Sudan University of Science and Technology

College of Graduate Studies

Assessment of Lipase enzyme, Cholesterol and Triglycerides among Sudanese post-menopausal women in Khartoum State

تقييم إنزيم الليباز ,الكوليسترول والدهون الثلاثية في مصل الدم لدى النساء السودانيات بعد سن اليأس في ولاية الخرطوم

A dissertation submitted in Partial fulfillment for the requirement of MSc degree in Medical Laboratory Sciences

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قال الله تعالى

" الْحَمْدُ لِلَّهِ الَّذِي أَنزَلَ عَلَى عَبْدِهِ الْكِتَابَ وَلَمْ يَجْعَل لَّهُ عِوَجَا {1} قَيِّمًا لِّيُنذِرَ بَأْسًا شَدِيدًا مِن لَّدُنْهُ وَيُبَشِّرَ الْمُؤْمِنِينَ الَّذِينَ يَعْمَلُونَ الصَّالِحَاتِ أَنَّ لَهُمْ أَجْرًا حَسَنًا {2} مَاكِثِينَ فِيهِ أَبَدًا {3} وَيُنذِرَ الَّذِينَ قَالُوا اتَّخَذَ اللَّهُ وَلَدًا {4} "

صدق الله العظيم , سورة الكهف

Dedication

I humbly dedicate this dissertation to my fathers and mothers for their grateful, supported, encouragement and constant keeping Allah in my life.

To every Teacher and Guardian during our educational career.

To every patient suffering from any disease on his miserable bed.

Acknowledgments

First I wish to thank Allah for granting me the Confidence and Success to complete this study. I would like to express my sincere gratitude and honest appreciation to my supervisor **Dr. Nuha Elgaili Abubaker**

Her kindness good guidance, valuable direction and generous advice that have kept me on the right track. My thanks are extended to my colleagues in the Clinical Chemistry Department, Faculty of Medical Laboratory Science, **Sudan University of Science and Technology**.

. Thanks for my friends, who helped me and mad my work wonderful. Last thanks for everyone helped me in my research.

Abstract

Background: Menopause is a natural event in the ageing process and signifies the end of reproductive years with cessation of cyclic ovarian function as manifested by cyclic menstruation. Lipid profile is altered in menopause because of various reasons.

Objectives: The aim of this study to evaluate cholesterol, triglycerides and lipase enzyme among Sudanese normal premenopausal women and postmenopausal women.

Materials and methods: This is a case control study carried out in Saba'a center-Khartoum State during September 2019, to evaluate the level of cholesterol, triglycerides and lipase in postmenopausal women and normal premenopausal women.

50 postmenopausal women and 50 healthy premenopausal women were enrolled in this study after taking their verbal consent.

Result: The results showed that, 68% of post menopause women over 55 years old, while 32% less than 55 years old. The result showed that, there were significant increase in triglyceride and cholesterol levels and significant decrease in hepatic lipase enzyme among postmenopausal women when compare with control group, (183.8±37.49 versus 82.12±12.33, p-value =0.00) for triglyceride.(238.9±30.08 versus 130.2±28.45 , p-value =0.00) for cholesterol. (89.70±28.38 versus 104.3±38.95, p-value =0.035) for lipase. There was significant positive correlation between lipase enzyme and age in post menopausal women (r=0.462, p- value=0.001) , There were no correlation between triglyceride , cholesterol and age (r=-0.201, p- value=0.162) (r=0.148,p- value= 0.306) respectively. Also there were no correlation between triglyceride, cholesterol and lipase (r=0.017, p- value=0.908) (r=0.141, p- value= 0.329) respectively.

Conclusion: The study was observed that postmenopausal women have a significant increase in triglyceride and cholesterol and significant decrease in lipase enzyme level. There was significant positive correlation between lipase enzyme and age in post menopausal women.

المستخلص

خلفية

انقطاع الطمث هو حدث طبيعي في عملية الشيخوخة و يدل على نهاية سنوات الانجاب مع وقف وظيفة المبيض الدوري كما يتضح من الحيض الدوري. يتم تغير البروتين الدهني و انزيم الليباز في سن الياس لأسباب المبيض.

الهدف من الدراسة

هو تقييم الكوليسترول, الدهون الثلاثية و انزيم الليباز بين النساء السودانيات قبل انقطاع الطمث الطبيعي و النساء بعد انقطاع الطمث.

المواد والطرق

هذه دراسة لحالات اجريت في مركز سبا في ولاية الخرطوم. لتقييم مستوى الكوليسترول, الدهون الثلاثية و انزيم الليباز بين النساء السودانيات قبل انقطاع الطمث و نساء ما بعد انقطاع الطمث.

النتيجة

اظهرت النتائج وجود ارتفاع ملحوظ في البروتين الدهني و انخفاض ملحوظ في مستويات انزيم الليباز لدى النساء بعد انقطاع الطمث عند مقارنتهم مع المجموعة الضابطة.

الخلاصة

قد لاحظت الدراسة ان لدى النساء بعد انقطاع الطمث ارتفاع ملحوظ في الكوليسترول, الدهون الثلاثية و انخفاض كبير في مستوى انزيم الليباز.

List of Abbreviations

ER	Endoplasmic Reticulum
HDL	High Density Lipoprotein
HL	Hepatic Lipase
LDL	Low Density Lipoprotein
LPL	Lipoprotein Lipase
TG	Triglyceride
VLDL	Very Low Density Lipoprotein

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Chapter One

Introduction, Rationale and Objectives

1.1 Introduction

Menopause is a natural event in the ageing process and signifies the end of reproductive years with cessation of cyclic ovarian functions as manifested by cyclic menstruation. It is heralded by menopausal transition, a period when the endocrine, biological and clinical features of approaching menopause begins (Burger *etal* ., 2002). The average age of menopause is 51 years and less than 1% of women experience it before the age of 40 years, with some women undergoing premature menopause at a very early age, affecting their ability to have children (Derek, 1990).

Dyslipidemia are disorders of lipoprotein metabolism, including lipoprotein overproduction or deficiency. These disorders may be manifested by elevation of the serum total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride concentrations, and a decrease in the high-density lipoprotein (HDL) cholesterol concentration. (Mohan *et al*., 2010)

Triglycerides are fats which are found in foods such as meats, dairy produce and cooking oils. Triglycerides are absorbed in the intestines and transported by the bloodstream to the tissues where they are either stored as fat or used to provide energy. Fat that is stored is also comprised of triglycerides (Albert *et al.*, 1998).

