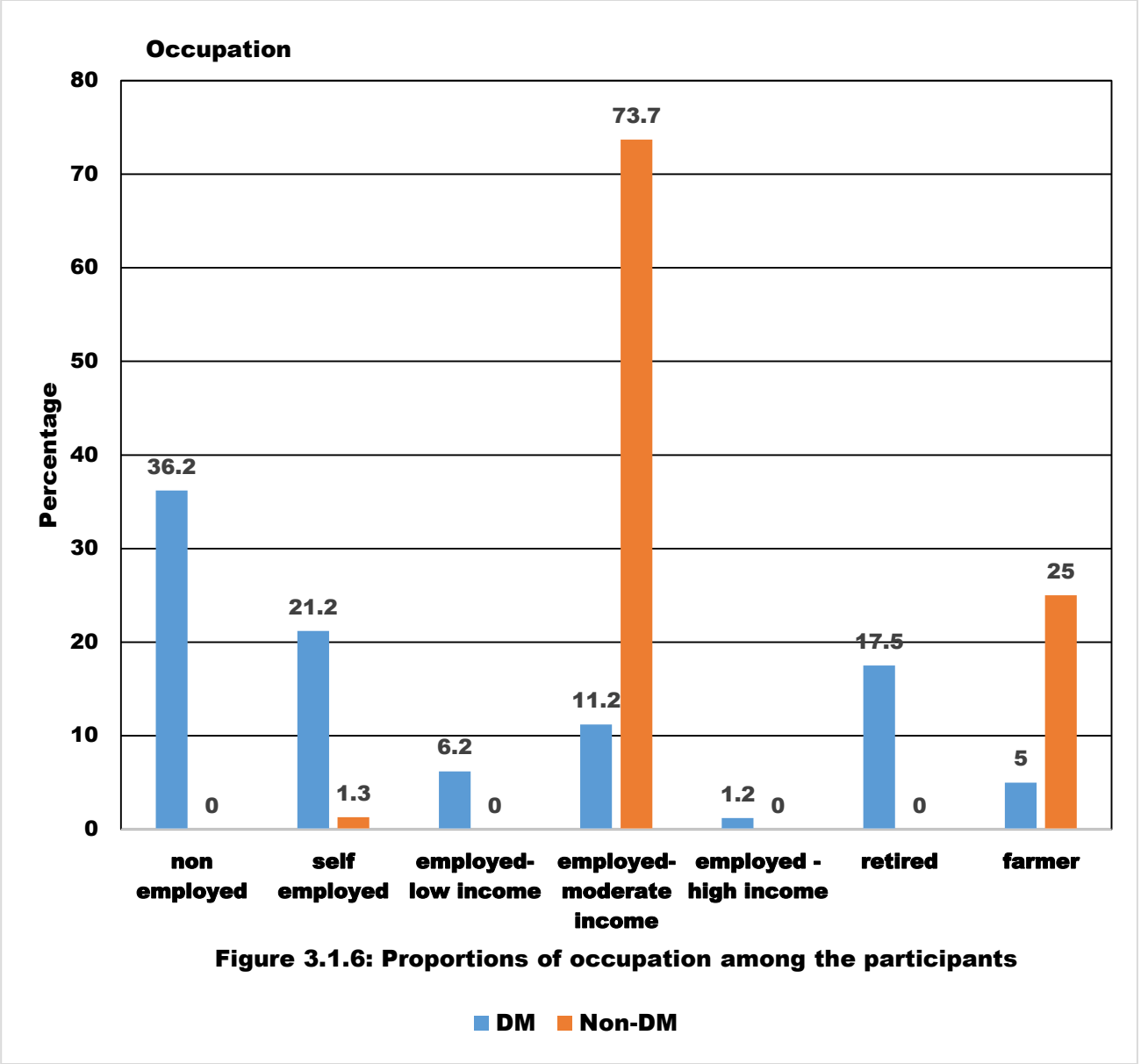

Figure 3.1.6: Proportions of occupation among the participants

p <0.001

Generally the results show that the healthy individuals were of a higher social economic status with majority of them (73.7%) being employed with a moderate income whereas the majority of the diabetics were not employed (36.2%), self-employed (21.2%) or retired (17.5%).

