

“ A correlative study to assess the effect of type 2 diabetes mellitus on lipid profile”



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**FULL TERM TRAINING REPORT SUBMITTED TO
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE
IN
CLINICAL BIOCHEMISTRY
SUBMITTED BY
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LOVELY PROFESSIONAL UNIVERSITY, PUNJAB INDIA**



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CERTIFICATE

This is to certify that the present full term training report entitled " **A correlative study to assess the effect of type 2 Diabetes Mellitus on lipid profile**" is the outcome of the original piece of work carried out by Mr ADNAN MAKHDOOMI (Reg No 11308450) himself under my guidance and the contents of his thesis did not form a basis of the award of any previous degree to him and to the best of my knowledge to anybody also. The thesis has not been submitted by the candidate for any research degree in any other University.

The dissertation is fit for submission to the partial fulfilment of the conditions for the award of **M.Sc. in Clinical Biochemistry**. Further, certified that the candidate in habit and character is a fit and proper person for the award.

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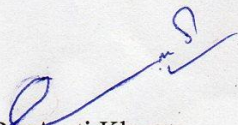
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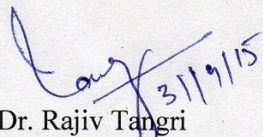
Place:- LPU, Punjab

CERTIFICATE

This is to certify that the work contained in this project report entitled “ **A correlative study to asses the effects of type 2 diabetes mellitus on lipid profile of both the sexes of age group 35-80 years in Delhi region**”. Submitted to the Lovely Professional University as one of the requirements for the Degree of Master of Clinical Biochemistry has been carried out during the academic period January 2015 to April 2015 by **Mr. Adnan Makhdoomi** at the Department of Biochemistry SRL Limited, Gurgaon Haryana, under the supervision of **Dr.Aarti Khanna** (senior Pathologist Biochemistry)

This is further stated that no part of this work has been submitted either in part or in full for any degree or diploma in Lovely professional university and it is original work by candidate


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Declaration

This is to submit that this written submission in the form of full term training report entitled "**A correlative study to assess the effect of type 2 Diabetes Mellitus on lipid profile**" represents original ideas in my own words and where others' ideas and words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the school and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

This thesis encompasses the information generated by me based on experimental work carried out in the **SRL limited Gurgaon**. I assure and hold full responsibility for its genuineness.

Date

Adnan Makhdoomi

Place: Punjab, India

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Place: LPU, Punjab

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Date

List of Abbreviations

DM	Diabetes Mellitus
CVD	Cardiovascular Disease
NEFA	Non Esterified Fatty Acids
CETP	Cholesterol Ester Transport Protein
FBG	Fasting Blood Glucose
S.D	Standard Deviation
LPL	Lipoprotein Lipase
HBA1c	Glycosylated Hemoglobin
HPLC	High Performance Liquid Chromatography
VCS	Variant Chromatographic Station
NAD	Nicotinamide adenine Dinucleotide
mg/dl	Milli Gram Per Deci Litre
nm	Nano meters
CAD	Coronary Artery Disease
NCEP	National Cholesterol Education Programme
ADA	American Diabetes Association

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ABSTRACT

Diabetic patients with consorted dyslipidemia are soft targets of cardiovascular deaths. An early arbitration to normalize circulating lipids shown to reduce cardiovascular intricacy and mortality. Glycated hemoglobin (HbA1c) is a routinely used marker for long term glycemic control. Diabetic patients with type 2 diabetes mellitus are at preeminent risk of developing vascular diseases because of lipid changes. This study was conducted on the Diabetic patients of SRL Limited Gurgaon to evaluate the correlation between the type 2 diabetes mellitus and lipid profile. Fifty (50) patients, 28 (Males), 22 (Females) were included in the study conducted from January through April 2015. The patients were clinically assessed and brief history taken with the aid of questionnaire. The lipid parameters studied were Triglycerides, Total cholesterol, Low Density lipoprotein and High Density lipoprotein. In Diabetes mellitus age and duration of illness are not decisive indices for lipid profile prophecy.

Conclusion:- Outcome of this study showed that most of the patients who participated in the study had their lipid levels perplexed.

Key Words:- Glycated hemoglobin, Diabetes Mellitus

CHAPTER 1

1. INTRODUCTION

Diabetes is a complex disease where the carbohydrate and fat metabolism are impaired. It is a group of metabolic disease characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Diabetes causes about 5% of all deaths globally each year [1]. Diabetes mellitus (DM) has been known to be associated with lipid disorders and cardiovascular intricacy. The risk of chronic aggravation increases as a function of duration of hyperglycemia. The complications usually become ostensible in the second decade of hyperglycemia [2].

Patients with type 2 DM have an atherogenic dyslipidemia characterized by three lipoprotein abnormalities elevated VLDL, elevated LDL and decreased HDL. Diabetic patients with accompanied dyslipidemia are soft targets of cardiovascular deaths. Patients with type 2 diabetes often exhibit an atherogenic lipid profile, which greatly increases their risk of CVD compared with people without diabetes [3]. The risk for CVD is higher in Diabetic subjects than non- diabetic subjects. Glycated hemoglobin (HbA1c) is an ordinarily used marker for long term glycemic control. Patients with type 2 diabetes have an increased preponderance of lipid abnormalities. A timely intervention to normalize circulating lipids could reduce the chances of cardiovascular intricacy. In consonance with its function as an indicator for the mean blood glucose level, HbA1c predicts the risk for the development of diabetic complication in diabetes patients [4].

The lipid abnormalities are prevalent in DM because insulin resistance or deficiency affects key enzymes and pathways in lipid metabolism. In particular, the following processes are affected: Apo-protein production, regulation of lipoprotein lipase, action of cholesterol ester, transfer proteins and hepatic and peripheral actions of insulin [5]. Apart from classical risk factors like dyslipidemia, elevated HbA1c has now been regarded as an independent risk

factor for CVD in subjects with or without diabetes. Estimated risk of CVD has shown to be increased by 18% for each 1% increase in absolute HbA1c value in diabetic population [6].

The aim of this study was to find out association between glycaemic control (HbA1c as a marker) and serum lipid profile of Indian type 2 diabetic patients.[7]. Diabetes is associated with the development of many cardiovascular diseases. Diabetes impairs the utilization of lipids and lipoproteins which cause diabetes induced atherogenic dyslipidemia that is one of the most important risks factor for the development of atherosclerosis in diabetic individuals [8]. Diabetic individuals are more prone to dyslipidemia as compared to normal individuals; therefore, the chance of mortality and morbidity is high in diabetic individuals [9]. Certain modifiable and non-modifiable risk factors contribute in the amelioration of atherosclerosis. Non-modifiable risk factors include age, gender and genetics, whereas modifiable risk factors include obesity, smoking, hypertension, diabetes and dyslipidemia [10].