Hyper triglyceridemia is a well-known cause of acute pancreatitis. Typically, the risk of acute pancreatitis increases progressively with fasting TG levels over 500 mg/dl, with a significant increase when TG levels exceed 1000 mg/dl. (Scherer *et al*, 2014)

Cholesterol is a fatty substance, vital for good health. It helps form cell membranes, various hormones, bile and vitamin D. some cholesterol get from diet but most is made in the liver.(Scherer *et al.*, 2014).

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. Lipases occur widely in nature, but only microbial lipases are commercially significant. (Nagar, 2001)

. Usoro *et al* (2006) were performed a study Lipid Profile of Postmenopausal Women in Calabar, Nigeria and found hyperlipidemia in post-menopausal women. (Usoro *et al.*, 2006)

1.2Rationale

Menopause is a natural event in the ageing process and signifies the end of reproductive years with cessation of cyclic ovarian function as manifested by cyclic menstruation. Lipid profile and enzymes such as lipase are altered in menopause because of various reasons. Increase in cardiovascular risk after menopause and in premature menopause may be partly attributable to the drop in sex hormones. This study high light to study the change in lipids profile and lipase enzyme in postmenopausal women and help to early detection of dyslipidemia , to avoid cardiovascular disease.

1.3 Objectives

1.3.1 General objective

To assess the plasma levels of Cholesterol, Triglycerides and lipase enzyme among Sudanese postmenopausal women.

1.3.2 Specific objectives

1-To measure and compare the mean concentration of Cholesterol , Triglycerides and lipase enzyme in study groups.

2- To correlate between lipase , cholesterol, triglyceride, and age

3- To correlate between cholesterol , triglyceride levels and lipase.

Chapter Two

Literature Review

Chapter Two

2-Literature Review

2.1 Menopause period

Menopause, a period during which the female sexual cycle ceases and female sex hormones diminish rapidly. It occurs between 45-55 years of age. It is characterized by hot flushes, night sweats and various other psychological and biochemical changes occur. (Murugan *et al.,* 2015)

The 3-5 years period before menopause when estrogen and hormones level begin to drop is called perimenopause.

Postmenopausal start after one year has passed since last menstrual cycle.

The hormonal changes associated with menopause e.g low plasma levels of estrogen and marked increase in follicle stimulating hormone level exert a significant effect on metabolism of plasma lipids and lipoproteins. Studies by (Swapnali *et al*., 2011) and (Kalavathi *et al*., 1991) have shown altered lipid profile in post-menopausal women.

2.1.1 Menopause and Coronary atherosclerosis

Coronary atherosclerosis is the most important cardiovascular disorder, and its incidence increases with age in both sexes. Risk factors have been less studied in women than in men. However, it is known that after menopause, women present an increase in the incidence rates of cardiovascular disease that rapidly rise to those in men. Numerous studies have demonstrated that the increase in cardiovascular risk after menopause and in premature menopause may be partly attributable to the drop in sex hormones. (Castelli *et al.*, 2009)

2.2 Lipid

In biology and biochemistry, a lipid is a bio molecule that is soluble in non polar solvents. Non-polar solvents are typically hydrocarbons used to dissolve other naturally occurring hydrocarbon lipid molecules that do not (or do not easily) dissolve in water, including fatty acids, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, and phospholipids. (Fahy *et al*., 2009)

2.2.1 Functions of lipid

The functions of lipids include storing energy, signaling, and acting as structural components of cell membranes. Lipids have applications in the cosmetic and food industries as well as in nanotechnology. (Subramaniam *et al.*, 2011)

2.2.2 Cholesterol

Cholesterol is one of the most highly recognized molecules in human biology, in part because of a direct relationship between its concentrations in blood and tissues and the development of atherosclerotic vascular disease. Cholesterol, which is transported in the blood in lipoproteins because of its absolute insolubility in water, serves as a stabilizing component of cell membrane and as a precursor of the bile salts and steroid hormones. Precursors of cholesterol are converted to ubiquinone, dolichol, and, in the skin, to cholecalciferol, the active form of vitamin D. As a major component of blood lipoproteins, cholesterol can appear in its free, unesterified form in the outer shell of these macromolecules and as cholesterol esters in the lipoprotein core. (Fadhil *et al.*, 2019)

2.2.2.1 Biosynthesis of Cholesterol

Slightly less than half of the cholesterol in the body derives from biosynthesis de novo(from the beginning) by virtually all tissues in humans, although liver, intestine, adrenal cortex, and reproductive tissues including ovaries, testes and placenta make the largest contributions to the body cholesterol pool. The liver plays a central role in the regulation of the body cholesterol balance which is a series of transport, biosynthetic, and regulatory mechanism. Biosynthesis in the liver accounts for approximately 10%, and in the intestines approximately 15%, of the amount produced each day. Cholesterol synthesis occurs in the cytoplasm and microsomes from the two-carbon acetate group of Acetyl-CoA.It was estimated that nearly 50% of the daily cholesterol production is converted to bile acids and salts and secreted in bile. Most of this re-absorbed and re-used by way of entero-hepatic circulation. About 0.5 – 1.0 g/day is used for the biosynthesis of steroid hormones (about 40 mg/day). A small part of cholesterol (about 50 mg/day) in adult is excreted also from the surface of the skin. Feeding a diet containing 0.5 % cholesterol to an animal resulting 95%inhibition of hepatic cholesterol biosynthesis. In

general, an adult on a low-cholesterol diet typically synthesizes about 800 mg of cholesterol/day in the liver cells as a major site and by the intestinal cells.(Fadhil *et al.*, 2019)

2.2.2.2 Regulating Cholesterol Biosynthesis

Normal healthy adults synthesize cholesterol at a rate of approximately (1.0g/day)and consume approximately (0.3 g/day). A relatively constant level of cholesterol in the body (150 - 200 mg/dl) is maintained primarily by controlling the level of de novo synthesis. The level of cholesterol synthesis is regulated in part by the dietary intake of cholesterol. Cholesterol from both diet and synthesis is utilized in the formation of membranes and in the synthesis of the steroid hormones and bile acids. The greatest proportion of cholesterol is used in bile acid synthesis (50%). (Fadhil *et al.*, 2019)