The type of residence also showed a similar picture as above in which the majority diabetics were observed to be from high density residence (53.2%) whereas the healthy counterparts were mostly from moderate density residence (71.1%).

Table 3.1.6: The proportions of the residence among the study participants

RESIDENCE			**Low density**	**Moderate density**	**High density**
Study participants	Diabetics (n=79)	Count	13	24	42
		Percentage	16.5%	30.4%	53.2%
	Healthy individuals (n=76)	Count	16	54	6
		Percentage	21.1%	71.1%	7.9%

$p<0.001$

3.2 Inferential analysis

3.2.1 Multiple regression analysis

A multiple regression model was done by use of the automatic linear modelling to identify the combination of the response variables that best predict serum Lp(a) levels as the target. The response variables were added into the model in a forward stepwise manner. 4 response variables were included in the final equation namely: glycaemic control (HbA1c), fasting blood glucose (FBS), triglycerides and social economic status (residence) $p<0.001$,$p=0.030$,$p=0.040$,$p=0.004$ respectively (table 3.2.1a). Adjusted R^2 was .0.357 and the intercept 1.643.

Glycemic control was the most important predictor of Lp(a) in the regression equation with a coefficient of 5.220 (table 3.2.1a). Hence every unit rise in HbA1c level predicts an increase in Lp(a) levels by a factor of 5.220.

Moderate social economic status (moderate density residence) is associated with lower Lp(a) levels. There was a positive association between FBS (glucose) and Lp(a) levels (coefficient 0.660), whereas the relationship between Lp(a) and triglyceride levels was inverse (coefficient -4.794).

Table 3.2.1a: list of significant predictors of Lp(a)

Coefficients

Target: Lipoprotein (a)

Model Term	Coefficient ▼	Std.Error	t	Sig.	95% Confidence Interval		Importance
					Lower	Upper	
HBA1c_transformed	5.220	0.980	5.328	.000	3.284	7.156	0.454
Residence_transformed=2	-7.165	2.462	-2.911	.004	-12.031	-2.299	0.136
FBS_transformed	0.660	0.300	2.197	.030	0.066	1.253	0.077
Trig_transformed	-4.794	2.310	-2.075	.040	-9.359	-0.228	0.069

CHAPTER 4

4.1. Discussion

4.1.1 Lipoprotein (a) levels in Diabetes Mellitus Type 2 patients

The results of this study showed that the median serum levels of Lp(a) in the T2DM patients were higher than those of healthy individuals (20.0 vs 13.6 mg/dl) and the difference was significant ($p<0.001$) with a moderate effect size ($r=0.28$). However, a total of 17 % of the T2DM patients had serum Lp(a) levels higher than 30mg/dl (figure 3.1.2a). The results show that Lp(a) levels were elevated in T2DM patients as compared to the healthy population. This is in agreement with findings from other studies[33, 34, 35].

The relationship between Lp(a) and diabetes mellitus has not been well established. Regarding type 1 diabetes mellitus, some studies have reported higher Lp(a) levels[36], which have not been confirmed by other studies [19]. Conflicting results have also been reported for type 2 diabetes mellitus. In a sub-study carried out from the San Antonio Heart Study, diabetic men and women showed no difference in Lp(a) concentrations when compared with non-diabetic individuals[37]. On the other hand, a prospective study carried out with 26,700 North-American women had shown a higher incidence of type 2 diabetes mellitus among those with lower Lp(a) levels[18].

The variations and controversies in the results observed in different studies may be as a consequence of both the study design and the analytical methods used [38] and also because plasma concentrations of Lp(a) are genetically influenced.

Plasma concentrations of Lp(a) have a hereditary character, with large interindividual variation, being not altered by environmental factors, and tending to remain constant throughout life. In the general population, Lp(a) concentrations can range from < 1 mg/dL to > 1,000 mg/dL [39].

