Sudan University of Science and Technology

College of Graduate Studies

Evaluation of Serum Triglyceride, Cholesterol and High Density Lipoprotein Cholesterol levels among Sudanese Females with Polycystic Ovary Syndrome in Aljazeera State.

تقييم مستويات الدهون ثلاثية الجلسريدات ، الكوليسترول والدهون عالية الكثافة لدى النساء السودانيات المصابات بمتلازمة تكيس المبايض بولاية الجزيرة.

A dissertation submitted in a partial fulfillment for the requirements of the master degree in Medical Laboratory Science - Clinical Chemistry

By

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الاية

قـــال تعـــالى:

وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ ۚ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا

سورة النساء الآية (113)

Dedication

The god firstly and finally who give force and support me till complete this research with safely and successfully.

To…..

The candle which burns to light my life my mothers who always encourages me and them oblivious…

To…..

My source of supercilious my father…

To….

My teachers who taught me throughout my life from the beginning till today…

To….

Anyone who help me by any way, my brothers, sisters, friends and colleagues…

To….

Funny fourth that shared happy and sad always stay with each other…

Acknowledgments

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Abstract

Poly Cystic Ovary Syndrome (PCOS) is a major health problem which affect women in reproductive age and lead to infertility and dyslipidemia.

This study was carried out to measure serum level of High Density Lipoprotein cholesterol (HDL-c), Triglyceride (TG) and Total cholesterol in PCOS patients. Fifty samples were collected from PCOS patients in period between February to May 2020, chosen randomly from Elhasahesa Educational Hospital for gynecological and obstetrics in Aljazeera State, and Fifty apparently healthy individuals as control group. HDL.c and TG were measured by ROCHE DIAGNOSTIC – COBAS INTEGRA400 PLUS, and the results were analyzed using Statistical Package of Social Science (SPSS), computer program.

The study showed that, serum level of TG and total serum cholesterol were increased and HDL.cwas decreased in PCOS patients compared to control group :TG(mean ± SD: 170 ± 8.5 mg/dl versus 87.3 ± 23.36 mg/dl, P.value (0.00),serumtotal cholesterol (mean ± SD: 213.44 ± 32.44 mg/dl versus 100.36 ± 27.7 mg/dl, P.value(0.00), HDL.-c(mean ± SD: 31.28 ± 4.2 mg/dl versus 66.7± 11.6 mg/dl, P.value(0.00). Also the finding of this study showed that, there were strong positive correlation TG, Total cholesterol and BMI(r-value 0.879, p-value 0.00, r- value 0.966, p-value 0.00)respectively, while there was negative correlation between HDL and BMI(r-value -0.863, p-value 0.00). There were no correlation between HDL.c, TG, total cholesterol and age of PCOS patients (r-value 0.073, p.value0.470) (r value -0.069, p.value 0.496, r-value -0.026, p-value 0.801) respectively.

It is conclude that, the serum level of HDL.c decreased while serum level of total cholesterol and TGincreased among PCOS patients.

المستخلص

مرض تكيس المبايض هو أحد الأمراض التي تصيب النساء في عمر الانجاب وتؤدي إلى العقم وتذبذب الدهون.أجريت هِذه الدراسة لتقييم مستويات الدهون عالية الكثافة الدهون ثلاثية الجلسريدات والكوليستيرول لدى النساء السودانيات المصابات بتكيس المبايض.

تم جمع خمسين عينة من المرضى عشوائياً في الفترة من فبراير الى مايو2020 ، من مستشفى الحصاحيصا التعليمي لأمراض النساء والتوليد في ولاية الجزيرة وخمسين من الاصحاء كمجموعة ضابطة، تم تقييم مستوياتالكوليسترول والدهون ثلاثية الجلسريداتROCHE DIAGNOSTICCOBAS INTERA والدهون عالية الكثافة في مصل الدم بإستخدام جهاز 400PLUS. تم تحليل البيانات بإستخدام الحزمة الإحصائية للعلوم الإجتماعية ، برنامج كمبيوتر.

أظهرت الدراسة إرتفاع معدل الكوليسترول والدهون ثلاثية الجلسريد وإنخفاض معدل الدهون عالية الكثافة لدى المرضى اللتي يعانين من تكيس المبايض عند مقارنتهن بالمجموعة الضابطة (المتوسط± الإنحراف المعياري) الدهون ثلاثية الجلسريد (170.64±8.5 مقابل87.3 ± 23.36مليجرام \ديسليتر وكان الإحتمال الإحصائي للمقارنة 0.00) الكوليسترول (213.44±32.44 مقابل100.36 ± 27.7مليجرام \ديسيليتروكان الإحتمال الإحصائي للمقارنة0.00) الدهون عالية الكثافة (31.28±4.2 مقابل66.7± 11.6مليجرام\ديسيليتروكان الإحتمال الإحصائي للمقارنة0.00) ، وأيضاً أوضحت الدراسة وجود إرتباط معنوي قوي ملحوظ بين الدهون ثلاثية الجلسريدات، الكوليسترول ومعامل كتلة الجسم (معامل بيرسون=0.00 ، قيمة الارتباط =0.879، معامل بيرسون= 0.00 ، قيمة الارتباط0.966) علي التوالي. بينما اوضحت الدراسة وجود ارتباط عكسي بين الدهون عالية الكثافة ومعامل كتلة الجسم (معامل بيرسون=0.00 ، قيمة الارتباط 0.863-) ، كما أوضحت الدراسة عدم وجود ارتباط معنوي بين عمر المرضى والدهون ثلاثية الجلسريدات والكوليسترول والدهون عالية الكثافة (معامل بيرسون0.069-، قيمة الارتباط 0.496)، (معامل بيرسون0.801، قيمة الارتباط -0.026، معامل بيرسون0.470 ، قيمة الارتباط0.073) على التوالي.

خلصت الدراسة الى أن مستويات الدهون عالية الكثافة تنخفض بينما مستويات الدهون ثلاثية الجلسريد والكوليسترول ترتفع لدي المرضى اللاتي يعانين من تكيس المبايض.

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Chapter One

Introduction, Rational and objectives

1.1 Introduction

Polycystic ovary syndrome which means that the ovaries become enlarged with multiple cysts and contain fluid filled sacs which surround the eggs, resulting in woman's ovaries or adrenal glands increased production of estrogen and androgen male hormone than normal, which can stop eggs from ovulation leading to oligomenorrhea, hirsutism, infertility and obesity in young women (Danish, 2010). Polycystic ovaries syndrome is more common in woman who have obesity or mother or sister with PCOS (Genazzani, 2008). According to Rotterdam criteria PCOS is defined by the existence of at least two of three criteria. Which are hyperandrogenism, chronic anovulation, and polycystic ovaries on ultrasound findings (RotterdamRevised, 2003).The prevalence of PCOS is estimated to be approximately 48.5 million women aged 20–44 years (Rojas *et al*, 2014).The prevalence of polycystic ovary syndrome in the Qatari population between 16–20% (Darghame*tal* ., 2017).The prevalence of PCOS in Australian women is estimated to be between 12-21% (Jacqueline and Helena, 2012).Increase incidence of insulin resistance and basal insulinemia which have been related to dislipidemia observed by Mcut 2008. Also study done by Homeira in 2018 suggest that there is strong relationship between PCOS and increase level of glucose and triglycerides and obesity. Depend on the results of above studies this study conduct to evaluate the plasma lipids in Sudanese females with poly cystic ovary syndrome.