Age is an important risk factor in atherosclerosis [11]. Cholesterol and lipoproteins levels increase with age in both genders, but the more conspicuous increase has been reported in females than in males [12]. Diabetes is considered a syndrome because of the many symptoms the individuals present with especially if management is not adhered to. Lack of insulin or relatively low insulin levels affects the metabolism of carbohydrate, protein, fat, water and electrolyte balance resulting in diabetes [13]. Patients with diabetes can have a reasonably normal life-style if they are well educated and instigated concerning the disease [14].

Physical exercise has antiatherogenic effects because of its potential benefits on blood pressure levels, glucose tolerance, body weight and composition, plasma lipids, and lipoprotein metabolism,[15]. Exercise reduces HbA1c by an amount that should decrease the risk of diabetic intricacy [16]. Early detection and treatment of hyperlipidaemia in diabetes mellitus can prevent the progression of lipid abnormalities and minimize the risk for

atherogenic cardiovascular disorder and cerebrovascular accident [17]. The worldwide prevalence of diabetes mellitus had risen dramatically. Basing on current trends, the International Diabetes Federation projects that 438 million individuals will have diabetes by the year 2030 [18]. It is estimated that 35 million in our country already have diabetes and it is expected to reach 70 to 80 million by 2030AD. In India the prevalence is 2-4% in rural and 4.0- 11.6% in urban areas [19]. The aim of this study was to find out association between glycemic control (HbA1c as a marker) and serum lipid profile in Delhi's type 2 diabetes patients.

1.1 BIOCHEMISTRY

Type 2 Diabetes mellitus associated lipid abnormalities due to insulin resistance

1.1.1 Hypertriglyceridemia

The reduced action of insulin on adipocytes in fasting and postprandial state reduces suppression of lipolysis, i.e. reduced suppression of disintegration of stored triglycerides, and so greater release of nonesterified fatty acids (NEFA). The increased NEFA delivery to the liver increases hepatic triglyceride production which in turn serves to ramble hepatic VLDL production [20]. Since NEFA from abdominal visceral adipocytes are released into the portal circulation and so pass directly to the liver, the reduced action of insulin on abdominal visceral adipocytes may be relevant. Type 2 diabetes is confederated with visceral adiposity so, sprouting carting of NEFA into portal circulation [21]. The reduced action of insulin in both the fasting and post-prandial states, on hepatocytes results in reduced suppression of VLDL production. In fasting state the major triglyceride carrying lipoprotein is VLDL, the production of VLDL particularly the large, triglyceride rich VLDL (VLDL1) is abolished by insulin [22]. The high circulating insulin level suppresses the VLDL production in post-prandial state. The abridged action of insulin at the hepatocytes level results in failure to burke VLDL production and therefore maximizing post-prandial lipaemia. Insulin

impediment also results in preeminent VLDL production and so hypertriglyceridemia in the fasting state. Hence, in the insulin resistant state, VLDL production is increased both by greater NEFA delivery to the liver, and by reduced insulin-mediated suppression. In both the fasting and post-prandial states, reduced action of insulin on the lipolytic enzyme lipoprotein lipase results in reduced clearance of the triglyceride-rich lipoproteins, VLDL and chylomicrons. This is an important contributing factor to Hypertriglyceridemia in type 2 diabetes. Lipoprotein lipase is an endothelium-bound lipase that hydrolyses the triglyceride carried on lipoprotein particles to release NEFA that are then carried intracellularly [23].

1.1.2 LOW HIGH DENSITY LIPOPROTEIN CHOLESTEROL

Lipoprotein particles are poised of a hydrophobic core subsisting of esterified cholesterol and triglyceride, and a hydrophilic surface, abiding of Apo lipoproteins un esterified cholesterol and phospholipid. The core decreases in size as the triglyceride present in the core promulgating lipoproteins is hydrolysed. This must be consorted by the interrelated loss of surface fixins so that the particle can remain spherical. Apo lipoprotein A1 and phospholipid shed from the surface of VLDL as lipoprotein lipase hydrolyses VLDLcore triglyceride can associate to form nascent HDL particles. This pathway of HDL production is therefore decreased in the insulin resistant state due to decreased lipoprotein lipase activity [24].

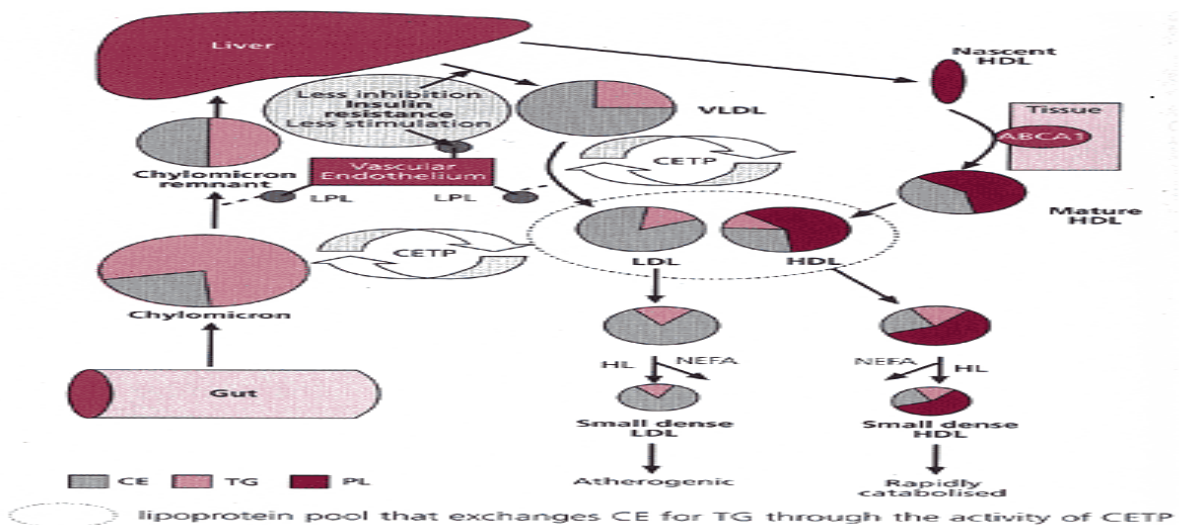


Fig:- Mechanism resulting in Diabetic Dyslipidemia [25].