2.2.2.3 The Utilization of Cholesterol

Cholesterol is transported in the plasma predominantly as cholesteryl esters associated with lipoproteins. Dietary cholesterol is transported from the small intestine to the liver within chylomicrons. Cholesterol synthesized by the liver, as well as any dietary cholesterol in the liver that exceeds hepatic needs, is transported in the serum within LDLs .The liver synthesizes VLDLs and these are converted to LDLs through the action of endothelial cell-associated lipoprotein lipase. Cholesterol found in plasma membranes can be extracted by HDLs and esterified by the HDL-associated enzyme LCAT. The cholesterol acquired from peripheral tissues by HDLs can then be transferred to VLDLs and LDLs via the action of cholesteryl ester transfer protein (apo-D) which is associated with HDLs. Reverse cholesterol transport allows peripheral cholesterol to be returned to the liver in LDLs. Ultimately, cholesterol is excreted in the bile as free cholesterol or as bile salts following conversion to bile acids in the liver. (Fadhil *et al.*, 2019)

2.2.2.4 Chylomicrons

Chylomicrons are assembled in the intestinal mucosa as a means to transport dietary cholesterol and TGs to the rest of the body. Chylomicrons are, therefore, the molecules formed to mobilize dietary exogenous lipids. The predominant lipids of chylomicrons are TGs (which contain long chain fatty acids). The Apo lipoproteins that predominate before the chylomicrons enter the circulation include apoB-48 and apoA-I, -A-II and IV. ApoB-48combines only with chylomicrons. The surface is a layer of phospholipids, with head

groups facing the aqueous phase. Triacylglycerol's sequestered in the interior (yellow) make up more than 80% of the mass. Several Apo lipoproteins that protrude from the surface (B-48, C-III, C-II) act as signals in the uptake and metabolism of chylomicron contents. The diameter of chylomicrons ranges from about 100 to 500 nm.). (Fadhil *et al.*, 2019)

2.2.2.5 Clinical Application

Serum cholesterol is increased in the following conditions:

1- Diabetes mellitus, because Acetyl-CoA pool is increased and more molecules are channeled to cholesterol.

2- Obstructive jaundice, where excretion of cholesterol through bile is blocked.

3- Hypothyroidism, where the receptors for HDL on liver cells are decreased, and so excretion is not effective.

4- Frederickson's type ll hyperlipoproteinemia which is a hereditary autosomal dominant condition.

5- In nephritic syndrome, where there is albumin loss through urine, globulins are increased as a compensatory mechanism. When lipoproteins are increased, cholesterol is correspondingly increased. (Fadhil *et al.*, 2019)

2.2.3 Triglyceride

Triglycerides are the main constituents of body fat in humans and other vertebrates, as well as vegetable fat. They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin. oils. There are many different types of triglyceride, with the main division between saturated and unsaturated types. Saturated fats are "saturated" with hydrogen — all available places where hydrogen atoms could be bonded to carbon atoms are occupied. These have a higher melting point and are more likely to be solid at room temperature. Unsaturated fats have double bonds between some of the carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms. These have a lower melting point and are more likely to be liquid at room temperature. (Nelson *et al.*, 2000)

2.2.3.1 Chemical structure Triglyceride

Triglycerides are tri-esters consisting of a glycerol bound to three fatty acid molecules. Alcohols have a hydroxyl (HO–) group. Organic acids have a carboxyl (–COOH) group. Alcohols and organic acids join to form esters. The glycerol molecule has three hydroxyl (HO–) groups and each fatty acid has a carboxyl group (–COOH). In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds:$HOCH_2CH(OH)CH_2OH + RCO_2H + R'CO_2H + R''CO_2H \rightarrow RCO_2CH_2CH(O_2CR')CH_2CO_2R'' + 3H_2O$. (White *et al.,* 2011)

2.2.3.2 Lipase and triglyceride

The pancreatic lipase acts at the ester bond, hydrolyzing the bond and "releasing" the fatty acid. In triglyceride form, lipids cannot be absorbed by the duodenum. Fatty acids, monoglycerides (one glycerol, one fatty acid), and some diglycerides are absorbed by the duodenum, once the triglycerides have been broken down. (Drummond *et al.,* 2014)

2.2.3.3 Role in disease

In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis, heart diseases and stroke. However, the relative negative impact of raised levels of triglycerides compared to that of LDL: HDL ratios is as yet unknown. The risk can be partly accounted for by a strong inverse relationship between triglyceride level and HDL-cholesterol level. But the risk is also due to high triglyceride levels increasing the quantity of small, dense LDL particles. (Ivanova *et al.,* 2017)

2.2.3.4 Triglyceride and Mechanism of Atherosclerosis

Triglycerides are transported from the liver and intestines by very-low-density lipoprotein (VLDL) and chylomicrons, respectively, and delivered to peripheral tissues to meet energy needs. Once the triglyceride core of these triglyceride-rich lipoproteins is hydrolyzed, the resulting VLDL and chylomicron become relatively cholesterol enriched. Because triglycerides do not accumulate in foam cells, the association of plasma triglycerides and ASCVD may be due to these remnant lipoproteins. Remnants have the potential to accumulate in the arterial endothelium, where they may be taken up by macrophages, promote foam cell formation, and, ultimately, fatty streak formation and subsequent plaque progression (Khetarpal *et al.,* 2015). A unique aspect of these

remnants compared with LDL particles is that they do not require oxidative modification to be taken up by arterial macrophages; they are also associated with a greater degree of inflammation. Hypertriglyceridemia is also associated with higher concentrations of small dense LDL particles (which may be more atherogenic than other LDL particles), reduced HDL particle and apolipoprotein (apo) A-I concentrations, and greater concentrations of apoCIII– containing particles. Changes in the structure of these lipoprotein particle subclasses may potentially accelerate atherosclerotic processes. Additionally, lipoprotein particles with higher triglyceride content may be more readily oxidizable, thereby enhancing their atherogenic potential. Furthermore, triglycerides may have more direct effects on inflammatory responses. Lipoprotein lipase at the endothelial cell surface and within the sub endothelial space hydrolyzes remnant triglycerides and generates proinflammatory mediators, including free fatty acids (Varbo *et al.,* 2013).

2.2.3.5 Hypertriglyceridemia

Hypertriglyceridemia is defined as an abnormal concentration of triglyceride in the blood. According to the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III) guidelines, a normal triglyceride level is

>150 mg/dl. It may be primary or secondary in nature. Primary hypertriglyceridemia is the result of various genetic defects leading to disordered triglyceride metabolism. Secondary causes are acquired causes, such as, high fat diet, obesity, diabetes, hypothyroidism, and certain medications. (Varbo *et al.,* 2013).