1.2 Rationale

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders that occur in 4% -7% of women of reproductive age. It is the one of the main cause of women infertility and hence potential risk to a woman's health over her lifestyle clearly remain a clinical investigational, genetic, and therapeutic challenge. PCOS is known to be associated with reproductive morbidity and increased risk for endometrial cancer, type 2 diabetes mellitus, and hypertension, metabolic and cardiovascular diseases (Galluzzo, 2008; Fakhoury*et al*., 2012).

Few Recent data showed that PCOS is related to abnormal lipids metabolism that have essential role in the regulation of ovarian follicle growth and ovulation rate. And suggest that may be

associated with PCOS complications. This study may help to support the Sudanese study suggested that abnormal lipoproteins levels is associated with the development of PCOS in Sudan.

1.3 Objectives:

1-3.1 General objective

To evaluate Serum levels of lipids among Female patients with Polycystic Ovarian Syndrome in Sudan.

1.3.2 Specific objectives:

1-To measure and compare the mean serum cholesterol, triglycerides and HDL-c levels in cases and control group.

2- To correlate between serum cholesterol, triglycerides and HDL-c levels and study variables (ages and BMI) in case group.

Chapter Tow

2. Literature review

2.1 polycystic ovary syndrome:

The polycystic ovary syndrome (PCOS) was first described by Stein and Leventhal in 1935(Azzi*et al*, 2006).Stein and Leventhal suggested that the ovarian change in bilateral cystic ovaries is most probably a result of some hormonal stimulation and that this stimulation was most likely the result of anterior pituitary secretions. PCOS is associated with several other diseases/morbidity-related factors such as obesity and other cardiovascular disease, which are becoming more prevalent among females today (Schmidt, 2011).

Poly cystic ovary syndrome has become one of the major public-health challengesit affects 5%–10% of women of reproductive age worldwide (Walters *et al.,* 2012).Woman with PCOS are higher risk of diabetes metabolic syndrome, heart disease, high blood pressure (Dunaif *et al.,*2008).

2.1.1. Etiology of PCOS:

The etiology of PCOS remains unclear, and abnormal ovarian steroidogenesis, hyperinsulinemia, and neuroendocrine abnormalities have been proposed as a primary underlying abnormality (Dunaif*et al.*, 2008). Abnormal steroidogenesis is suggested by studies showing that ovarian theca cells produce excessive androgens and show abnormal ovarian steroid responses to gonadotropin (Rojas *et al.*, 2014). The etiology of PCOS is complex and seem to be influenced by multiple genetic and environmental factors. Among the genetic causes, mutation in genes involved in the synthesis, transport and regulation of androgens have been pointed out. Increasing evidence support relevant role of insulin resistance in blood promote the increased synthesis and secretion of androgens by theca cells in the ovaries through the insulin receptor and insulin growth factor-1, also is note is that insulin decreases hepatic synthesis of sexual hormone – binding globulin (SHBG), which in turn increases the free fraction and the biological activity of androgen and estrogens (William, 2012).

2.1.2 Pathophysiology of PCOS:

The pathophysiology of PCOS involves primary defects in the hypothalamic–pituitary axis,

insulin secretion and action, and ovarian function. (Shannon, 2012) although the cause of PCOS is unknown, PCOS has been linked to insulin resistance and obesity. The association with insulin function is expected; insulin helps to regulate ovarian function, and the ovaries respond to excess insulin by producing androgens, which can lead to anovulation.(Diamenti *et,al.*,2006) Follicular maturation arrest is a hallmark sign that an ovarian abnormality exists. Clinical signs of PCOS include elevated luteinizing hormone (LH) and gonadotropin–releasing hormone (GnRH) levels, whereas follicular-stimulating hormone (FSH) levels are muted or unchanged. As a result of the increase in GnRH, stimulation of the ovarian theca cells, in turn, produces more androgens. (Urbanek, 2007) Follicular arrest can be corrected by elevating endogenous FSH levels or by providing exogenous FSH. (Shannon, 2012) some studies suggest that PCOS is a primary defect in young girls who are entering puberty and who have a family history of the disorder. Approximately 25% of patients with PCOS have elevated prolactin levels (Marx, 2003).

2.1.3 Clinical presentation of PCOS:

PCOS is a hormonal disorder with a potential to lead to various diseases. It also continues to be a common cause of infertility among women, although signs and symptoms vary, the three most common factors associated with PCOS include ovulation irregularities, increased androgen levels, and cystic ovaries. Problems with ovulation and elevated androgen levels occur in the majority of women with PCOS. hirsutism, acne, and alopecia are directly associated with elevated androgen levels, and the prevalence.1f polycystic ovaries on pelvic ultrasound exceeds 70% in patients with PCOS (Azziz *et al.*, 2006).

2.1.4 Complications of PCOS:

(A) Infertility:

Infertility is defined as absence of pregnancy after two years of regular intercourse, without using any contraception method**.** PCOS is characterized by anovulation due to menstrual alteration by oligmenorrhea. in70%of patient's oligmenorrhea patterns with bleeding at intervals of more than 45 days or fewer that nine annual menstruation periods. alternated with the interval of secondary amenorrhea (absence of menstruation for at least 3consecutive months).this absence of ovulation or dys ovulation implies and alteration in fertility present in 30% to 70% of the patients, infertility are related to the hyper secretion of LH (70) present in women with hyper androgenism an ovulatory women, which entails a longer period of time to achieve pregnancy

(Izzo, 2013).infertility has been considered by world health organization(WHO)as public health problem.one of the central goals of the UN conference program on action on population and development in2015was to guarantee, for all individuals, access to quality reproductive health services(piazza, 2015).Treatment in these women with fertility problems is focused on inducting ovulation. The objective is to increase levels of endogenous FSH that stimulate follicular development. This can be done in one of two ways; by increasing endogenous production with the use of anti-estrogens drugs or aromatase inhibitors. Through exogenous administration of the hormone (legro *et al., 2013*).

(B)Hirsutism:

Hirsutism defined as an excessive growth of terminal hair in androgen dependent areas of women. Is one of the most widely used clinical criteria for the diagnosis of androgen excess and is observed in 50% - 80% of patients with hyperandrogenism (yildiz, 2006*).*

(C)Acne:

Acne is a disorder of the pilosebaceous unit, with lesions on the face,neck,back and chest area. The importance of androgens in the acne pathogenesis is well - know and authenticated. As vulgaris acne, the androgen levels are usually normal. It is believed that the local conversion has been increased for a greater receptors sensibility for androgens in patients with acne in relations to normal population. Perhaps, it represents the most important cause in the disease activation (Fraser *et al*., 2004).

(D)Androgenicalopecia:

The androgenic alopecia in women is characterized by hair loss in the central region of the scalp, with important psychosocial repercussions. In the presence of androgens with increased level of 5-alpha-reductase, the higher concentration of androgen receptors, and lower levels of cytochrome p450enzyme, is shortened anagen phase and terminal follicles suffer miniaturization, becoming vellus hairs (lee *et al., 2007*). Most patients with androgenetic alopecia have the normal endocrine function. The anamnesis and physical examination are important to search of other signs of hyper androgenism(yildiz, 2006).