Cholesterol ester transfer protein (CETP) revamps triglyceride and esterified cholesterol between different lipoprotein species, and between different particles within indivisible lipoprotein species, by reciprocating triglyceride for esterified cholesterol. Triglyceride levels (substrate) are exceeding determinant of CETP activity [26]. Therefore, in the presence of increased triglyceride-rich lipoproteins, CETP activity is increased so that all circulating lipoproteins become enriched in triglyceride, in particular HDL and LDL particles. In the insulin resistant Hypertriglyceridemia state, HDL particles therefore conduce to be small and dense and so more likely to abide catabolism, so that HDL particle numbers and HDL-c concentrations are reduced [27].

1.1.3 PIDDLING DENSE LDL PARTICLES

LDL particles that are triglyceride fortified due to the Hypertriglyceridemia and elevated CETP exertion, are also indoctrinated by the triglyceride lipase activity of hepatic lipase into smaller and crammed particles, whereas large buoyant LDL is unloaded expeditiously by the LDL receptor pathway, small dense LDL is expunged more slowly. Small dense LDL particles are more competently altered by oxidation and, particularly in type 2 diabetes, by glycation, and are more atherogenic [28].

CHAPTER 2

AIMS

AND

OBJECTIVE

CHAPTER 2

AIM

- A correlative study to assess the effect of type 2 diabetes mellitus on lipid profile

Objective

- To study in detail the correlation between Diabetes Mellitus and lipid profile.
- To evaluate effect of type 2 Diabetes Mellitus on Lipid Profile.
- To Assess the complications caused by Diabetes mellitus.
- To assess the complications caused by increased Lipid level

CHAPTER 3
REVIEW
OF
LITERATURE

CHAPTER 3

3.0 Review of Literature

In type 2 diabetes mellitus lipid disturbances are of great importance for the development of cardiovascular diseases which are the arching cause of mortality and morbidity [29]. Dyslipidemia, concorded with type 2 diabetes, is individualized by increased levels of triglycerides (TG), reduced levels of high-density lipoprotein (HDL) cholesterol, while total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol may be either normal or elevated [30]. Diabetes enervates the utilization of lipids and lipoproteins which cause diabetes lured atherogenic dyslipidemia that is one of the most importunate risk factor for the development of atherosclerosis in diabetic individuals [31, 32]. Obdurate hyperglycaemia causes glycosylation of all proteins of arterial wall. This eventually causes proteins, especially collagen cross linking and matrix endothelial cell dysfunction, bequeathing further to atherosclerosis. The preponderance of dyslipidemia in diabetes mellitus is 95% [33].

Nasir et al, (2008) [34]. Type 2 diabetes patients who were admitted to the out ward patient were chosen for the study. It was assured that they were fulfilling inclusion criteria. A total of 100 patients were included in the study ageing >40 years and had no evidence of cardiac disorder. Fasting blood glucose (FBG) and glycosylated haemoglobin (HbA1c) were estimated to know about good glycemic control. Blood samples were also taken for estimation of lipids. After analysing all the parameters it was found that people with type 2 diabetes mellitus had their lipid levels deranged. It was found that the patients were having high LDL- CHOLESTEROL, while as TG was as per the recommendations. HDL- Cholesterol was above required levels in all patients. Majority of the patients had Hypertriglyceridemia and LDL was border line high i.e. 94% which is consistent with other studies. In patients with good glycemic control only 64% patients were having

Hypertriglyceridemia as compared to patients who were having poor glycemic control 94% patients were having Hypertriglyceridemia. There are studies showing that improved control of hyperglycemia do modify diabetes associated dyslipidemia [35]. The outcome of this study showed that the patients who were diabetic were having their lipid levels deranged

Shaikh et al (2010) [36]. In this study a total of one thousand patients were taken for the study. Blood samples were taken and analysed for various parameters such as FBG and Lipid Profile. Patients with type 2 diabetes mellitus had a mean age of 40+10.56 yrs. The analysis of results showed that raised cholesterol was seen in 38% patients. Triglyceride was increased in 60% patients. The HDL was decreased in 20% patients while LDL was raised in 29%. This study indicates that these numbers of diabetics are prone in future for developing cardiovascular and cerebrovascular complications. Outcome of this study showed that majority of type 2 diabetes mellitus had uncontrolled blood sugar levels and most had their lipid levels perplexed [37].

Mahato et al,(2010) [38]. The study involved a total of 294 patients . Fasting serum samples were analysed for FBG and lipid profile panel tests. Lipid reference levels were set according to National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP). According to this panel hypercholesterolemia is defined as TC>200 mg/dl, high LDL-C when value >100 mg/dl, hypertriglyceridemia as TAG >150 mg/dl and low HDL-C when value <40 mg/dl. The mean age \pm SEM of male and female subjects were 62.72 \pm 10.24 and 55.86 \pm 12.08 years respectively. After analysing all the results it was found that the level of FBG and HbA1c was slightly higher in females than in males but the differences were not significant. Hypercholesterolemia was found in 27.89% individuals. Hypertriglyceridemia was found in 63.26% patients, low HDL-C was found in 15.6% and increased level of LDL-C was found in 47.6% patients. The outcome of this study showed that there was high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL-C and low HDL-C levels which are

well known risk factors for cardiovascular diseases. The main disorder of lipid metabolism was hypertriglyceridemia [39].

Khursheed et al,(2011) [40].The study was carried out to determine the frequency and pattern of hyperlipidaemia in patients with diabetes mellitus. A total of 100 patients were included in the study out of which 72 were males and 28 were females. The patients were taken randomly. The history was taken relevant clinical examination and all routine investigations were performed. In the early morning 5ml venous blood samples were collected for FBG and Lipid profile examination. . The lipid profile was evaluated by National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) classification of lipid profile. The mean \pm SD for age of patients with type 2diabetes mellitus was 53. 73 \pm 7. 88. After analysing all the results it was found that 72% of diabetes patients were dyslipidemic [41]. In this study it was found that males had higher levels of LDL-C than females. 19% diabetic males had LDL-C>160mg/dl. This finding was consistent with that by Ahmad et al [42]. It was also found that serum TG levels were found to be much raised among diabetic females as compared to males whereas serum cholesterol and LDL-C levels were higher among male diabetics. Hyperlipidaemia is the commonest complication of diabetes mellitus and it predisposes them to premature atherosclerosis and macro vascular complications. Common lipid abnormalities in diabetes are raised triglycerides, LDL-C serum cholesterol and low raised triglycerides, HDL-C. Therefore good glycemic control can prevent development and progression of lipid-abnormalities among patients with diabetes mellitus [43]. The results of this study showed that diabetic patients were having at least one lipid abnormality. The study also showed that females were having more lipid abnormalities than males. The level of TG was much raised in females than in males.