2.2.4 Lipase

Lipases (EC 3.1.1.3 triacylglycerol acyl hydrolase) are a group of water soluble enzymes, which exhibit the ability of acting at the interface between aqueous and organic phases. They primarily catalyze the hydrolysis of ester bonds in water insoluble lipid substrates. However, some lipases are also able to catalyze the processes of esterification, interesterification, transesterification, acidolysis, aminolysis and may show enantio selective properties. (Hasan *et al.,* 2009)

is a lipolytic enzyme synthesized mostly by hepatocytes and found localized at the surface of liver sinusoidal capillaries. It can be considered as a lipase of the vascular compartment, together with lipoprotein lipase (LPL), with which it shares number of structural and functional similarities. HL exerts both triglyceride lipase and

phospholipase A1 activities, and is involved at different steps of lipoprotein metabolism. During recent years, cellular and molecular biology approaches, as well as studies in transgenic animals, have been useful in elucidating the enzyme structure and functional domains, its metabolic roles, and the mechanisms regulating its expression. This article will focus on the structure/function relationship of HL, its synthesis, and the regulation of its expression in liver and steroidogenic tissues. The metabolic and patho-physiological roles of HL are developed in a joint review. (Cai *et al.,* 1989)

2.2.4.1 Synthesis and secretion

HL is a secreted glycoprotein and synthesized in the endoplasmic reticulum (ER). An NH 2 -terminal leader peptide is lost after crossing through the ER membrane. Kinetics of endogenously labeled enzyme and use of glycosidases or inhibitors have demonstrated that HL is first synthesized as a high mannose form (52–55 kDa in the rat), and further acquires sialic acid-containing complex oligosaccharides during transit through the Golgi cisternae. The mature HL (57–59 kDa, in the rat) is then rapidly secreted. The residence half-time of HL in hepatocyte is about 60 min. The rat enzyme contains two *N* -glycosylation sites. (Cisar *et al.,* 1987)

2.2.4.2 Regulation of Hepatic Lipase Expression

Several putative regulatory elements have been identified in the rat HL promoter, allowing the definition of partners potentially involved in the regulation of the enzyme expression. Among them, responsive elements for cholesterol (SRE), estrogens (ERE), thyroid hormones (TRE), and glucocorticoids (GRE), and for cAMP. In addition, another motif possibly responsive for glucose and/or insulin (proximal E-box), has been since described in humans. (Sensel *et al.,* 1990)

2.2.4.3 Lipase polymorphisms

Apart from hormonal modulations, HL activity is largely influenced by genetic factors. According to recent studies, the polymorphism C-514T in the promoter region would explain up to 38% of the variability of the HL activity. Functionally, the variant allele, as compared with wild-type, drives a decreased transcriptional activity of a promoter/reporter construct in murine hepatoma cells and it has been recently associated with fasting hyperinsulinemia and insulin resistance (Pihlajamaki *et al.*, 2000). It is noteworthy that the_ 514 site is at the center of a CAC*GGG sequence, almost analogous

to the CACGTG motif characteristic of an E-box onto which can bind the upstream stimulatory factors USF1/2. The latter are transcription factors involved in the regulation of glucose and lipid metabolism in the liver. For instance, USFs are part of the insulin response complexes that interact with the fatty acid synthase gene. It is tempting to speculate that the C*–514T substitution would disrupt the E-box analogous sequence and impair the stimulatory regulation exerted by insulin. Interestingly, it has been recently reported that USF proteins can bind to the _ 514 regions, and that the affinity is 4-fold reduced by the C-514T substitution (Botma *et al*., 2001).

2.3 Lipid and lipase in postmenopausal period

The decrease in estrogens is associated with the decrease in the number of low density lipoprotein receptors, named B:E receptors, which may explain the high levels of atherogenic lipoproteins, intermediate density lipoprotein (IDL) and low density lipoprotein (LDL), described after menopause. Plasma concentration of IDL and LDL cholesterol depends on the amount of their respective precursors, very low density lipoprotein (VLDL) and IDL, and on the activity of lipoprotein lipase (LPL). On the other hand, hepatic lipase (HL) is a key enzyme in the conversion of IDL to LDL and of HDL2 to HDL3. HL activity may alter the pathway of reverse cholesterol transport because it has been postulated that increased HDL2 cholesterol evidences a more effective antiatherogenic mechanism. This enzyme is subject to hormonal control. (Arca *et al*., 2010)

Sex hormones strongly influence body fat distribution and adipocyte differentiation. Estrogens and testosterone differentially affect adipocyte physiology and function of lipase enzyme, but the importance of estrogens in the development of metabolic diseases during menopause is disputed. Estrogens and estrogen receptors regulate various aspects of glucose and lipid metabolism. Disturbances of this metabolic signal lead to the development of metabolic syndrome and a higher cardiovascular risk in women. The absence of estrogens is a clue factor in the onset of cardiovascular disease during the menopausal period, which is characterized by lipid profile and enzymes such as lipase variations and predominant abdominal fat accumulation. (Olszowy *et al*., 2012)

Chapter Three

Materials and Methods

Chapter three

3- Materials and Methods

3.1 Materials

3.1.1 Study approach

A quantitative method was used to measure serum cholesterol, triglyceride and lipase enzyme in postmenopausal Sudanese women during the period from April to December 2019.

3.1.2 Study design

This was analytical case control, hospital based study.

3.1.3 Study area

The study was conducted in Khartoum State.

3.1.4 Study population

The study included 50 randomly selected postmenopausal women as case and 50apparently healthy premenopausal women as control group.

3.1.5 Inclusion criteria

Sudanese Postmenopausal women (45-60 years old) and healthy premenopausal women as control group were included in this study.

3.1.6 Exclusion criteria

- Women with- Obesity- Genetic disorders related to lipid metabolism.
- Heart disease.- liver disease –Alcohol and any disease alter lipid or lipase enzyme were excluded from this study.

3.1.7 Ethical consideration

Verbal consent was taken regarding acceptance to participate in the study and reassurance of confidentiality. Before the specimen was collected, the donors knew that, this specimen was collected for research purpose.

3.1.8 Data collection

Data was collected using structural interviewing questionnaire, which was designed to collect and maintain all valuable information concerning each case examined**.**

3.1.9 Specimen collection

About 3ml of venous blood were collected by using sterile disposal plastic syringe and applying aseptic standard non traumatic vein puncture technique. And informed consent was obtained verbally before blood sample collection every sample was emptied in Lithium heparin anticoagulant container and then centrifuged (3000 rpm) for 10 minutes, the plasma was stored at - 20◦c until analyzed.

3.2 Method

3.2.1 Estimation of cholesterol level:

3.2.1.1 Principle of cholesterol level:

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration.

3.2.1.2 Procedure of cholesterol level: Appendix 1

3.2.2 Estimation of Triglycerides level

3.2.2.1 Principle of Triglycerides level

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm.