(E)Acantosenigricans:

The acanthosisnigricansis characterized by the presence of a brown and velvety plate with

accentuation in the furrows of skin. The dermatopathology is the most commonly observed in the neck and intertriginous areas such as armpits. Groin and inframammary region and it is reported in 5% of patient PCOS (Araujo *et al., 2002*).Although to be Acnto associated with obesity, PCOS and diabetes, may be present in genetic disease, drug reaction (nicotinic acid), and malignancies. The presence of Acanto indicates the glucose tolerance test. When severe, extensive and progressive, may be associated with malignancy, especially when the mucus is also involved (Araujo *et al, 2002*).

2.2. Plasma Lipids:

The main plasma lipid transport forms are free fatty acid, triglyceride and cholesteryl ester. Free fatty acid derived primarily from adipocyte Triglycerides, is transported as a physical complex with plasma albumin. Triglycerides and cholesterol ester are transported in the core of plasma lipoproteins. The intestine secretes dietary fat in chylomicrons, lipoproteins that transport triglyceride to tissue for storage dietary cholesterol is transported to the liver by chylomicron remnants which are formed from chylomicrons. HDL.c take up cholesterol from tissues and other plasma lipoproteins (Spector, 1984).

2.2.1. Triglyceride:

Triglycerides are the main constituents of body fat in human and other Animals, as well as vegetable fat. They are also present in the blood to enable the bidirectional transference of adipose fat and the blood glucose from the liver, and are a major component of human skin oils (Nelson and Cox, 2000).

2.2.1.1. Triglyceride Metabolism:

The pancreatic lipase acts at the ester bond, hydrolyzing the bond and realizing the fatty acid. Fatty acids, monoglycerides and some diglyceridesare absorbed by duodenum, once the triglyceride have been broken down. In the intestine, following the secretion of lipases and bile, triglyceride are split in to monoacylglycerol and free fatty acids in the process called lipolysis. They are subsequently moved to absorptive enterocyte cells lining the intestines. The triglyceride are rebuilt in the enterocyte from their fragments and packaged together with cholesterol and proteins to form chylomicrons. These are excreted from the cells and collected by lymph system and transported to the large vessels near the heat before being mixed in to the blood. Various

tissues can capture the chylomicrons releasing the triglyceride tobe used as a source of energy. Liver cells can synthesize and store triglycerides. When the body requires fatty acids as an energy source, the hormone glucagon signals the breakdown of triglycerides by hormone sensitive-lipase to release fatty acids. As the brain cannot utilize fatty acids as an energy source (unless converted to a ketone), (White and Vankatesh, 2011) the glycerol component of TG can be converted into glucose, via gluconeogenesis by conversion into dihydroxyacetone phosphate and then into glyceraldehyde 3-phosphate, for brain fuel when it is broken down. Fat cells may also be broken down for that reason if the brain's needs ever outweigh the bodies. Triglycerides cannot pass through the cell membranes freely. Special enzymes on the walls of blood vessels called lipoprotein lipases must break down TG in to free fatty acids and glycerol. Fatty acids can then be taken up by cells via the fatty acid transporter (FAT).Triglycerides, as major component of very-low-density lipoprotein(VLDL) and chylomicrons, play an important role in metabolisms energy sources and transporters of dietary fat (Drummond and Brefere,2014).

2.2.1.2 Clinical significance of Triglyceride:

Disorder of TG metabolism are separated in to those causing Hypertriglyceridemia and hypertriglyceridemia.

A- Hypertriglyceridemia:

Denotes high (hyper-) blood level (emia) of triglycerides the most abundant fatty molecule in most organisms. Elevated level of TG are associated withatherosclerosis, even in the absence of hypercholesterolemia (high cholesterol levels) and predispose to cardiovascular disease. Very high TG levels also increase the risk of acute pancreatitis. Hypertriglyceridemia itself is usually symptomless, although high levels may be associated with skin lesions known as xanthomas (Berglund*et al.,* 2012) causes of high TG include: Overeating, obesity, Diabetes mellitus and insulin resistance, excess alcohol consumption, kidney failure, nephritic syndrome, genetic predisposition. Some form of familial hyperlipidemia such as familial combined hyperlipidemia i.e. type II hyperlipidemia. Lipoprotein lipase deficiency-Deficiency of this water-soluble enzyme that hydrolyzes triglycerides in lipoproteins, lead to elevated level of TG in the blood, lysosomal acid lipase deficiency, and certain medications e.g.: isotrtionin, propofol, HIV medications, hypothyroidism (underactive thyroid), SLE and associated autoimmune responses Glycogen storage disease type I

B-Hypotriglyceridemia:

Causes of low TG level include: Hyperthyroidism refers to overactive thyroid, disorder contribute to Malnutrition (Improper absorption of nutrients) can lead to malnutrition, certain medications and certain drugs, low fat diet and malabsorption syndrome (Beigneux*et al.,* 2017).

2.2.2Cholesterol:

Cholesterol is an unsaturated steroid alcohol containing four rings (A, B, C, and D), and it has single C–H side chain tail similar to a fatty acid in its physical properties. The only hydrophilic part of cholesterol is the hydroxyl group in the A-ring. Cholesterol is, therefore, also an amphipathic lipid and is found on the surface of lipid layers along with phospholipids. Cholesterol is oriented in lipid layers so that the four rings and the side chain tail are buried in the membrane in a parallel orientation to the fatty acid acyl chains on adjacent phospholipid molecules. The polar hydroxyl group on the cholesterol A-ring faces outward, away from the lipid layer, allowing it to interact with water by non-covalent hydrogen bonding or with the polar head groups of phospholipids. Cholesterol can also exist in an esterified form called cholesteryl ester, with the hydroxyl group conjugated by an ester bond to a fatty acid, in the same way as in triglycerides. In contrast to free cholesterol, there are no polar groups on cholesteryl esters, makingthem very hydrophobic. Because it is not charged, cholesteryl esters are also classified as a neutral lipid and are not found on the surface of lipid layers but instead are located in the center of intracellular lipid droplets or in the hydrophobic core of lipoproteins, along with triglycerides. Cholesterol is almost exclusively synthesized by animals, but plants do contain other sterols (called phytosterols) similar in structure to cholesterol. Dietary phytosterols are known to lower plasma total cholesterol and LDL cholesterol (LDL-C) and raise HDL cholesterol (HDL-C) levels most likely by competing with the intestinal absorption of cholesterol. Cholesterol is synthesized in most tissues of the body from acetyl-CoA in the microsomal and cytosolic compartments of the cell. More than 25 enzymes are involved in the formation of cholesterol from acetyl-CoA. The principal steps include the conversion of acetyl-CoA derived from either the β-oxidation of fatty acids or the oxidative decarboxylation of pyruvate to β-hydroxy β-methyl glutaryl CoA (HMG-CoA).3HMG-CoA is then converted to mevalonic acid by the enzyme HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis.3 Mevalonic acid is phosphorylated, isomerized, and converted to geranyl- and farnesyl pyrophosphate, which, in turn, forms squalene.3 Cyclization of squalene occurs with oxidation, methyl group transfer

reactions, saturation of the side chain, and double bond shifting to producecholesterol.3 Cholesterol is also unique in that,unlike other lipids, it is not readily catabolized by most cells and, therefore, does not serve as a source of fuel. Cholesterol can, however, be converted in the liver to primary bile acids, such as cholic acid and chenodeoxycholic acid, which promote fat absorption in the intestine by acting as detergents. A small amount of cholesterol can also be converted by some tissue, such as the adrenal gland,testis, and ovary, to steroid hormones, such as glucocorticoids, mineralocorticoids, and estrogens. Finally, a small amount of cholesterol, after first being converted to 7-dehydrocholesterol, can also be transformed to vitamin D3 in the skin by irradiation from sunlight (Michael, 2016).