Mahajan et al, (2013) [44]. The study involved a total of 150 patients, 100 cases of uncontrolled type 2 diabetes mellitus were taken. Out of these 100 cases, 50 were having

DM for less than 10 years, categorized as group I and 50 were having DM for more than 10 years, categorized as group II. Fasting venous blood samples were analysed for serum TC, Triglyceride (TG), high density lipoproteins (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C). After all the analysing it was found that serum total cholesterol (TC), LDL cholesterol (LDL-C) and VLDL cholesterol levels show increase in group I and highly increased in group II as compared to controls. The decrease in HDL-C was highly significant in group II as compared to group I. Patients with type 2 diabetes are usually dyslipidemic. The high levels of insulin resistance associated with type 2 diabetes have multiple effects on fat metabolism. A decrease in lipoprotein lipase (LPL) activity resulting in reduced catabolism of chylomicrons and VLDL, an increase in the release of free fatty acid from the adipose tissue, an increase in fatty acid synthesis by liver, an increase in hepatic VLDL production [45]. Patients with type 2 diabetes have several lipid abnormalities including elevated plasma triglycerides (due to increased VLDL and lipoprotein remnants), elevated oxidized LDL and decreased HDL cholesterol. It was found that the people with diabetes with more than eight years had an increased risk of cardiovascular disease death compared with those who had diabetes for less than two years. From this study it was conclude that the dyslipidemia in type 2 diabetes become more abnormal with increase in duration of diabetes [46].

Fatima et al, (2014) [47]. The study included 576 subjects of age group 45-75 of both sexes and they were divided in to three groups of equal number. One group whose blood glucose levels was normal N (192), another group with normal blood glucose level and atherosclerosis NA (192), and the third group with diabetes and atherosclerosis DA (192). The Diabetes of the patients was diagnosed by analysing glycosylated haemoglobin HbA1c. Serum samples were collected and analysed for FBG, LDL-C, HDL-C, TG, TC, and VLDL-C was calculated using Freidwald's formula. After analysing all the results it was found that patients of diabetes and atherosclerosis group had significant increase in the levels of TC, TG

LDL and VLDL as compared to the normal blood glucose and atherosclerotic patients TC TG, LDL and VLDL and N group for TC, TG and VLDL. Whereas, DA group males and females showed significantly lower level of HDL in comparison to NA group and N group. Among DA group subjects, females have significantly higher level of TC, TG, LDL, and VLDL and significantly lower level of HDL as compared to males. Diabetic individuals are at an increased risk of CVD compared to no diabetic individuals, therefore diabetic subject have high mortality rate [48]. The results correlated with that Ghazanfari, et al.HbA1c level enhances the risk of diabetes induced mortality risk in subjects having CVD [49]. The outcome of this study showed that the patients with diabetes and atherosclerosis were having increased levels of TC, TG, LDL and VLDL were as HDL was decreased, while the other group with normal blood glucose and atherosclerosis didn't had too much increase in the TC, TG, LDL and VLDL levels. it was found that females had higher levels of TC, TG, LDL and VLDL than males.

CHAPTER 4
MATERIALS
AND
METHODS

CHAPTER 4

4.0 Material and Method

A case control study was carried out in Super Religare Limited (SRL) Gurgaon in the Department of Biochemistry from Jan 2015 to April 2015. The study included a total of 50 subjects. All the subjects were the case of type 2 diabetes mellitus. They were diagnosed on the basis of elevated HbA1c (glycosylated haemoglobin) level $>6.5\%$. History of all the patients was recorded. Blood samples were collected in Fasting state for lipid estimation. Apart from lipid estimation patients FBG and glycosylated haemoglobin was also estimated to know about glycemic control. Fasting Lipid profile tests included total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol. For serum lipid reference level, National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) guideline was referred. According to NCEP- ATP III guideline, hypercholesterolemia is defined as $TC > 200$ mg/dl, high LDL-C when value > 100 mg/dl, hypertriglyceridemia as $TAG > 150$ mg/dl and low HDL-C when value < 40 mg/dl. Diabetes was defined as per American Diabetes Association (ADA) criteria.

4.1 METHOD ANALYSIS

All the serum samples were analysed by automation using the DADE Behring- Dimension RxL Max Auto Analyser and blood samples were analysed using LH 750 auto analyser. The precision of instrument was checked on many occasions. . All the analytical procedures were standardized the reagents were calibrated to the instrument before sample analysis was done.

4.1.1 Estimation of HbA1c by Bio-Rad VARIANT 2

Principle

This programme utilizes principle of ion-exchange high performance liquid chromatography (HPLC). The samples are automatically mixed and diluted on the variant 2 sampling station

(VSS) and injected into analytical cartridge. The variant 2 chromatographic station (VCS) dual pumps deliver a programmed buffer gradient of increasing ionic strength to the cartridge, where the haemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins are then passed through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. An additional filter at 690 nm corrects for background absorbance.

4.1.2. Estimation of Triglyceride by Enzymatic method

The TGL method used on Dade Dimension® clinical chemistry system is an in vitro diagnostic test intended for the quantitative estimation of triglyceride in human serum and plasma.

Principle

The triglycerides method is based on enzymatic procedure in which a combination of enzymes is employed for the measurement of serum or plasma triglycerides. The sample is incubated with lipoprotein lipase enzyme reagent that converts triglyceride into free glycerol and fatty acids. Glycerol kinase catalyses the phosphorylation of glycerol by adenosine-5-triphosphate to Glycerol-3-phosphate. Glycerol-3-phosphate-oxidase oxidizes glycerol-3-phosphate to Dihydroxyacetone phosphate and Hydrogen Peroxide (H₂O₂). The catalytic action of peroxidase (POD) forms quinoneimine from H₂O₂, aminoantipyrine and 4-chlorophenol. The change in the absorbance due to the formation of quinoneimine is directly proportional to the total amount of glycerol and its precursors in the sample and is measured using a bichromatic (510-700) endpoint technique.

Procedure

The TGL Flex® reagent cartridge, Cat. No DF69A is required to perform the TGL test. The test was performed on the Dade Dimension® clinical chemistry system (auto analyser) after the method was calibrated using the appropriate enzyme verifiers. Sampling, reagent,

delivery, mixing, processing and printing of results are automatically performed by the dimensions®system.

4.1.3 Estimation of Cholesterol by Cholesterol esterase method

The CHOL method used on the Dimension® clinical chemistry system is an in vitro diagnostic test intended for the quantitative estimation of total cholesterol in human serum and plasma

Summary

The CHOL method is based on the principle first described by Stadman and later adapted by other workers including Rautela and Liedtke.