3.2.2.2 Procedure of Triglycerides level: Appendix 11

3.2.3 Estimation of lipase

3.2.3.1 Principle of lipase:

The spectrophotometric methods for lipase activity determination make use of synthetic lipase substrates transformed upon enzyme catalyzed hydrolysis into products able to be detected spectrophotemetrically. The predominant substrates are p-nitro phenyl and naphthyl esters of the long chain fatty acids, and thioesters. The lipolysis of the p-nitro

phenyl esters (laureates, palmitates, oleates) gives rise to the yellow colored p-nitro phenol, measured at 405-410 nm.

3.2.3.2 Procedure of lipase: Appendix 111

3.3 Quality control

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of test and control samples.

3.4 Statistical analysis

Data obtained from this study was analyzed using statistical package for the social science (SPSS version16). Independent t test was used for comparison and persons correlation was used for correlation**.**

Chapter Four

Results

Chapter Four

4.Results

Hundred participants were enrolled in this study; 50 Sudanese Postmenopausal women (45-60 years old) as control group, with mean age +SD 57.2±6.69 years and 50 controls with mean age +SD 55.2±4.15 years. The results of biochemical parameters of plasma cholesterol, triglyceride levels and lipase enzyme activity in postmenopausal women (case group) and premenopausal women (control group) are given in figures and tables .

Figure (4.1) shows age distribution among case group. 32% were< 55 years old, and 68% were > 55 years old.

Table (4.1) represent the comparison mean ± SD of plasma cholesterol, triglyceride and lipase enzyme in case versus control group, the result showed there were significantly increased in plasma cholesterol, triglyceride levels and significant decrease in lipase enzyme in case group compared to control group(183.8±37.49 versus 82.12±12.33, p-value =0.00) for triglyceride.(238.9±30.08 versus 130.2±28.45 , p-value =0.00) for cholesterol. (89.70±28.38 versus 104.3±38.95, p-value =0.035) for lipase

Figure (4.2) shows correlation between lipase and age, there was significant positive correlation (r=0.462, p-value 0.001).

Figure (4.3) shows correlation between triglyceride and age, there was no correlation (r=-0.201, p- value=0.162).

Figure (4.4) shows correlation between cholesterol and age, there was no correlation (r=0.148,p- value= 0.306) .

Figure (4.5) shows correlation between triglyceride and lipase level, there was no correlation (r=0.017, p- value=0.908).

Figure (4.6) shows correlation between cholesterol and lipase levels, there was no correlation (r=0.141, p- value= 0.329).

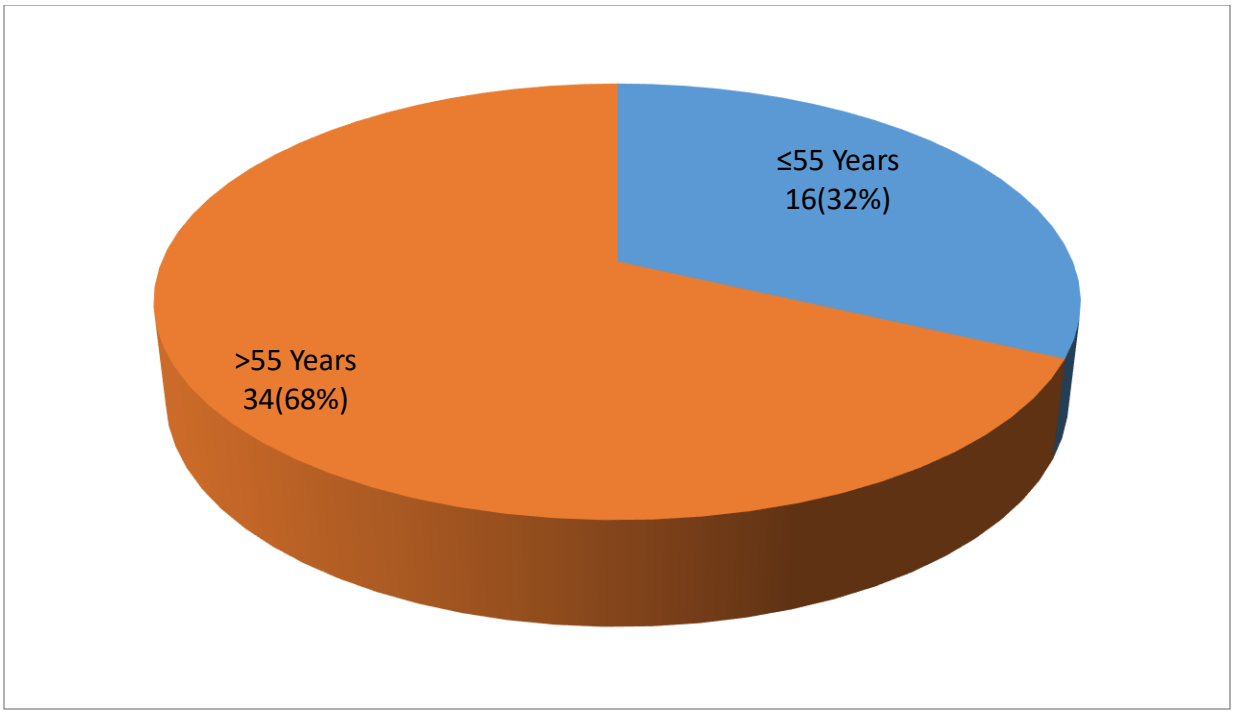

Figure (4.1) Age distribution among case group.

Table 4.1 Mean concentration of triglyceride, cholesterol and lipase in case and control group.

Parameters	Case (Mean±SD)	Control (Mean±SD)	*P-value*
Triglycerides (mg/dl)	183.8±37.49	82.12±12.33	0.000
Cholesterol (mg/dl)	238.9±30.08	130.2±28.45	0.000
Lipase (u/l)	89.70±28.38	104.3±38.95	0.035

Result given in mean ±SD, p- value ≤ 0.05 considered significant.

Independent sample T test was used for comparison.