LP(a) has also been identified as a strong independent risk factor for CVD in many different populations and ethnicities [46]. High serum Lp(a) levels are a powerful risk factor for coronary artery disease both in the general population and patients with diabetes[47]. However, despite advances made in the prevention and management of

cardiovascular disease, people with diabetes mellitus continue to have alarmingly high morbidity and mortality secondary to cardiovascular disease [48].

Epidemiologic studies have demonstrated that diabetes mellitus is an independent risk factor for cardiovascular disease and that it amplifies the effects of other common risk factors, such as smoking, metabolic syndrome[49], [50] and elevated Lp(a) levels [47] The mortality associated with a coronary event in people with diabetes mellitus is significantly higher than the mortality in non-diabetic individuals [51] .

Lipoprotein (a) levels over 20-30 mg/dL are associated with a two-fold risk of developing coronary artery disease [52.] Several mechanisms of Lp(a) participation in atherogenesis have been proposed. One of them consists in the direct deposition of that lipoprotein on arterial wall, similarly to that which happens with LDL and oxidized LDL. The fact that Lp(a) is more likely to undergo oxidation than LDL itself might facilitate uptake by macrophages via scavenger receptors[53]. That is the most universal mechanism of atherogenesis, in which macrophages 'indulge themselves' in the cholesterol from LDL, and eventually from Lp(a), transforming themselves into foam cells, precursors of atherosclerosis.

The pathogenicity of Lp(a) in cardiovascular disease is due to its strong atherogenic and pro- inflammatory properties [54] .

Its ability to stimulate smooth muscle proliferation appears to contribute to atherosclerotic plaque formation [55]. Another pro-atherogenic mechanism of Lp(a) would relate to the inverse correlation between that lipoprotein levels and vascular reactivity, in which case the increase in Lp(a) plasma levels would induce endothelial dysfunction [39] . Due to its structural resemblance to plasminogen and tissue plasminogen activator it has the ability to competitively inhibit fibrinolysis and this property together with its ability to promote thrombogenesis may contribute to its association with myocardial infarction (MI) and ischaemic stroke [56,57].

4.1.2 Factors influencing the serum levels of Lipoprotein (a) in Diabetes Mellitus Type 2 patients.

Biochemical factors

The plasma Lp(a) concentration is predominantly, considered to be genetically determined[58,59] , but a number of factors may also influence an increase or decrease of the plasma concentrations.

Increases in Lp(a) levels can be transient in the presence of inflammatory processes or tissue damages, such as those occurring with other acute phase proteins (haptoglobin, alpha-1-antitripsin, and C-reactive protein)[60]. This can follow an episode of acute myocardial infarction, in which Lp(a) levels increase considerably in the first 24 hours, returning to baseline values in approximately 30 days[61]. Lp(a) levels are increased in chronic inflammatory disease, such as rheumatoid arthritis[62], systemic lupus erythematosus[63], and acquired immunodeficiency syndrome[64], and under some conditions, such as after heart transplantation[65], chronic renal failure[66], and pulmonary arterial hypertension[67] On the other hand, liver diseases and abusive use of steroid hormones decrease Lp(a) levels [66].

The results of our study shows FBS levels are positively associated with Lp(a) levels ($p=0.030$, $r=0.660$). Hence a series of elevated FBS measurements could therefore predict levels of Lp(a). This is supported in some findings[68,69] though others do not agree[35]. However, poor glycemic control which is assessed by serum levels of HbA1c, was seen to be positively associated with elevated Lp(a) ($p<0.001$,$r=5.220$). Lp(a) levels were elevated in poorly controlled glycaemia. Our study showed that glycemic control was the strongest predictor of serum Lp(a) levels. This is in agreement with a few studies but contradicted by many.

It was reported that poorly controlled diabetes mellitus was associated with a high Lp (a) levels[70] it has also been observed that the prolonged hyperglycemia significantly increased the concentration of Lp(a)[71]. On the contrary, other workers[72,73, 74,] report that there is no association between glycemic control and Lp(a)levels.