Principle

Cholesterol esterase catalyse the hydrolysis of cholesterol esters to produce free cholesterol which, along with pre-existing free cholesterol, is oxidized in a reaction catalysed by cholesterol oxidase to form cholest-4-ene-3-one and hydrogen peroxide. In presence of horseradish peroxidase, the hydrogen peroxide thus formed is used to oxidize N, N diethylaniline-HCL/4-aminoantipyrine to produce a chromophore that absorbs at 540 nm. The absorbance due to oxidized is directly proportional to the total cholesterol and is measured using a polychromatic (452,540,700 nm) endpoint technique.

Procedure

The CHOL Flex ®reagent cartridge Cat No, DF27 is required to perform CHOL test. The test was performed on the Dade Dimension® clinical chemistry system (auto analyser) after the method was calibrated using the appropriate enzyme verifiers. Sampling, reagent, delivery, mixing, processing and printing of results are automatically performed by the dimensions®system.

4.1.4. Estimation of HDL-C by two reagent method

The AHDL method is an in vitro diagnostic test for the quantitative measurement of High Density lipoprotein cholesterol (HDL-C) in human serum and plasma on Dimension® clinical chemistry system. Measurement of HDL-C is used as an aid in the diagnosis of lipid disorders such as Diabetes Mellitus, various liver and renal diseases and in the assessment of the risk of CD.

Principle

The AHDL assay measures HDL cholesterol levels directly without the need for sample pre-treatment or specialized centrifugation steps, using a two reagent format. In the first reaction chylomicrons, VLDL and LDL form water soluble complexes with dextran sulphate in the presence of magnesium sulphate. These complexes are resistant to the polyethylene glycol modified cholesterol esterase and cholesterol oxidase that react with HDL cholesterol. In the presence of oxygen, the HDL cholesterol is oxidized to Δ^4 -cholestenone and hydrogen peroxide. The generated hydrogen peroxide reacts with 4-aminoantipyrine and sodium N-(2-hydroxy-3-sulfoethyl)-3,5-dimethoxyaniline in the presence of peroxidase to form a coloured dye that is measured using a bichromatic (600/700 nm) endpoint technique. The colour intensity of the dye is directly proportional to the serum HDL-C concentration.

Procedure

The

AHDL Flex® reagent cartridge, Cat No DF488 is required to perform the test. The test was performed on the Dade Dimension® clinical chemistry system (auto analyser) after the method was calibrated using the appropriate enzyme verifiers. Sampling, reagent, delivery, mixing, processing and printing of results are automatically performed by the Dimension® system.

4.1.5. Estimation of ALDL by two reagent format

The ALDL method on the Dimension® clinical chemistry system is an in vitro diagnostic test intended for the quantitative estimation of low density lipoprotein cholesterol in human serum and plasma. LDL-C measurements are used in the diagnosis and treatment of lipid disorder such as diabetes mellitus, atherosclerosis and various liver and renal diseases.

Principle

The ALDL cholesterol assay is a homogenous method for directly measuring LDL-C levels in human serum or plasma, without the need for any off-line pre-treatment or centrifugation steps.

The method is in a two reagent format and depends on the properties of detergent 1 which solubilizes only non-LDL particles. Cholesterol released is oxidised by cholesterol esterase and cholesterol oxidase in a non-colour forming reaction. Detergent 2 solubilizes the remaining LDL particles. The soluble LDL-C is then oxidized by the action of cholesterol esterase and cholesterol oxidase forming cholestenone and hydrogen peroxide. The enzymatic action of peroxidase on hydrogen peroxide colour in the presence of N,N-bis(4-sulfobutyl)-m-toulidine, disodium salt (DSBmt) and 4-aminoantipyrine that is measured using a bichromatic (540,700 nm) end point technique. The colour produced is directly proportional to the amount of LDL-C present in the sample.

Procedure

The ALDL Flex ®reagent cartridge Cat No, DF131 is required to perform the test. The test was performed on the Dade Dimension® clinical chemistry system (auto analyser) after the method was calibrated using the appropriate enzyme verifiers. Sampling, reagent, delivery, mixing, processing and printing of results are automatically performed by the dimensions®system.

4.1.6. Estimation of Glucose by Hexokinase method

The GLUC method for the method on the Dimension® clinical chemistry system is an in vitro diagnostic test intended for the quantitative estimation of glucose in human serum, plasma, urine and cerebrospinal fluid.

Summary

The glucose(GLUC) method is an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method presented as a general clinic laboratory method by kunst et al. The hexokinase method is the generally accepted reference method for measuring glucose. Glucose measurements are used in the diagnosis and treatment of disorders of carbohydrate metabolism such as diabetes mellitus, neonatal hypoglycemia and insulinoma.

Principle

Hexokinase catalyses the phosphorylation of glucose in the presence of adenosine-5-triphosphate and magnesium to form glucose-6-phosphate and adenosine diphosphate(ADP).G-6-p is the oxidized by glucose-6-phosphate dehydrogenase in the presence of Nicotinamide adenine dinucleotide to produce 6-phosphogluconate and NADH. One mole of NADH for each mole of glucose present. The absorbance due to NADH is determined using a bichromatic (340 and 383 nm) endpoint technique.

Procedure

The GLUC Flex ®reagent cartridge Cat No, DF40 is required to perform the test. The test was performed on the Dade Dimension® clinical chemistry system (auto analyser) after the method was calibrated using the appropriate enzyme verifiers. Sampling, reagent, delivery, mixing, processing and printing of results are automatically performed by the dimensions®system.

CHAPTER 5

RESULT

AND

DISCUSSION

CHAPTER 5

5.0. Result and Discussion

In order to evaluate the effect of type 2 diabetes mellitus on HBA1c, TC, TG, ALDL, AHDL and VLDL in all subjects, investigations were performed on Type 2 diabetes mellitus patients for the estimation of TC, TG, ALDL, AHDL and VLDL by clinical laboratory.

5.1. Effect on lipid profile of Males

The study demonstrated that HBA1c has significant influence on FBG ($P < 0.05$). The FBG level was increased.

A total of 28 male patients were included in the study which had the mean age of 52.14 ± 26 years, range of 35-80 years. After analysis of results showed that cholesterol was raised in % (n=26). Triglyceride level was increased in 96% of patients. HDL was decreased in 78% of the patients (n=22). LDL was increased in 89% of male patients. VLDL was increased in almost 98% of the patients. Apart from this the male subjects were divided into two age groups ranging from 35-55 years and 56-80 years. it was found that the male subjects ageing between 35-55 years had high levels of Cholesterol, triglyceride, Low density lipoproteins while High density lipoprotein level was low. in the male subject of age ranging from 56-80 years it was found that Fasting blood glucose level was higher than the patients of other age group. other parameters were slightly different as that of other group.