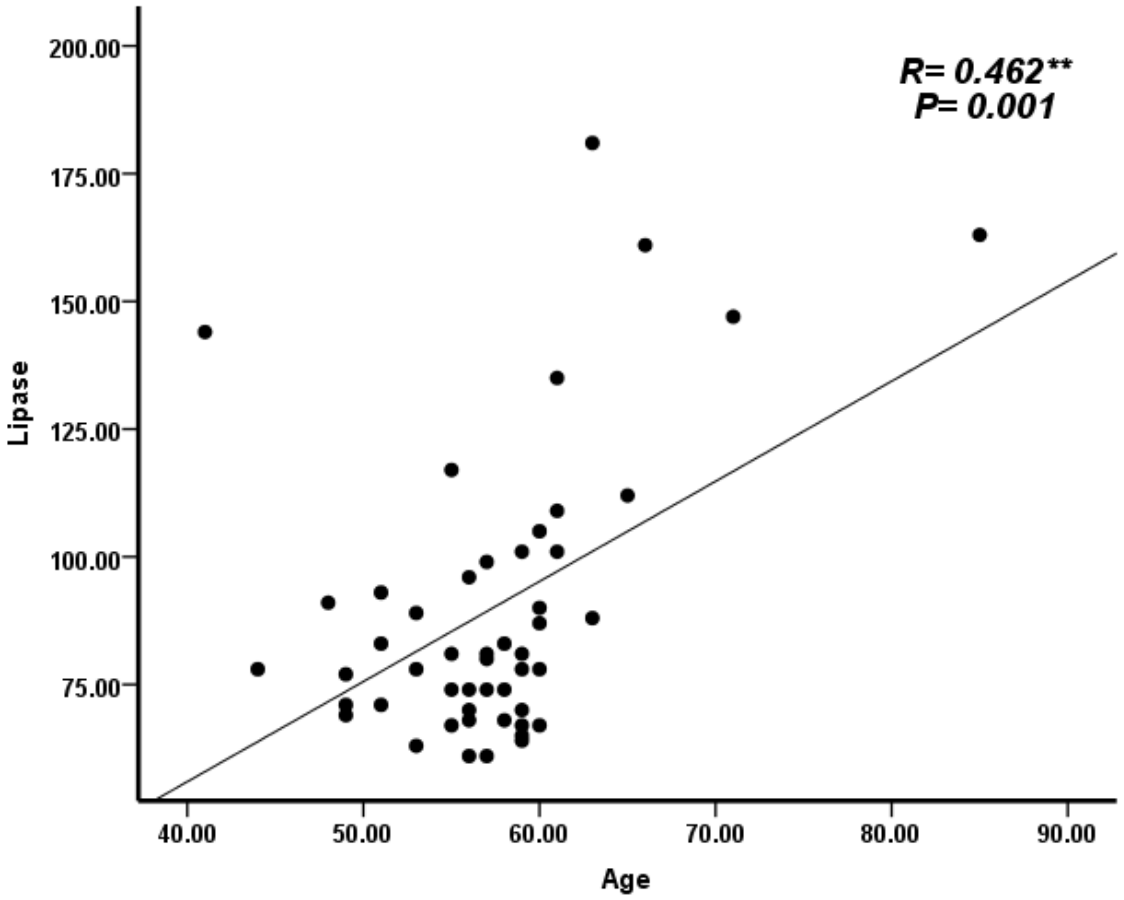

Figure (4.2) Correlation between lipase and age.(R=0.462 , P=0.001)

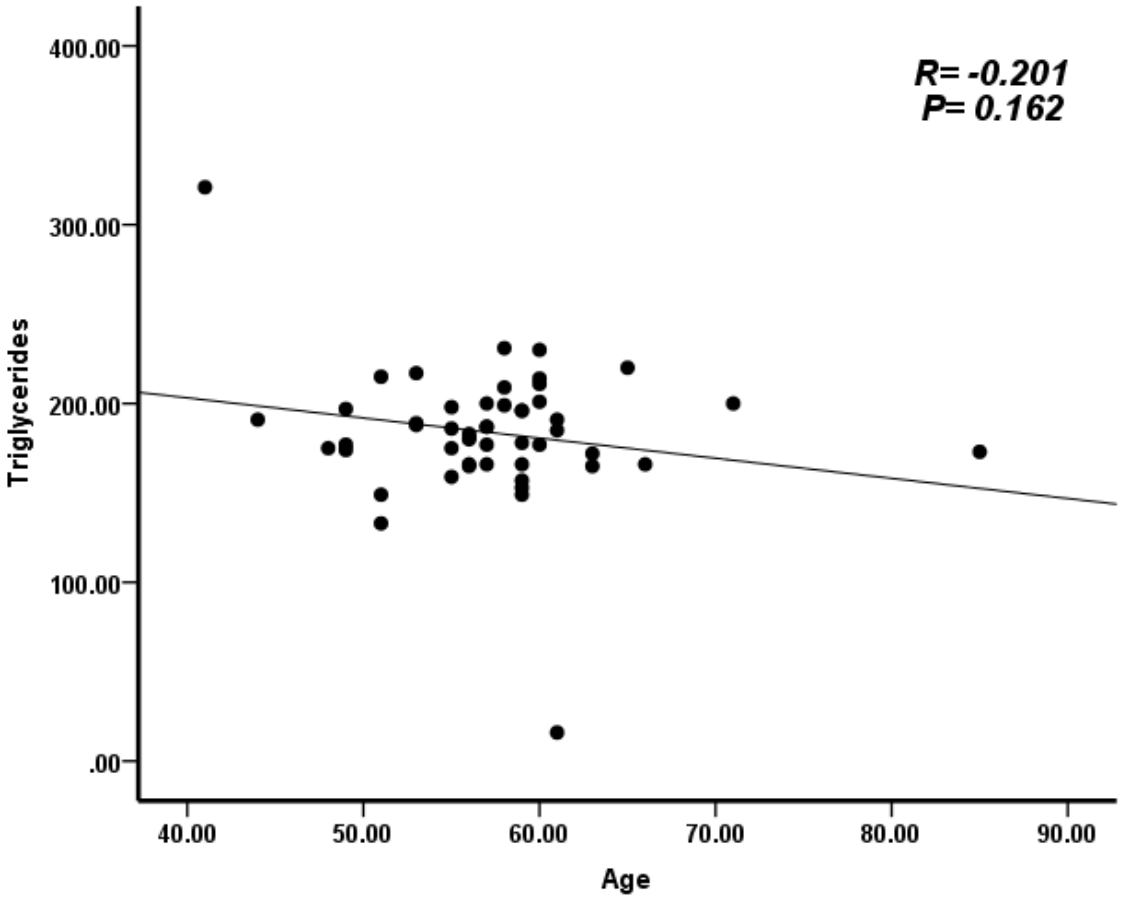

Figure (4.3) Correlation between triglyceride and age, (R=0.201, P=0.162)