2.2.2.1Hypercholesterolemia:

Hypercholesterolemia is the lipid abnormality most closely linked to heart disease (castell, 1986).One form of the disease, which is associated with genetic abnormalities that predispose affected individuals to elevated cholesterol levels, is called FH. Homozygotes for FH are fortunately rare (1:1million in the population) and can have total cholesterol concentrations as high as 800to 1000 mg/dL(20 to 26 mmol/L).These patients can have their first heart attack when still in their teenage years (Marals, 2004). Heterozygotes for the disease are seen much more frequently (1:500 in the population), because it is an autosomal codominant disorder; a defect in just one of the two copies of the LDL receptor can adversely affect lipid levels. Heterozygotes tend to have total cholesterol concentrations in the range of300 to 600 mg/dL (8to15mmol/L) and, if not treated, become symptomatic for heart disease in their twenties to fifties. Approximately 5% of patients younger than age 50 with CAD are FH heterozygotes. Other symptoms associated with FH include tendinous and tuberous xanthomas, which are cholesterol deposits under the skin, and arcus, which are cholesterol deposits in the cornea (Schaefer, 1997) in both homozygotes and heterozygotes, the cholesterol elevation is primarily associated with an increase in LDL-C. These individuals synthesize intracellular cholesterol normally but lack, or are deficient in, active LDL receptors. There are several classes of defects in the LDL receptor gene that are associated with FH. Class1 mutations result in null allele where no LDL receptor protein is identified. Class2 mutation, which accounts for the majority of clinical mutations in FH, creates a membrane protein that is synthesized but not transported to the Golgi efficiently and, thus, is degraded. Class3 mutations result in an LDL receptor that cannot bind LDL. Class 4 mutations create an LDL receptor that cannot internalize the LDL and transport it to the Golgi,

and class 5 mutations create LDL receptors that do not recycle (changed, 2003).Mutationsin the LDL receptor gene promoter region have also been described. Consequently, LDL builds up in the circulation, because there are insufficient receptors to bind the LDL and transfer the cholesterol into the cells. Cells, however, which require cholesterol for use in cell membrane and hormone production, synthesize cholesterol intra cellular at an increased rate to compensate for the lack of cholesterol from the receptor-mediated mechanism. (Marals, 2004).

2.2.3 High Density Lipoprotein-c:

High-density lipoprotein (HDL) is one of the five major groups of lipoproteins. Lipoproteins are complex particles composed of multiple proteins which transport all fat molecules (lipid) around the body within the water outside cells. They are typically composed of 80-100 proteins per particles (organized by one, two or three Apo A; more as the particles enlarge picking up and carrying more fat molecules) and transporting up to hundreds of fat molecules per particles. HDL particles are sometimes referred to as "Good cholesterol" because they can transport fat molecules out of artery walls, reduce macrophage accumulation and thus help prevent or even regress atherosclerosis, but studies have shown that HDL-lackingn mice still have the ability to transport cholesterol to bile, suggesting that there are alternative mechanism for cholesterol removal.(Betteridge*et al.,* 2008).

2.2.3.1 High Density Lipoprotein.c Metabolism:

The mechanism by which HDL metabolism to occur cannot act alone there for I want to talk about all lipoproteins metabolism. The mechanism by which lipids are used, transported, and removed from the body are complex. Lipoproteins transport lipids in three separate but interacting path ways through the body.

A- Exogenous Pathway:

The newly synthesized chylomicrons in the intestine are initially secreted into the lacteals and then pass into the lymphatic duct and eventually enter the circulation by way of thoracic duct.(Zannis*et al.,*2004).

B- Endogenous Pathway:

Most TG in the liver that are packaged in to VLDL are derived from diet and LDL which responsible for delivery of exogenous cholesterol to peripheral tissues. (Parhofer and Barrett, 2006).

C- Reverse Cholesterol Transport Pathway:

As previously described, one of the major roles of HDL is to maintain the equilibrium of cholesterol in peripheral cells by reverse cholesterol transport pathway (Toth, 2003; Lewis and Rader, 2005).

2.2.3.2 Recommended ranges:

The American Heart Association, NIH and NCEP provide asset of guidelines for fasting HDL level and risk for heart disease.

Table 2.1: Guidelines for fasting HDL level and risk for heart diseases:

Level mg/dl	Level mmol/L	Interpretation
<40/50 Men/women	<1.03	Low HDL cholesterol, heightened risk considered correlated for heart disease
40-59	1.03-1.55	Medium HDL level
>59	>1.55	High level, optimal condition correlated against heart disease

High LDL-c with low HDL-c level is an additional risk factor for cardiovascular disease (Ashwood*et al.*, 2008)

2.2.3.3 Clinical significance of High Density Lipoprotein.c:

2.2.3.3.1. Elevated level of High Density Lipoprotein.c:

Elevated HDL.c level usually correlate with decreased cardiovascular risk however, high HDL cholesterol levels caused by some genetic disorders may not protect against cardiovascular

disease, probably because of accompanying lipid and metabolic abnormalities (Price *et al.,* 2014).

A- Primary causes:

Single or multiple genetic mutations that result in overproduction or decreased clearance of HDL-c (Price *et al.,* 2014).

.B-Secondary causes:

Chronic alcoholism without cirrhosis- primary biliary cirrhosis, hyperthyroidism and drugs (e.g. Corticosteroids, insulin, phenytoin, estrogen)(Price *et al.,* 2014).

2.2.3.3.2 Decreased level of High Density Lipoprotein.c:

Diets that reduce atherosclerosis risk lower levels of HDL.c, but the significance of this is unclear (Brinton, 1990).

Low level of HDL.c, or hypo alpha lipoproteinemia (HA), include a variety of conditions, ranging from mild to severe, in which concentrations of alpha lipoproteins or high-density lipoprotein (HDL) are reduced. The etiology of HDL deficiencies ranges from secondary causes, such as smoking, to specific genetic mutations, such as Tangier disease and fish eye disease (Yamakawa*et al.,* 1999).

Chapter Three

3. Materials and Methods

3.1. Materials:

3.1.1. Study approach: Quantitative methods used to estimate cholesterol, triglyceride and HDL -c in Sudanese females with PCOS in Aljazeera state during the period from February to May 2020.

3.1.2. Study design:

This is comparative hospital based case control study.

3.1.3. Study area:

This study was conducted in Elhasahesa Educational Hospital for gynecological and obstetrics in Aljazeera State.

3.1.4. Study population:

The study included females with polycystic ovary syndrome and healthy females as control group.

3.1.5. Sample size:

A total of 100 samples were collected (50 patients and 50 apparently healthy individual serve as control (age matched with test group).

3.1.6. Inclusion criteria:

Sudanese females with PCOS and healthy individuals serve as control were included.

3.1.7. Exclusion criteria:

Any patients with cardiovascular disease and obesity and any disease lead to obesity formation were excluded.

3.1.8. Ethical considerations:

Verbal informed consent was obtained from all participants in this study.

3.1.9. Data collection:

The clinical data were obtained and recorded on questionnaire sheet.

3.1.10. Sample collection and processing:

From fasting patients local 70% antiseptic for the skin was used 4ml blood was collected by standard vein puncture method, directly in centrifuge tube (no anticoagulant container) and the serum was separated after centrifugation for 5 minutes at 3000 r. p. m at room temperature then used for estimation of triglyceride, HDL-c and total cholesterol.