	HBA1c (%)	FBG (mg/dl)	TC (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
MEAN	6.9	188.9	227.06	228.16	35.02	161.46	44.62
S.D	0.6	0.8	0.9	0.9	0.3	0.7	0.4
F-VALUE	0.08	0.004	0.003	0.003	0.008	0.004	0.008
P-VALUE	0.01	0.005	0.003	0.003	0.02	0.005	0.02

Fig:-The study demonstrates the significant difference $P < 0.05$. using one way ANOVA

5.2. Effect on Lipid Profile of Females

	HBA1c (%)	FBG (mg/dl)	TC (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
MEAN	7.02	191.08	228.51	228.49	34.97	161.23	44.53
S.D	0.16	0.9	1.01	1.01	0.3	0.8	0.4
F-VALUE	0.03	0.004	0.004	0.004	0.008	0.004	0.008
P-VALUE	0.03	0.04	0.003	0.003	0.02	0.005	0.02

Fig:- The study demonstrates the significant difference $P < 0.05$, Using One way ANOVA

After calculating the P-value of all the variables it showed that there is a significant difference $P < 0.05$ which means there is the increase in the values of the biochemical variables.

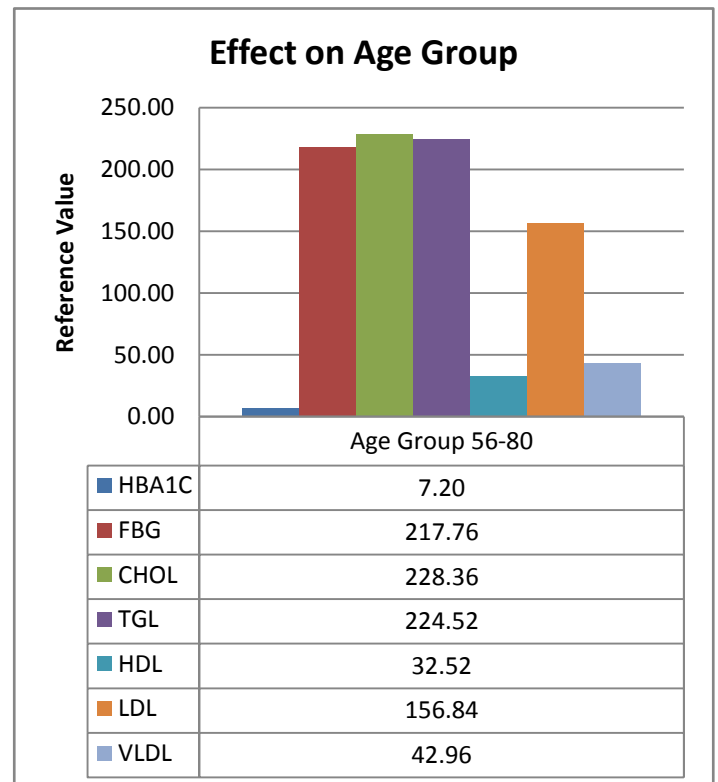
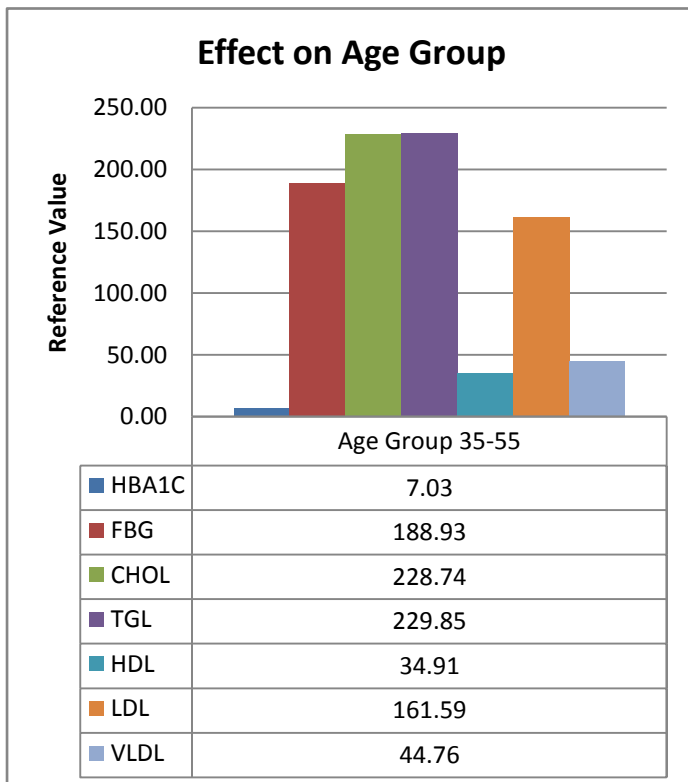
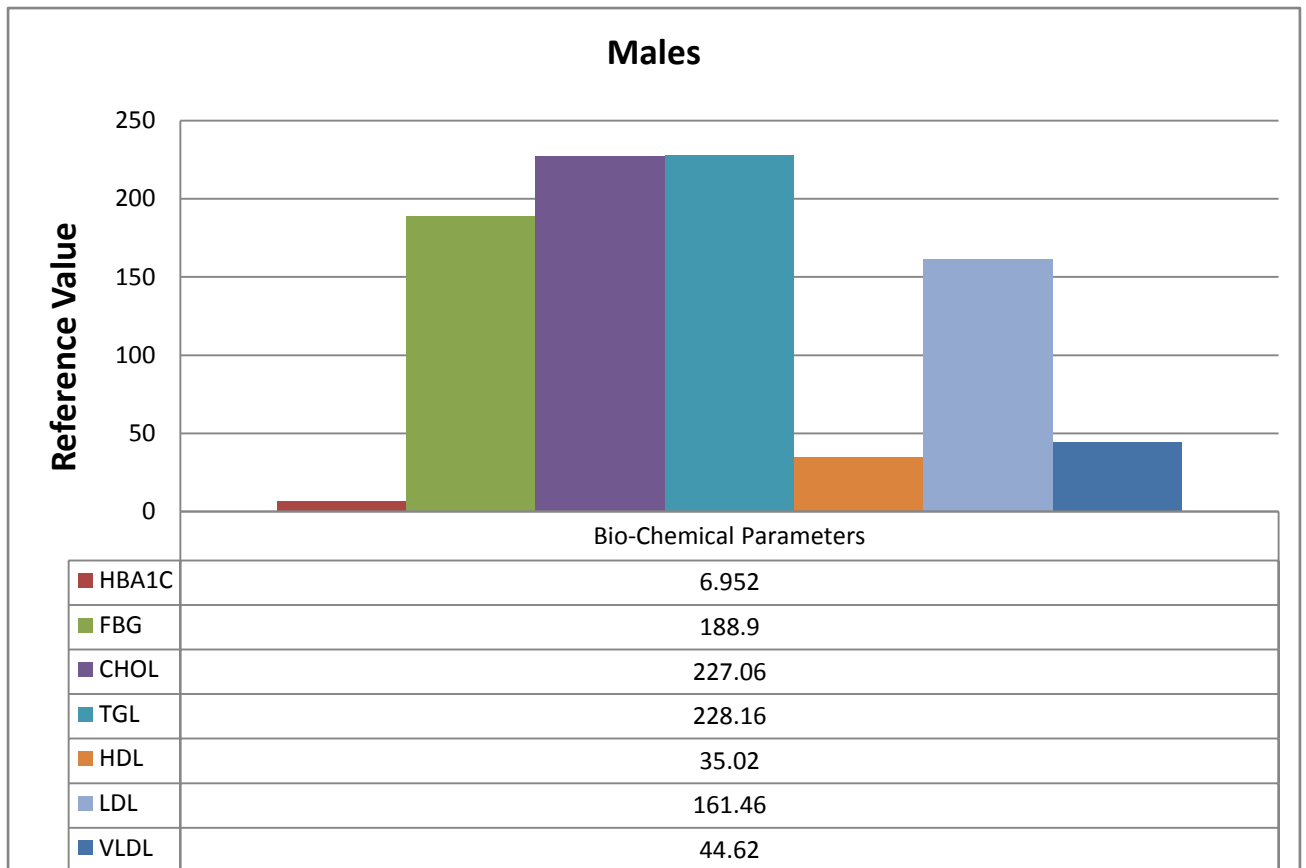