The effect of hyperglycemia on the rate of synthesis, transcription, and translation of apo(a) is still not exactly known. However, this association maybe due to the increase in the non-enzymatic glycation of proteins seen in chronic hyperglyceamia. It has been observed that the concentration of glycosylated Lp(a) is increased in the circulation in diabetic subjects [75,76] and it is evident from many studies that glycosylation prolongs the half-life of lipoproteins and this may be true for Lp(a), which may lead to higher levels of Lp(a) in diabetes.

Diabetes type 2 is characterized by an impairment of the ability of insulin to stimulate glucose uptake and inadequate compensation for insulin insensitivity [77]. The increased risk of atherosclerosis in T2DM consists of multiple factors. Diabetes-related changes in plasma lipid levels are among the key factors that are amenable to intervention. The spectrum of dyslipidemia in T2DM can include all the various types of dyslipidemia identified in the general population [78] however, one phenotype is particularly common in T2DM, which is attributed mostly to insulin resistance and insulin deficiency[79–82]. The characteristic features of this phenotype are a high plasma triglyceride concentration, low HDL-C concentration and increased concentration of small dense LDL–C particles.

Hypertriglyceridemia in T2DM, results from increased plasma concentrations of (Very Low Density Lipoprotein) VLDL [83], deficient lipoprotein lipase activity; increased cholesteryl ester transfer protein activity; and increased flux of free fatty acids to the liver[84].

The results of our study showed that there was an inverse relationship between the serum Lp(a) and triglyceride levels ($p=0.040$, $r= -4.794$). This is in agreement with the findings of a study in which they reported that Lp(a) concentrations were negatively correlated with triglyceride levels and directly with LDL-C levels[85]. A similar report indicated that decreased plasma Lp(a) levels in hypertriglyceremic patients as compared to the controls[57]. The precise mechanism underlying this association is not well known.

However, we assume that this inverse relationship could suggest that increased levels of triglyceride-rich lipoproteins may influence the metabolism of Lp(a)[57].

The median triglyceride levels among the diabetics was 1.1 (0.7-1.4) mmol/l, whereas the concentrations of TC, LDL-C, and HDL-C were also within the normal range (table 3.1.2a). According to WHO and NCEP (National Cholesterol Education Program) the desirable levels for the lipid profile are as follows: TG < 2.26, TC< 5.17, LDL-C< 3.36, HDL-C< 1.55. This suggests that these patients could be responding to their lipid lowering drugs, very well. However the elevated Lp(a) levels confirms reports that this analyte does not readily respond to the traditional lipid lowering drugs [26,28,34,39] hence we assume that effective management of diabetic dyslipidaemia is not associated with reduced Lp(a) concentrations, thereby, predisposing a patient to cardiovascular complications despite proper glycemic and lipid control.

Social economic factors

Socioeconomic status (SES) is an economic and sociological combined total measure of a person's work experience and of an individual's or family's economic and social position in relation to others, based on income, education, and occupation[86] . Socioeconomic status is typically broken into three categories, high SES, middle SES, and low SES Socioeconomic status underlies three major determinants of health: health care, environmental exposure, and health behavior.

Low income and little education have shown to be strong predictors of a range of physical and mental health problems, ranging from respiratory viruses, arthritis, coronary disease, and schizophrenia[2] whereas the more affluent are more likely to suffer from non-communicable diseases such as Hypertension and diabetes merely due to sedentary lifestyle and obesity[2].

Our findings showed that there was a significant relationship between Low Lp(a) and medium density residence which is associated to the middle SES. The middle SES individuals consist of individuals of medium income and a good education. Hence it is believed that this group of people are well informed concerning their health conditions and are likely to be in a better position to control their glycaemia very well, through effective life style modifications and consistent health care access.

4.2. Conclusion

The study showed that the plasma levels of Lp(a) in the T2DM were significantly higher than the non-diabetic healthy individuals. Glycemic control (HbA1c) , triglycerides (TG), fasting blood sugar (FBS) and social economic status (medium density residence) were predictors of serum levels of Lp(a). However, serum levels of L(a) were shown to be positively related to HbA1c and FBS but negatively related to TG. Whereas medium density residence was associated with low serum levels of Lp(a)

4.3. Recommendations

The mainstay of T2DM management is glycemic and lipid control. However, there is evidence that serum levels of Lp(a) do not respond to the traditional lipid lowering drugs . Hence amid tight management, T2DM patients are still at risk of developing atherocardiovascular complications. It is hereby proposed that Lp(a) be included on the lipid profile and the use of elevated HbA1c and FBS as effective markers of elevated serum levels of Lp(a).