3.2. Methodology:

3.2.1. Estimation of triglyceride:

3.2.1.1. Principle of the method:

Free glycerol is removed from the reaction mixture by pre-incubation with glycerol phosphate oxidase and peroxidase. The subsequent addition of lipase and the chromogen, 4-aminoantipyrin, results in the formation of color proportional to the amount of triglyceride in serum.

3.2.1.2. Procedure of Triglyceride:

The samples analyzed by full automated ROCHE DIAGNOSTIC – COBAS INTEGRA400 PLUS. (Appendix II).

3.2.2. Estimation of HDL-C:

3.2.2.1. Principle of the method:

The cholesterol concentration of HDL.C is determined enzymatically by cholesterol esterase and oxidase coupled with PEG to the amino groups (approximately 40%) cholesterol esters are broken down quantitatively into free cholesterol and fatty acid by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to cholestenone and hydrogen peroxide.

3.2.2.2. Procedure of HDL-C:

The samples analyzed by full automated full automated ROCHE DIAGNOSTIC – COBAS INTEGRA400 PLUS. (Appendix II).

3.2.3. Estimation of total serum cholesterol:

3.2.3.1. Principle of the method:

Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids, free cholesterol is then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide, the hydrogen peroxide then combines with phenol and para amino-

antipyrinein presence of peroxidase enzyme to form achromophore(quinoneimine dye) which can be measure at filter 500-550nm.

3.2.3.2. Procedure of total serum cholesterol:

The samples analyzed by full automated ROCHE DIAGNOSTIC – COBAS INTEGRA400 PLUS. (Appendix1I).

3.2.4. Measurement of BMI:

Body mass index (BMI) was calculated according following formula as: BMI = Body weight (kg) / (High (m)) ^{2}.

3.3 Quality control:

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

3.4 Statistical analysis:

Data obtained from this study was analyzed using statistical package of the social science (SPSS) version- 25, T test and person correlate were used for comparison and correlation.

Chapter Four

4. Results

This study included 50 female with PCOS and 50 without PCOS age matched ,no significant different between the mean of age in case and control (28.82±3.09) (28.90±3.3) respectively with(p value 0.901).

The mean of body mass index was significantly increased among PCOS patients (31.2±3.45) in comparison with (21.99±1.91) with (p- value 0.00) which represent in **table 4.1**.

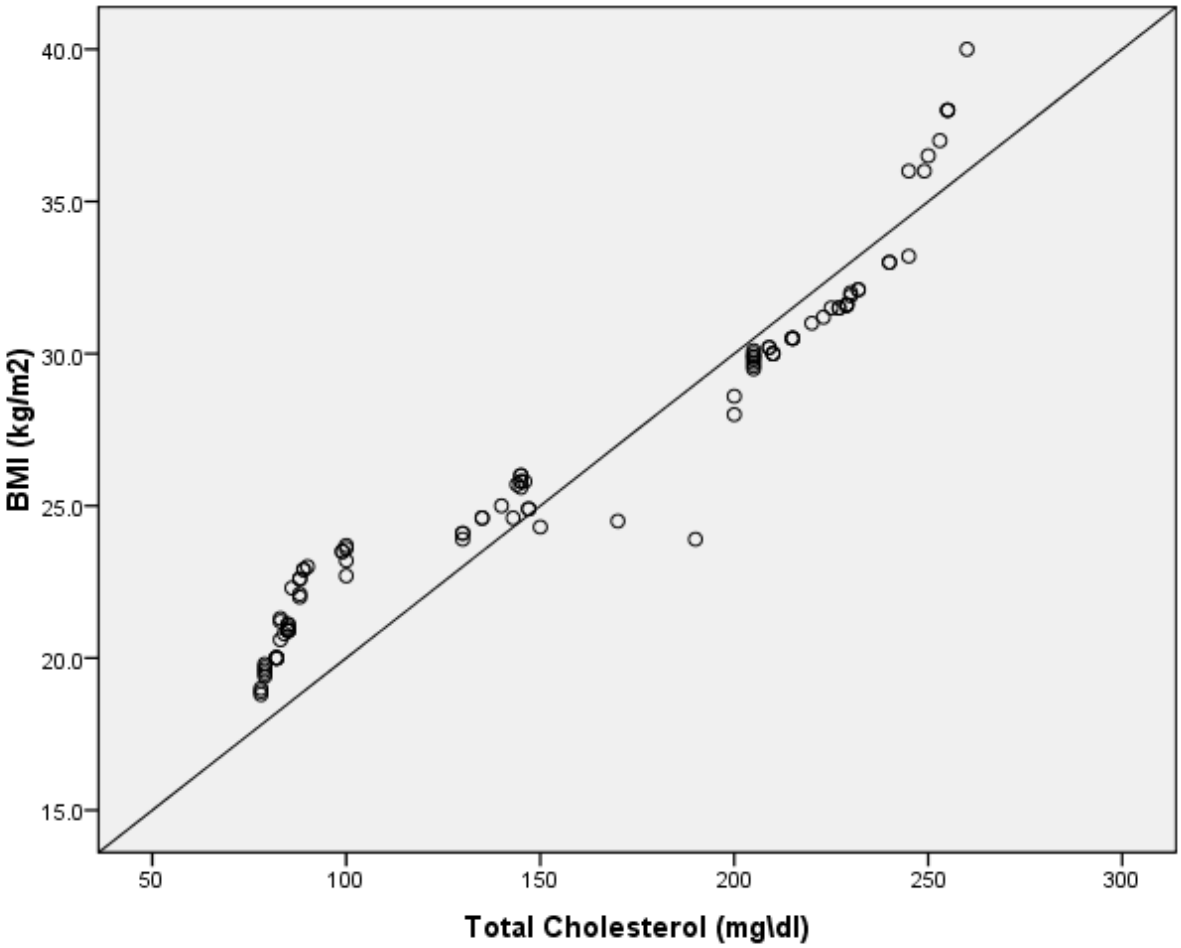
The mean concentration of cholesterol was significantly increased among PCOS patients (213.44±32.44) in comparison with (100.36±27.7) non PCOS with p value (0.00). The mean of triglyceride level showed significant increase in PCOS patients (170.64±8.5) versus non PCOS female (87.3±23.36) with p-value (0.00). Also our results revealed significant decrease in mean concentration of HDL-c in PCOS (31.28±4.2) in comparison with non PCOS (66.7±11.6) with (p value=0.00) which presented in **table4.2.**

Person's correlation showed, statistically significant strong positive correlation of serum cholesterol level when correlated with body mass index of PCOS patients (p-value 0.00,r-value 0.966)(Correlation is significant at the 0.01 level (2-tailed). Presented in **figure 4.1**, also statistically significant strong positive correlation observed when associate serum triglyceride with BMI of PCOS patient (p-value 0.00, r-value 0.879) Correlation is significant at the 0.01 level (2-tailed) presented in **figure4.2**. and statistically significant negative correlation observed when associate HDL-c with BMI of PCOS patients (p- value 0.00, r-value (-0.863) presented in **figure 4.3**.no correlation observed when associated serum cholesterol with age of PCOS patient (p value 0.801,r-value 0.026) presented in **figure 4.4** Also no correlation observed when associate serum triglyceride and HDL-c with age of PCOS patient (p-value 0.496, r-value -0.069) (p-value0.47, r-value 0.073) respectively presented in **figure 4.5** and **figure 4.6**.