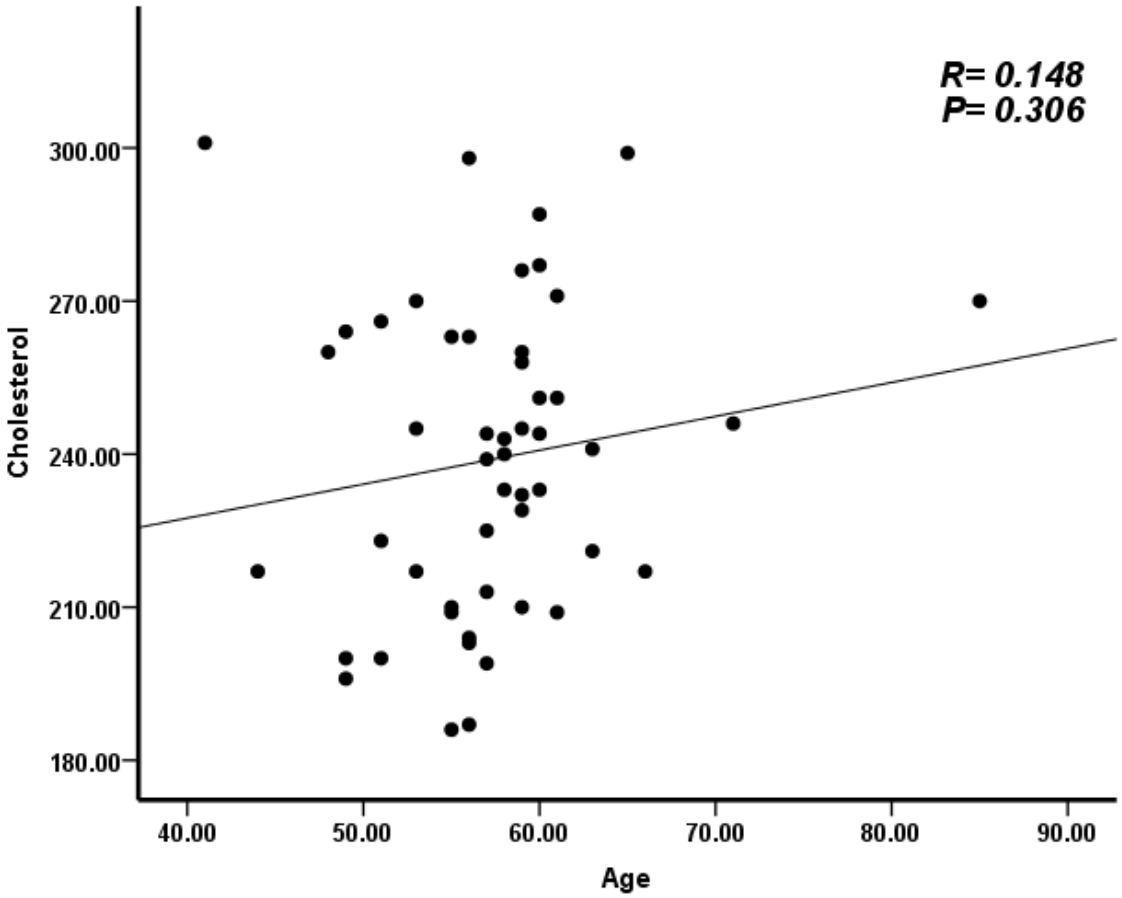

Figure (4.4) Correlation between cholesterol and age, (R=0.148, P=0.306)

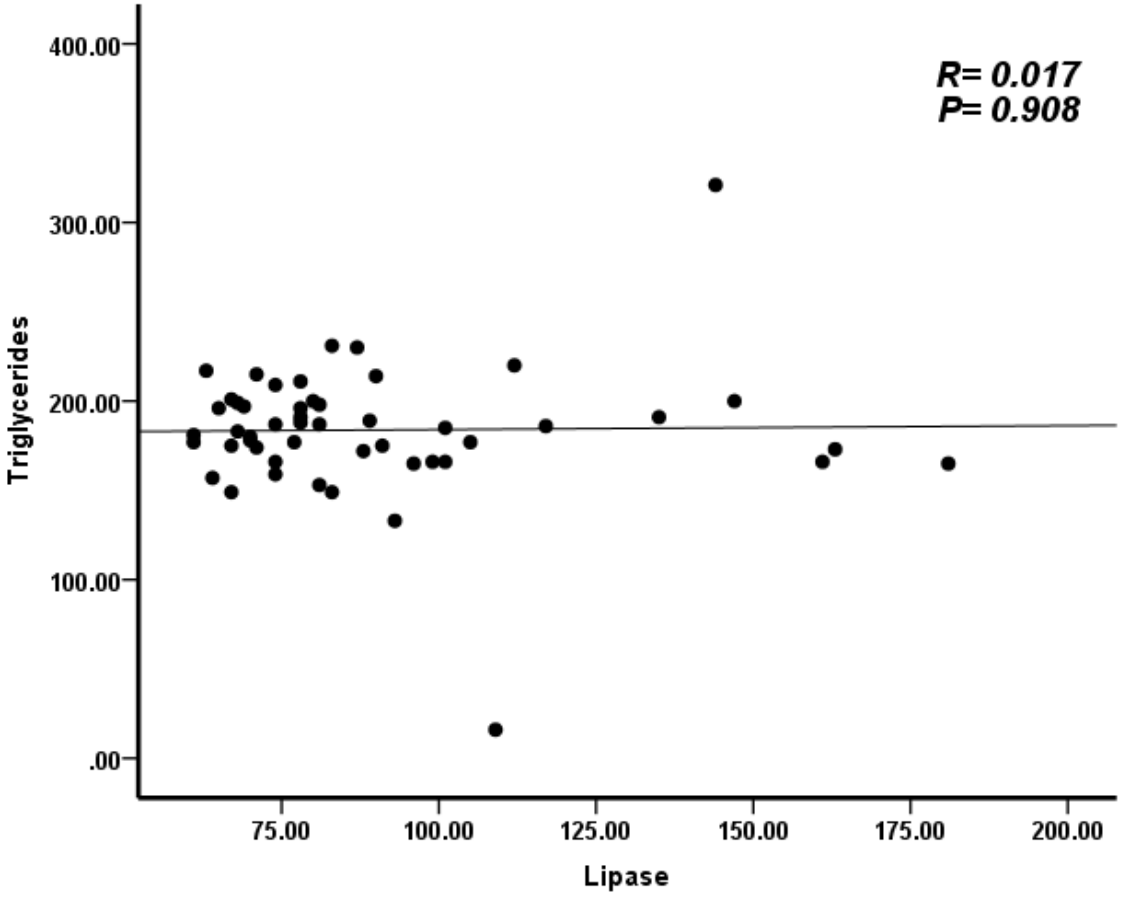

Figure (4.5) Correlation between triglyceride and lipase, (R=0.017, P=0.908)