4.4. Future study prospects

This was an analytical cross-sectional study. However, the study group and the controls were not matched on the basis of age and social economic status (occupation, income, education). It is hereby suggested that a larger cohort study with tightly matched study participants, though usually difficult to do, be conducted in order to fully understand the factors that influence the serum levels of this Lipoprotein(a). Furthermore, it is generally accepted that 30mg/dl of serum Lp(a) is the cut-off value for cardiovascular risk. However there is need to establish whether at this value, in our population, cardiovascular problems have occurred or not and if so, determine a different level.

4.5. References

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4.6 APPENDICES

4.6.1 Exclusion criterion explanations

7.1.1 Renal failure will be considered if, Serum Creatinine > 240umol/l and/or Urea > 30 umol/l.

7.1.2 Severe liver dysfunction will be considered if ALT > 200 IU/l, AST > 200 IU/l and Albumin < 28g/l (*According to Child-Pugh Classification of Severity of Liver Disease*)

7.1.3 Major surgery includes; laparotomy, hysterectomy, cholecystectomy, Thyroidectomy, Abdominal operations involved with cutting and resecting.

7.1.4 Pregnancy will be determined through questioning the Last Menstrual Period (LMP) for women in child bearing age.

4.6.2 Information sheet

I am Sinyani Angela conducting a research in fulfilment of my project requirements for the Master of Science in Pathology (Chemical Pathology) at the University of Zambia.

My study is looking at blood levels of a special compound called Lipoprotein (a). This substance is high in some patients who do not control their sugar very well. When it is high it gives us a sign that blood vessels are being damaged and the patient must control his/her sugar before a stroke or heart attack a sore on the leg occurs.

To do this, I will ask you a few questions using the form I have. If you are agreeable some small amount of blood (4 mils- show bottle), will then be collected from you. Lipoprotein (a) is measured in blood.

You have been identified to take part in this study because you are a diabetic patient attending this clinic and also based on the clinician's assessment

according to the study's selection criteria which includes lack of liver and renal dysfunction.

This is entirely voluntary and be assured that the information you will provide and also the results of the test will be strictly confidential. The results of the blood tests will be communicated to you through the clinician in the next appointment and they will be helpful in the management of your diabetes.

Nevertheless you have the right to seek further clarification or to withdraw. For further information you may contact the following;

a) Sinyani Angela :
The researcher
TEL; 0979-313630 Email Address; sinyanim@gmail.com

b) Dr Soka Nyirenda:
Principle Supervisor
TEL; 0977-842692 Email Address; so_kany@yahoo.com

c) Theresa Chisoso:
The Secretary
University of Zambia Biomedical Research Ethics Committee (UNZABREC):
TEL; 260-1-256067 Email Address; unzarec@zamtel.zm

4.6.3 Consent form.

PATIENT CONSENT (for patients aged 16 and above) .

I ……have agreed to take part in this research which is studying *The levels of Lipoprotein (a) and its Determinants among Diabetes Mellitus type 2 patients* in order to prevent or limit cardiovascular diseases. I agree to provide necessary information and a small amount of blood.

Participant signature……

Date……..

Participant thumbprint in the box above

Name of researcher: Sinyani Angela

Sign……

For further clarification you may contact the following:

d) Sinyani Angela :
The researcher
TEL; 0979-313630 Email Address; sinyanim@gmail.com

e) Dr Soka Nyirenda:
Principle Supervisor
TEL; 0977-842692 Email Address; so_kany@yahoo.com

f) Theresa Chisoso:
The Secretary
University of Zambia Biomedical Research Ethics Committee (UNZABREC):
TEL; 260-1-256067 Email Address; unzarec@zamtel.zm

4.6.4 Assent form

PARENT/ GUARDIAN ASSENT (for patients below the age of 16.)