fig:- Graph showing values of lipid in Males and in different age groups of Males

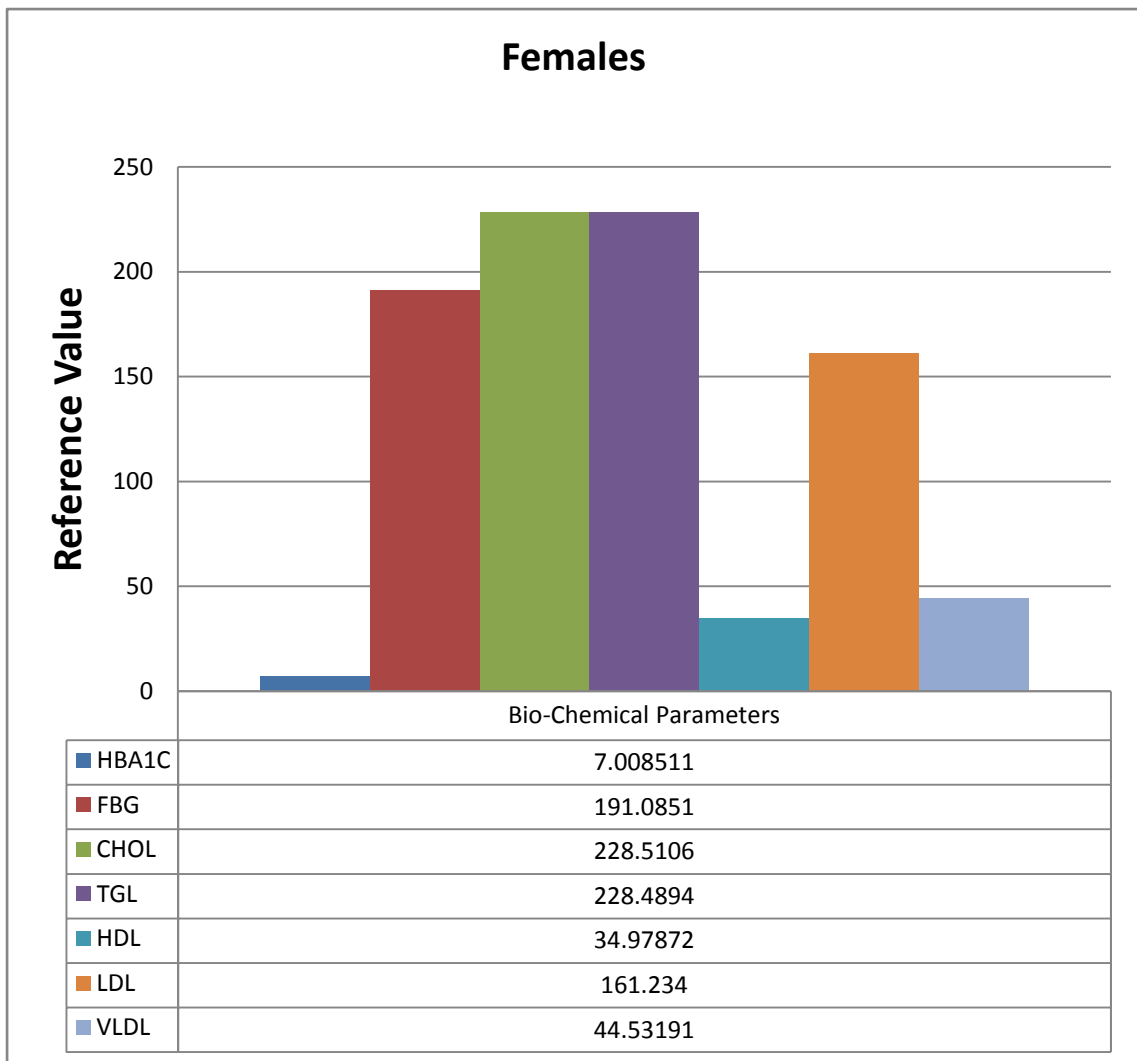
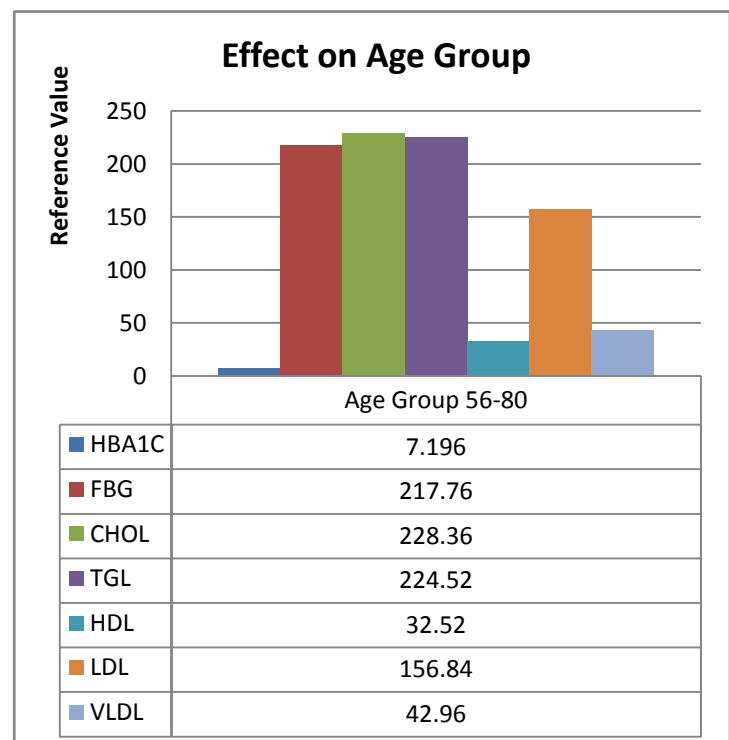
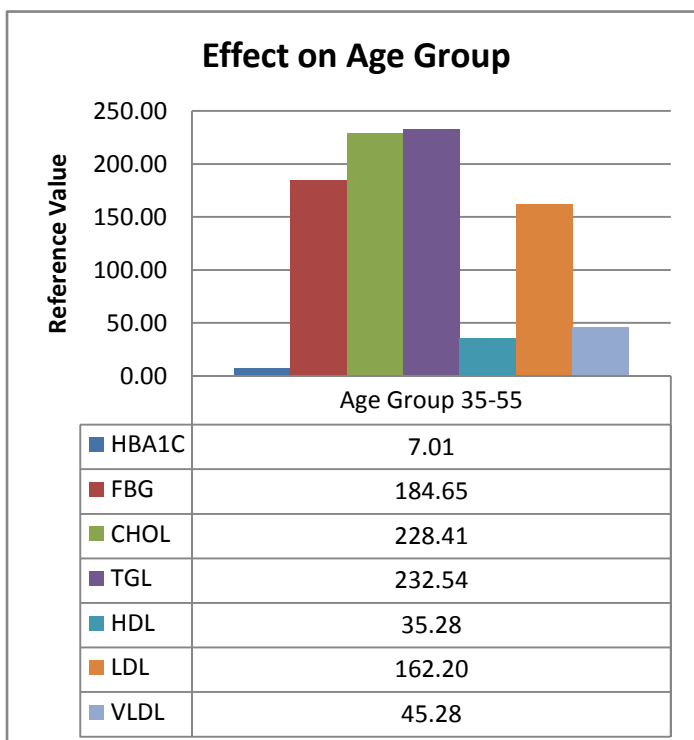