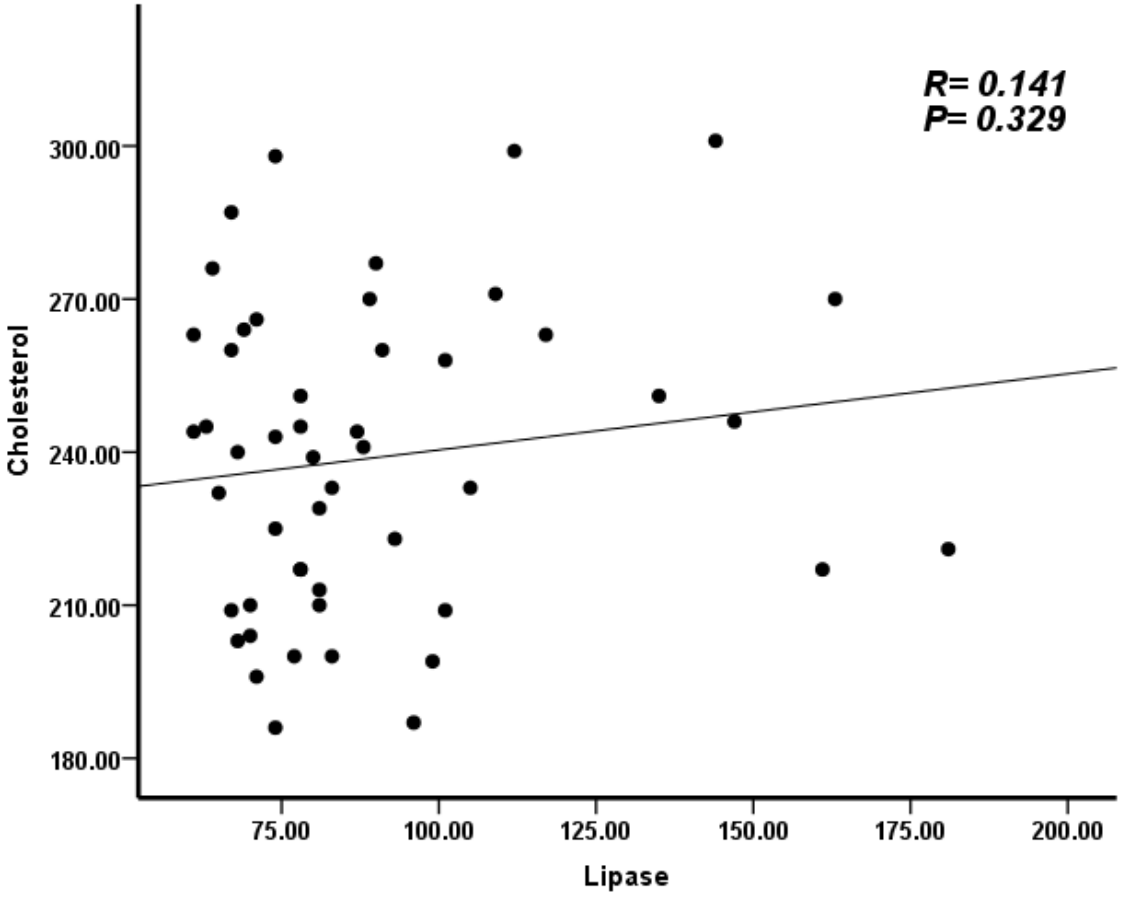

Figure (4.6) Correlation between cholesterol and lipase,(R=0.141, P=0.329)

Chapter Five

Discussion, Conclusion and Recommendation

5.1 Discussion

Menopausal statuses on Lipid profiles are affected by metabolic conditions, and alterations in lipid metabolism have been implicated in atherosclerosis and coronary heart disease. Results of this study showed that, there were significant increase in triglyceride and cholesterol levels in postmenopausal women when compare with control group. (p-value = 0.000), this result agreed with another result which finding confirmed that ; menopause alters the lipid profile in women. (Winder *et al* 1994). Also this result Parallel to another result which reported there were significant increase in cholesterol and triglyceride levels in post menopausal women, this may be due to the decrease in estrogens level in postmenopausal women is associated with the decrease in the number of low density lipoprotein receptors, named B:E receptors, which may explain the high levels of atherogenic lipoproteins (Arca *et al.,* 2010) . This result is similar to another result done by Swati Shah (2016),which reported that , the elevation levels of cholesterol and triglyceride in postmenopausal women happened due to the difference in hormonal status of women.(Shah,2016).

This study shows a significant decrease in lipase enzyme in case group versus control group with p-value 0.035. This result in agreement with another result performed by weise *et al.,* (2005), which showed significant decrease in lipase enzyme level due to the effect of estrogen on the liver enzymes. (weise *et al* ., 2005). Also this result like another result which finding confirmed that; Sex hormones strongly influence body fat distribution and adipocyte differentiation. Estrogens and testosterone differentially affect adipocyte physiology and function of lipase enzyme (Olszowy *et al.,* 2012).

The result of this study showed that, there was significant positive correlation between lipase enzyme and age in post menopausal women (r=0.462, p- value=0.00).This result is similar to another result which reported; there was significant positive correlation between lipase enzyme and age of postmenopausal women (Applebaum-Bowden., *et al*, 1989)

There were no correlation between triglyceride, cholesterol and age (r=-0.201, p-value=0.162) (r=0.148,p- value= 0.306) respectively . This result agree with another result done by Grandjean., *et al* (2004) whom deduced a same result (Grandjean *et al*., 2004). Also there were no correlation between triglyceride, cholesterol and lipase

(r=0.017, p- value=0.908) (r=0.141, p- value= 0.329) respectively. This result similar to another result carried out by American Heart Associations which showed there were no correlation between triglyceride, cholesterol and lipase. (Parke davis., 1986). This result disagreed with another result which found positive correlation between triglyceride, cholesterol and lipase. (South Bend Medical Foundation,1987).

5.2 Conclusion

This study concluded that:

Serum cholesterol and triglyceride levels are increased and lipase is decreased in postmenopausal women. There was positive correlation between lipase enzyme and age of postmenopausal women.

5.3 Recommendation

- Estimation of cholesterol and triglyceride should be done in postmenopausal women to avoid cardiovascular disease.
- Health education, good and healthy diets are important factors to achieve good levels of lipid profile, enzymes and other chemistries.
- Oral medicines that controlling level of lipid profile and lipase enzyme necessary for postmenopausal women.

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