I ……have allowed my child/ dependant to take part in this research which is studying *The levels of Lipoprotein (a) and its Determinants among Diabetes Mellitus type 2 patients* in order to prevent or limit cardiovascular diseases. I agree that he/she provide necessary information and a small amount of blood.

Signature……

Date……

Participant Thumbprint in the box above

Name of researcher: Sinyani Angela

Sign……

For further clarification you may contact the following:

g) Sinyani Angela :
The researcher
TEL; 0979-313630 Email Address; sinyanim@gmail.com

h) Dr Soka Nyirenda:
Principle Supervisor
TEL; 0977-842692 Email Address; so_kany@yahoo.com

i) Theresa Chisoso:
The Secretary
University of Zambia Biomedical Research Ethics Committee (UNZABREC): TEL; 260-1-256067 Email Address; unzarec@zamtel.zm

4.6.5 Questionaire

THE UNIVERSITY OF ZAMBIA
PATHOLOGY AND MICROBIOLOGY DEPARTMENT
MSc. Chemical Pathology Research Questionnaire

Title:

FACTORS ASSOCIATED WITH ELEVATED PLASMA LEVELS OF LIPOPROTEIN (a) IN INDIGENOUS BLACK ZAMBIANS WITH DIABETES MELLITUS TYPE 2 IN THE OUTPATIENT MEDICAL CLINIC AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA..

Date……..

Patient ID…… Serial #**.....**

Sex…….. Age……..... Fbs…….. Weight…….

Height……... BMI……....

Demographic Data

A)Marital status……B)Occupation……D)Residence……

Medical History

A) Date of diagnosis of diabetes……

B) Treatment (Drug And Dose):

-Upon diagnosis;……

-Currently; ……**.....**

Life Style

A) Have you ever exercised?

(A) Never…… (B) Rare……(C) Sometimes…… (D) Often……

B) DIET TYPE:

a) fat……. b) protein source…….c) carbohydrate source……

c) Last meal: how long ago was the last meal (hours)……

4.6.6 Specifications of Olympus AU400.

The Olympus AU400 is a fully automated, random access chemistry system. Its analytical principle is spectrophotometry and potentiometry and the analytical methods being: Colorimetry, turbidimetry, latex agglutination, homogeneous Immuno- Assays, indirect ISE (Ion Selective Electrolytes). Sample types can be Serum, plasma, urine, CSF to mention but a few.

It can run 400 photometric tests per hour. The reagent chamber is refrigerated (4-12° C) and accommodates 72 reagent bottles. 46 parameters can be analysed at a time. The instrument has two permanent probes: a sample and a reagent probe. The probes have good accuracy and precision (CV<3%). The reaction temperature is 37° C. it analyses at 13 different wavelengths between 340-800 nm. The results can be stored and printed.

4.6.7 Reagent properties

This table summarises the properties of the reagents for each test, the types of calibrators used and the levels of quality control selected. The storage of the reagents affects their stability and performance whilst the calibration and quality control affect the precision and accuracy of the tests respectively. Monitoring these properties ensures the validity of the results.

TEST	STORAGE/STABILITY	CALIBRATION	QUALITY CONTROL
Lp(a)	Stable until expiry date at 2-8°C protected from sunlight	LP(a) calibrator series cat # LP 3404	-Lp(a) control level 3 cat # LP 3406 -Lipid controls level 1 and 3
Triglycerides	Stable until expiry date when unopened and protected from sunlight at 2-8° C. -once open 30 days on board the instrument	System calibrator cat #66300	2 controls cat # ODC0002, ODC0003
Cholesterol	-Stable until expiry date when unopened and protected from sunlight	System calibrator cat	2 controls cat # ODC0002, ODC0003