Fig:- Graphs showing levels of lipid profile of Females and different age groups



5.3. RESULTS

A total of 22 female subjects were included in the study with the mean age of 51.89 ± 19 . After the analysis of results showed the women who had their HBA1c levels >6.5 were having increased levels of lipid profile. The cholesterol level was estimated as 228.56 ± 54 mg/dl, Triglyceride as 229.14 ± 45 mg/dl, HDL level was decreased as 34.97 ± 9 were as LDL level were increased as 161.23 ± 53 were as VLDL level showed increase 44.53 ± 8 . The increased level of all the lipid parameters with respect to males is due to less physical activity and obesity. Different age groups showed increase in different biochemical parameters. In the patients in between age group 35-55 years showed increase in the levels of TC, TGL VLDL and decrease in HDL levels, while as the patients in the age group of 56-80 years showed increased Fasting blood glucose (FBG) and HBA1c levels.

5.4 DISCUSSION

Diabetes mellitus escalates the risk for atherosclerotic vascular disease. The risk is greatest in people who have other known risk factors, such as, dyslipidemia, hypertension, smoking and obesity. There is a twofold to fourfold exuberance risk of coronary artery disease in type 2 diabetes mellitus compared with non-diabetic patients [50]. Literally 75–80% of adult diabetic patients die of coronary artery disease, cerebrovascular disease, peripheral vascular disease or a combination of these conditions. Patients with type 2 diabetes can have many lipid abnormalities, including hyperchylomicronaemia, exalted levels of very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides; and low levels of high-density lipoprotein cholesterol (HDL-C) [51]. Lipid abnormalities may be the result of the wobbly metabolic state of diabetes and enhanced control of hyperglycaemia does tame diabetes-associated dyslipidemia [52]. In type 2 diabetes the major brawl in lipoprotein metabolism are reflected by an increase in plasma

triglyceride and a low HDL- Cholesterol with normal or near normal LDL- Cholesterol levels. However in diabetics this LDL portion contains a greater ratio of small, dense LDL particles which are believed to be more atherogenic [53]. Lipid abnormalities are due to defiance to insulin and hyperglycemia which are decreased high density lipoprotein and increased and more small dense low density lipoprotein and elevated triglycerides [54]. This study revealed that females had much higher lipid levels than males. nearly 95% of patients of type 2 has increased Cholesterol level. LDL was increased in 86% of patients and HDL was decreased in 78% of the patients, this indicates that these number are prone in future for developing cardiovascular diseases. Management of high cholesterol in diabetes has improved in last few years and further hard work is required [55]. Insulin resistance is important factor in diabetic patients of type 2. Which leads to increased release of free fatty acids from fatty tissue, impaired insulin dependent muscle uptake of free fatty acids and increase fatty acid release to the hepatic tissue [56]. The finding is in agree with the previous study.

5.6 Conclusion

There was difference in glycemic control between males and females as measured by Fasting Blood Glucose and HBA1c levels. HBA1c showed positive correlation with TGL, LDL, TC and VLDL and negative correlation was seen between HBA1c and HDL levels. In this study it was found out that every patient had at least one type of dyslipidemia and 95.5% combined dyslipidemia. We found statistically significant difference between male and female patients. Females were having more lipid abnormalities than males. Type 2 DM patients in this study had elevated levels of TG, reduced levels of HDL with either normal or elevated levels of LDL. This indicates the influence of Type 2 DM on abnormal lipid profile of patients with its associated danger of elevated CVD risk Thus Lipid profile analysis must be made an integral part of Type 2 DM patients' clinical reviews and treatment. Type 2 DM and other diabetics

must be educated on the risks they face as a result of their condition and the necessary steps they need to manage it. From this study we conclude that that the dyslipidemia in type 2 diabetes become more abnormal with increase in duration of diabetes. Therefore, there is an urgent need for screening and therapeutic intervention for dyslipidemia in the diabetics which may help to decrease the morbidity and mortality from CAD. Overall diabetes mellitus is closely associated with Dyslipidemia but age group and DM duration may not be strong indices for lipid profile prediction especially with respect to subjects under management. further a large sample size and its study with stastical analysis is required to give a scientific judgement.

CHAPTER 6

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