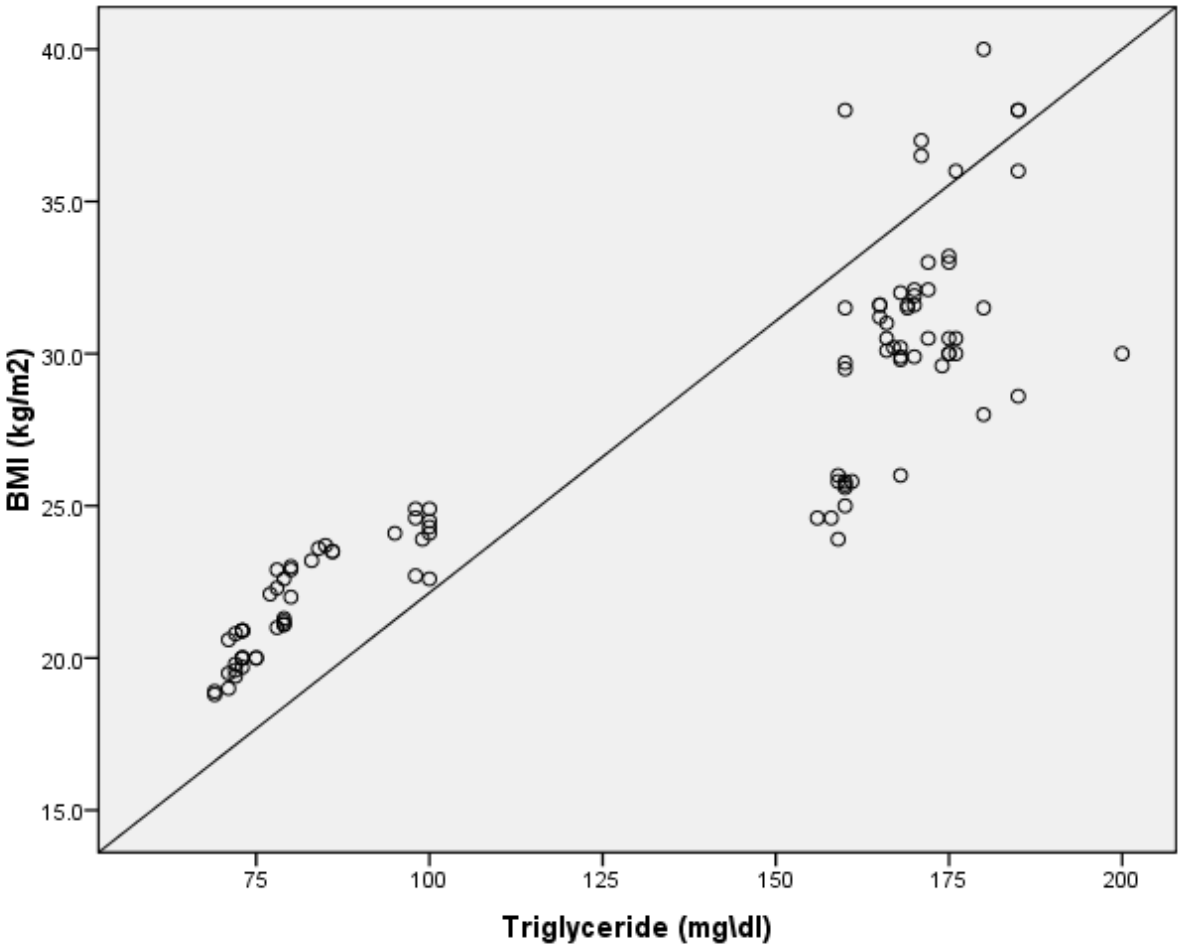
Table 4.1:

Comparison between the mean of total cholesterol, triglycerides and HDL-C in case and control:

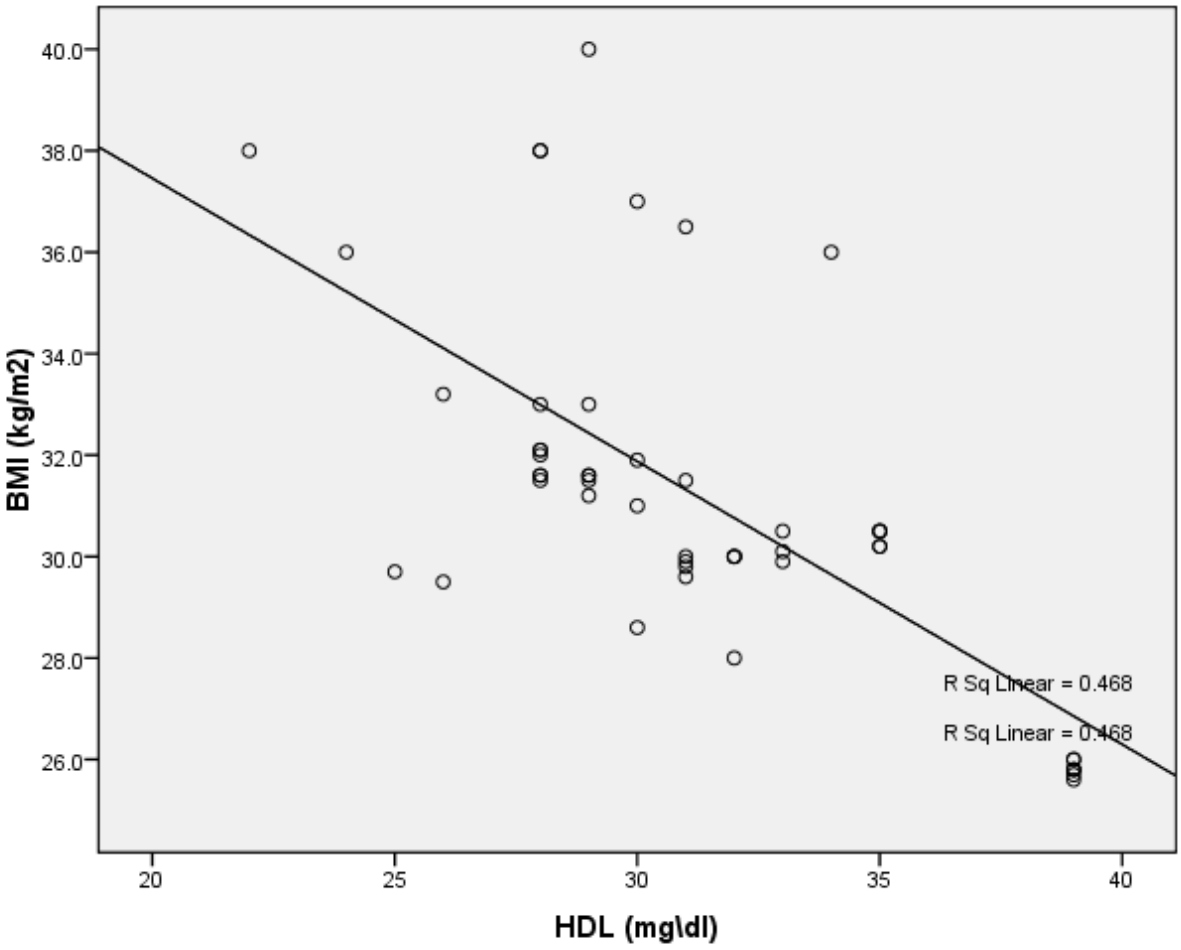
Variable	**Case (n=50) Mean** ±SD	**Control (n=50) Mean** ±SD	**P value**
Cholesterol mg/dl	**213.44**±32.44	**100.36**±27.7	**0.00**
Triglycerides mg/dl	**170.64**±8.5	**87.28**±23.36	**0.00**
HDL-C mg/dl	**31.28**±4.2	**66.7**±11.6	**0.00**