	at 2-8° C. -once open 90 days on board the instrument	#66300	
LDL	Stable until expiry date when unopened and protected from sunlight at 2-8° C. -once open 30 days on board the instrument	HDL cholesterol calibrator ODC0012	HDL/LDL cholesterol control serum ODC005
HDL	-Stable until expiry date when unopened and protected from sunlight at 2-8° C. -once open 30 days on board the instrument	HDL cholesterol calibrator ODC001	HDL/LDL cholesterol control serum ODC0005
AST	-Stable until expiry date when unopened and protected from sunlight at 2-8° C. -once open 30 days on board the instrument	System calibrator cat #66300	2 controls cat # ODC0002, ODC0003
ALT	-Stable until expiry date when unopened and protected from sunlight at 2-8° C. -once open 30 days on board the instrument	System calibrator cat #66300	2 controls cat # ODC0002, ODC0003
Urea	-Stable until expiry date when unopened and protected from sunlight at 2-8° C. -once open 30 days on board the instrument	System calibrator cat #66300	2 controls cat # ODC0002, ODC0003
Creatinine	Stable until expiry date when unopened and protected from sunlight at 2-8° C. -once open 7 days on	System calibrator cat #66300	2 controls cat # ODC0002, ODC0003

	board the instrument		
Albumin	Stable until expiry date when unopened at 2-25° C. -once open 90 days on board the instrument	System calibrator cat #66300	2 controls cat # ODC0003, ODC0004
$HbA1_c$	Stable until expiry date when unopened and protected from sunlight at 2-8° C. -once open 30 days on board the instrument	HbA1c Calibrators (1-6) (ODR 3032)	HbA1c controls 1& 2 cat # ODC0022
CRP	Stable until expiry date when unopened and protected from sunlight at 2-8° C. -once open 90 days on board the instrument	Serum protein multi-calibrator; ODR 30213	ITA control sera: ODC0014.ODC0015,ODC0016

4.6.8 Test properties

This table summarises the properties of the tests in terms of: the sample type/specimen tube used for each test, the storage/stability of the samples, the analytical methods used for each test, the sensitivity/specificity of the analytical method and the reference ranges of the assays. Each test has specific properties and these properties affect the performance of the test and the accuracy of the results e.g. Sample storage.

Test	**Analytical method**	**Sensitivity/specificity**	**Reference range**	**Sample storage/stability**	**Sample type/ tube**
Lp(a)	immunoturbidimetry	-Lowest detectable concentration 2.4mg/dl	The assay range :2.4-90mg/dl	-14 days-2-8° C	Plasma
Triglycerides	Enzymatic color test	0.01mmol/L	≥5.65mg/dl-very high	-7 days-2-8° C/-2 days 15-25° C	Plasma

Cholesterol	Enzymatic color test	0.07mmol/L	≥240mg/dl -high	7 days-2-8° C	plasma
LDL-C	Enzymatic color test	0.012mmol/L	≥190mg/dl – very high	-7 days-2-8° C/-1 day-15-25° C	Plasma
HDL-C	Enzymatic color test	0.002mmol/L	<40mg/dl- High Cardiovascular risk	-7 days-2-8° C/ -2 days-15-25° C	Plasma
AST	UV Kinetic test	1U/L	-Male- < 50 U/L -Female- < 35 U	-7 days- 2-8℃/-4 days-15-25℃	Plasma
ALT	UV Kinetic test	1 U/L	-Male- < 50 U/L -Female- < 35 U/L	-7 days- 2-8℃ -3 days-15-25℃	Plasma
Urea	UV Kinetic test	0.38mmol/L	17-43mg/l	7 days-2-25° C	Plasma
Creatinine	Jaffe method	Lowest detectable level-	70-129mg/dL	7 days- 2-25℃	Plasma
Albumin	Photometric color test	0.07g/L	35-52g/L	-30 days- 2-8℃ /-7 days-15-25℃	Plasma (separate from cells immediately)
$HbA1_c$	Latex agglutination inhibition assay	Lowest detectable level-0.03g/dl	2.0-4.4%	-7 days- 25° C/ -14 days-2-8℃/ -6 months- ≤ -70℃	Whole blood/ K_2-EDTA
CRP	immunoturbidimetry	Lowest detectable level is estimated at 1.57mg/l	Upper limit 5mg/L	Stable at 2-8℃ for 2 months and at 15-25℃ for 11 days.	Plasma