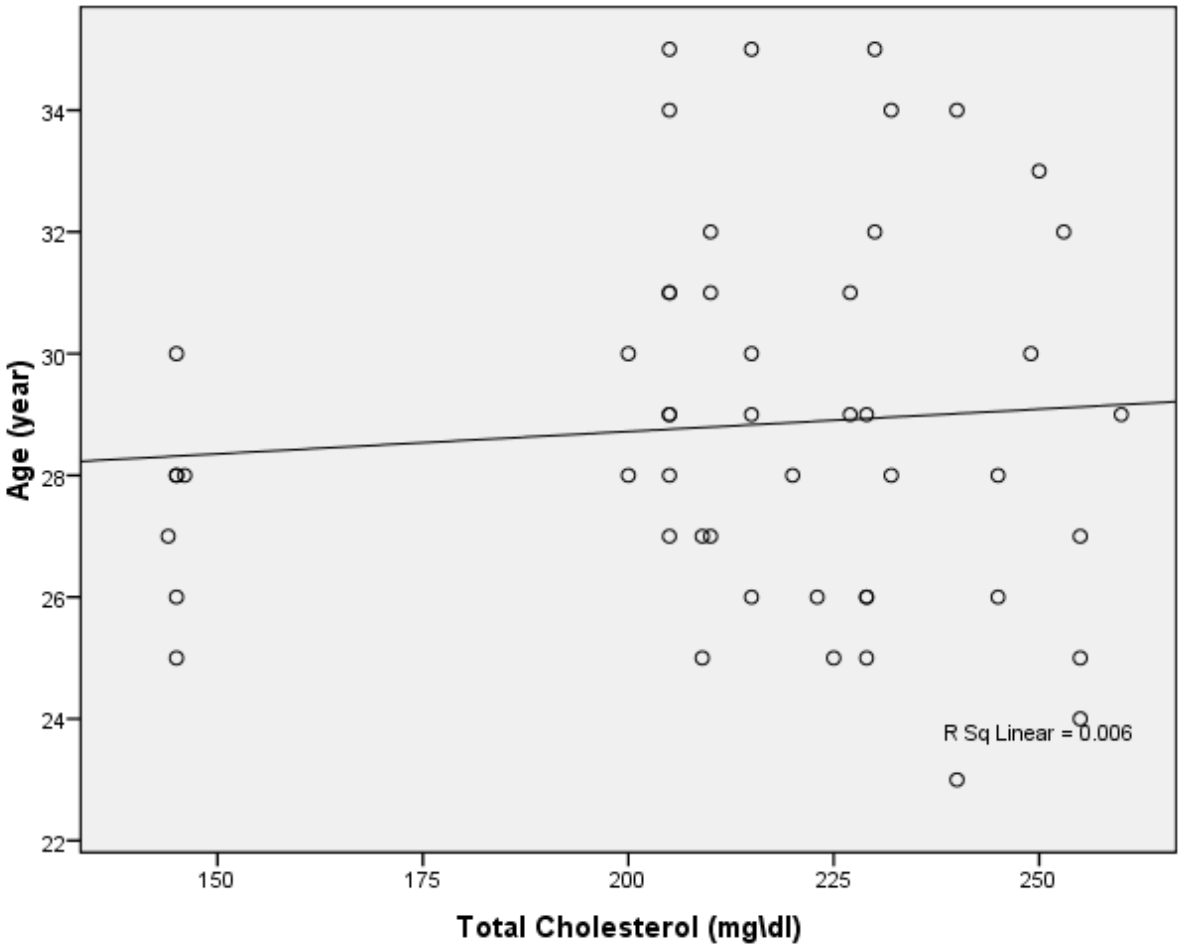
4.1 Figure: Correlation between BMI and total cholesterol,(r- value0.966, p-value= 0.00)



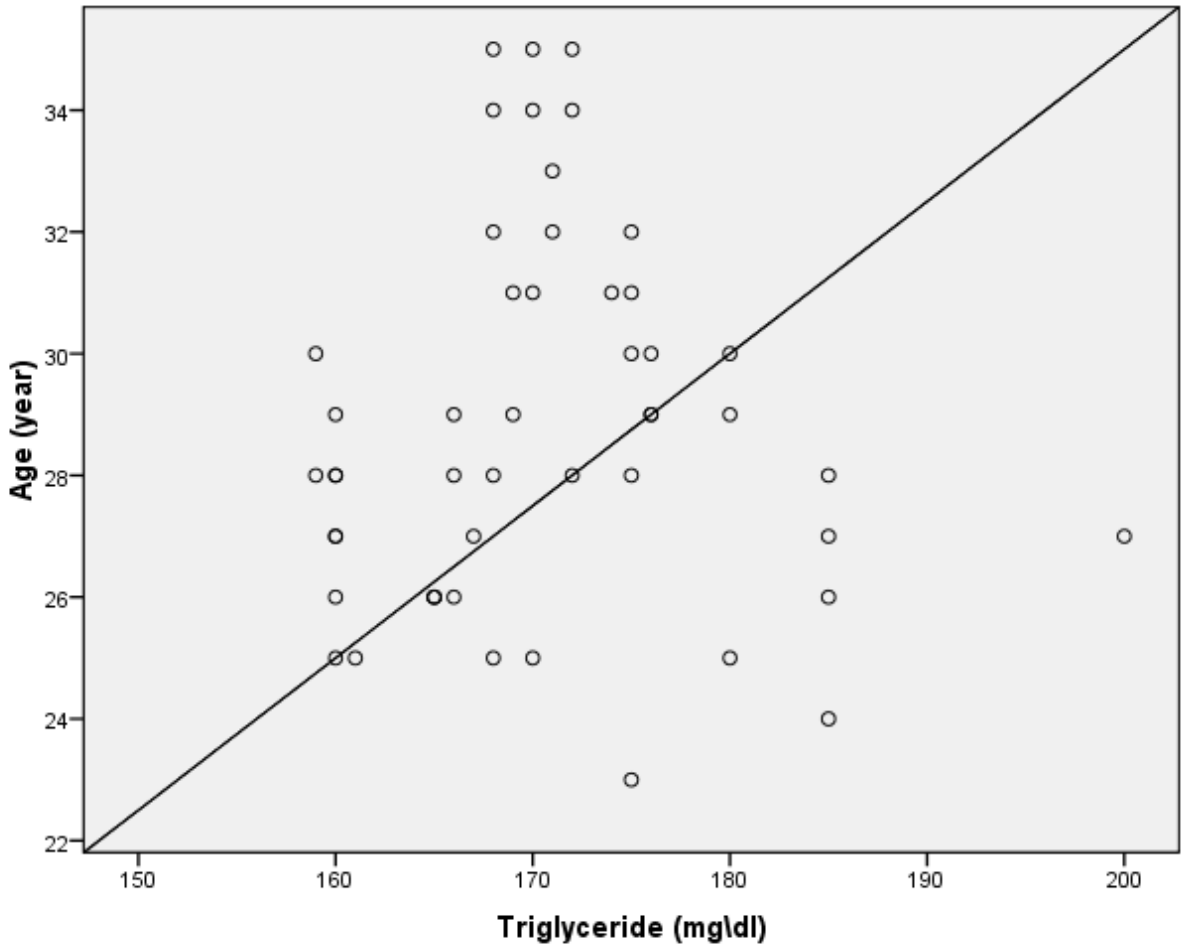
4.2Figure: Correlation between BMI and triglyceride (r-value 0.879,p-value 0.00)



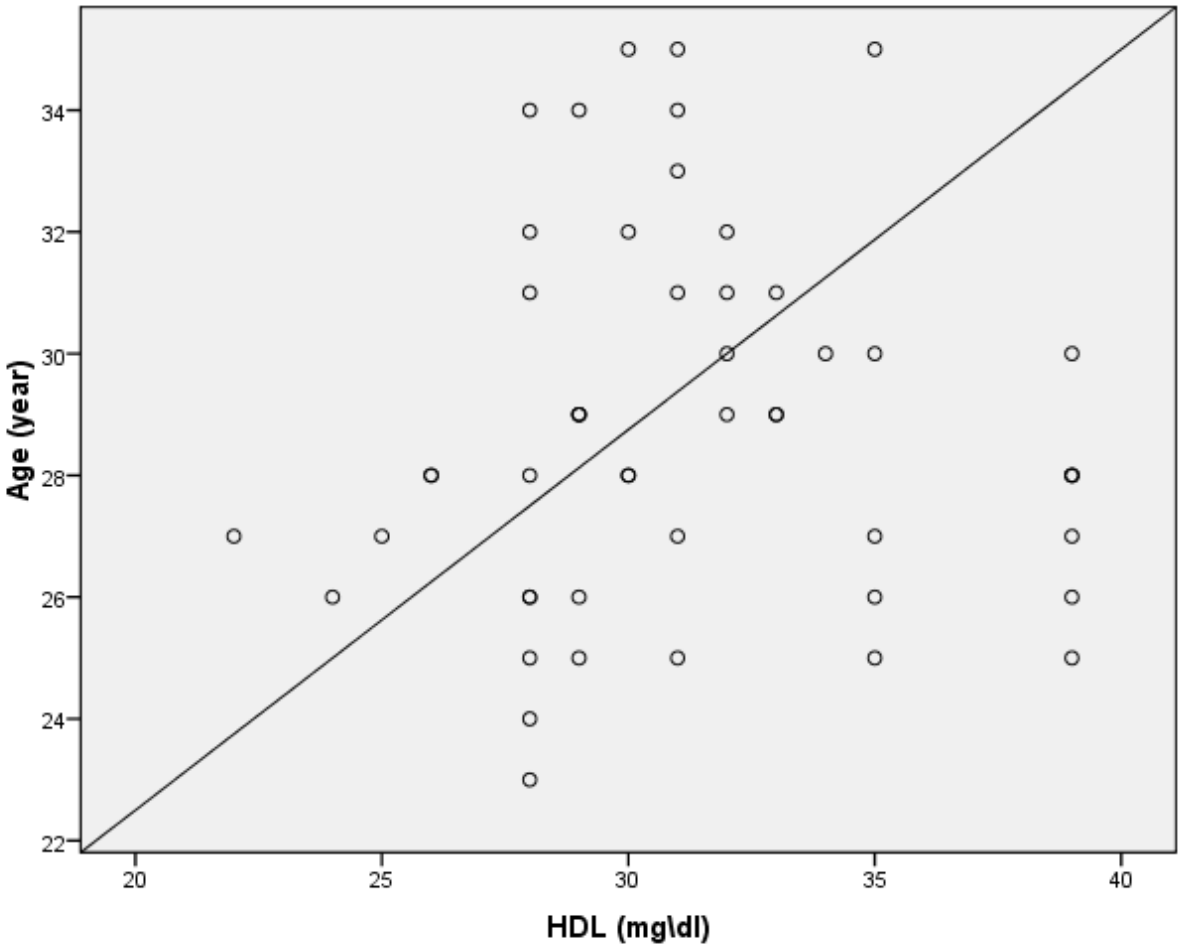
4.3 Figure: Correlation between BMI and HDL-C (r-value -0.863, p- value 0.00)



4.4 Figure correlation between cholesterol and age(r-value -0.026, p value 0.801)



4.5 Figure: Correlation between age and triglyceride: (r-value 0.069, p-value 0.496)



4.6 Figure: Correlation between age and HDL-C (r-value 0.073, p-value 0.470)

Chapter five

Discussion- Conclusion - Recommendations

5.1 Discussion:

PCOS is a condition characterized by large ovaries with multiple cysts resulting in increased production of estrogen and androgen leading to oligomenorrhea, hirsutism, acne, weight gain, excess hair growth on the face and body, thinning scalp hair, ovarian cysts and fertility impairment (Danish, 2010. William, 2012). PCOS is associated with several other diseases/morbidity-related factors such as obesity and other cardiovascular disease, which are becoming more prevalent among females today(Schmidt, 2011).therefore case control study was carried out to assessment status of cholesterol , triglyceride, HDL and BMI Sudanese's female with PCOS.

The present study revealed that, the mean of body mass index was significantly increase among PCOS patients (p- value 0.015) this finding indicate that the PCOS is associated with increased BMI or weight gain when compared with women without PCOS.Our finding was in agreement with some studies who stated that, PCOS is associated with increased BMI , weight gain (Danish, 2010 ; Genazzani, 2008; William, 2012; Martinez *et al*, 2007).

This result showed that, the mean concentration of cholesterol and triglyceride were significantly increasing among PCOS patients in comparison with non PCOS with p value 0.00. This data is similar to data obtain by some studies which reveal that increased mean concentration of cholesterol triglyceride among PCOS patients when compared with non PCOS due to insulin resistance which lead to dislipidemia in PCOS patients (farhan, 2016 ; Ain*et al.*,2019). This study disagreed with study done by Amini*et al* 2014 which showed no significant difference in mean concentrations of lipids between females with PCOS and others without PCOS. Person's correlation revealed positive correlation between serum cholesterol and BMI, serum cholesterol level directly correlated with body mass index of PCOS patients (p-value 0.00), this Data is similar to data obtain by (Mustafa, 2016), which found cholesterol correlated directly with BMI. The result showed; positive correlation observed when associate serum triglyceride with BMI of PCOS patient (p- value 0.00), which agree with (Mustafa, 2016). Also negative correlation observed when associate HDL-C with BMI of PCOS patients(r = -0.863 p- value 0.00), this Data

is similar to data obtain by (farhan, 2016) which show; negative correlation between HDL and BMI (r value- 0.802, p-value 0.01).

5.2 Conclusion

The study was concluded that, serum level of cholesterol, triglyceride are increased and HDL-C is decreased among PCOS patients. There were statistically significant strong positive correlation between cholesterol and Triglyceride with BMI while statistically negative correlation seen between HDL-C and BMI among PCOS patients.

5.3 Recommendations:

- From the findings of this study it is recommended that:
- Life style modifications program such as exercise, healthy diets, low calories intake, and physical activities to be implemented in females especially over weight to reduce the susceptibility to polycystic ovary syndrome.
- Further studies should be done to investigate insulin receptor gene, Aromatase enzyme gene, adiponectin genes among PCOS patient.
- Biochemical test “LH; FSH Rito or androgen or AMH” should be done as early screening tests especially to female have irregular menstrual cycle or over weight to prevent PCOS complications.

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