بسم الله الرحمن الرحيم

Sudan University of Science and Technology
Collage of Graduate Studies

Lipid profile Among Sudanese Women using Combined Oral contraceptives in Algazeera State

مستويات الدهون في السيدات اللائي يستخدمن حبوب منع الحمل مزدوجة الهرمون

A dissertation submitted for partial fulfillment for the requirement of M.Sc Degree in Medical Laboratory science- Clinical chemistry

By:

Samah Abdelateef Fadlalmola Mohammed

B.Sc in Medical Laboratory Sciences-Clinical Chemistry

Om Durman Islamic University

Supervisor:

Dr. Ghada Abdurrahman Alfadil

Assistant professor of clinical chemistry

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الآية

﴿وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلَّا قَلِيلًا﴾

صدق الله العظيم

سورة الاسراء الاية(85)

Dedication

To my parents mother and father
To my sons and my daughter
To my sister and my husband
To all my friends and all my teachers

Acknowledgments

All praises to Allah, for all countless gifts that he gave us, it is a great pleasure to acknowledge my deepest thanks and gratitude to **Dr. Ghada,** for her kind supervision, for her creative and comprehensive advice until this work came to existence, it is a great honor to work under her supervision.

 I would like to thank the **Alreyada heath laboratory for** helping me in samples analysis, my thank extend to the   participants who agreed to give me their sample to encourage reach.

Finally, I must express my very profound gratitude to my parents, my sister, and my friends for providing me with unfailing support and continuous encouragement throughout my study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

Abstract

Background: Combined oral contraceptive are medication administrated by mouth that prevent pregnancy primarily by inhibiting ovulation .long term using of c0mbined oral contraceptive lead to increase lipid profile, body mass index and increase risk of hypertension and cardiovascular diseases, so this study aim to evaluate lipid profile among women using combined oral contraceptive more than one year in Elgazera state.

Materials and methods: this study was included (100) women, (50) women using combined oral contraceptive and (50) women non using combined oral contraceptive at same age, blood specimen was collected from both groups and total plasma cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol were analyzed by using full automated analyzer(DIRUI .CS.T 240).

Results: the result revealed that there was significant increase of the means of total plasma cholesterol in combined oral contraceptive group (182± 24.8 mg/dL) when compared with control group (140.2± 24.4 mg/dL), p value (0.00),there was insignificant decrease of the means of serum HDL-C in combined oral contraceptive group (51.9± 11.7 mg/dL) compared with control group (54.1±9.4 mg/dL), p value (0.289), and insignificant increase of the means of LDL-C in combined oral contraceptive (96.7± 24.4 mg/dL) compared with control group (91.8± 15.8 mg/dL), p value (0.230), also there was significant increase of the mean of body mass index in combined oral contraceptive group (31.5± 2.8 mg/dL) compared with control group (28.1±2.5 mg/dL) , with p value (0.00). There were significant positive correlations between total cholesterol and (age, BMI, and duration of using contraceptive).

Conclusion: this study concludes that, women received combined oral contraceptive had higher level of plasma total cholesterol , plasma LDL-C, and lower level of plasma HDL-C, and significantly positive correlations between plasma total cholesterol and (age, body mass index and duration among women using combined oral contraceptive).

المستخلص

خلفية الدراسة : حبوب منع الحمل مزدوجة الهرمون هي عبارة عن عقار يستخدم لمنع الحمل عن طريق إيقاف عملية التبويض , إستخدام هذا العقار لفترة طويلة يؤدي إلي زيادة نسبة الدهون في الدم وزيادة في مؤشر كتلة الجسم مما يزيد من الإصابة بأمراض ضغط الدم وأمراض القلب وتصلب الشرائين.

الهدف من هذه الدراسة هو قياس نسبة الدهون في الدم لدي السيدات اللائي يستخدمن حبوب منع الحمل مزدوجة الهرمون لأكثر من عام بإستمرار.

الطرق والمواد: شملت هذه الدراسه 100 سيدة في سن الإنجاب وصنفن إلي مجموعتين ,مجموعة إستخدمت حبوب منع الحمل مزدوجة الهرمون وتضم 50 سيدة ومجموعه أخري لم تستخدم هذا العقار وتضم أيضا 50سيدة ومن ثم قياس نسبة الدهون في الدم لكل من المجموعتين والمقارنة بينهما.

النتائج : أوضحت الدراسة أن هناك زيادة واضحة في متوسط بلازما الكولسترول لدي المجموعة المستخدمة لحبوب منع الحمل مزدوجة الهرمون بالمقارنة مع السيدات اللائي لم يستخدمن هذا العقار ($p\ value\ =\ 0.00*$) قيمة إحتمالية . وأوضحت الدراسة ايضا أن هناك نقصان غير واضح في متوسط البروتين الدهني مرتفع الكثافة (HDL-C) لدي المجموعة المستخدمة لحبوب منع الحمل مزدوجة الهرمون بالمقارنة مع السيدات اللائي لم يستخدمن هذا العقار ($p\ value=0.289$) ,بينما هناك نقصان غير واضح في متوسط البروتين الدهني منخفض الكثافة (LDL-C) لدي المجموعة المستخدمة للعقار بالمقارنة مع المجموعة غير المستخدمة ($p\ value\ =0.230$). وأيضا أ ثبتت الدراسة أن هناك علاقة طردية بين نسبة الكولسترول مع العمر ومؤشر كتله الجسم والفترة الزمنية لهذا العقار.

الناتج النهائي : خلصت هذه الدراسة أن هناك زيادة في نسبة الكولسترول ومؤشر كتلة الجسم لدي المجموعة المستخدمة لهذا العقار , وأن هناك علاقة طردية بين نسبة الكولسترول والعمر ومؤشر كتلة الجسم والفترة الزمنية لإستخدام هذا العقار.

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1. Introduction, Rationale, and objectives

1.1 Introduction

Combined oral contraceptives consist of synthetic estrogen and progestin preparations that prevent pregnancy by suppressing ovulation, thickening the cervical mucus, and altering the endometrial.combined oral contraceptive use is not recommended for breast-feeding women until at least 6 months postpartum. Non–breast-feeding women should wait 3 weeks postpartum before beginning combined oral contraceptive use because of the increased risk of thromboembolic disease. If one pill is missed in a cycle, two pills should be taken at the next scheduled time, and the pack should be completed as usual. If two or more consecutive pills are missed, the package of pills should be completed as prescribed, and an alternative contraceptive method should be used for the remainder of the cycle. there are several contra indications to combined oral contraceptive use and several side effects (Bankowski., 2002).

Many authors of different countries reported dyslipidemia effects of long term use of oral contraceptives. they reported increased serum total cholesterol, low density lipoprotein (LDL-C) and triglyceride but decreased high density lipoprotein (HDL-C) (yasmin *et al*., 2013).

Dyslipidemia represent as spectrum of abnormalities resulting from aberrant lipid metabolism leading to excessive entry of lipoproteins into to the blood stream. dyslipidemia can be genetic in origin and/or result from or be exacerbated by a variety of secondary factors including life style other medical conditions and use of certain medication. elevations in total cholesterol, (LDL-C) and triglycerides as well as depressions in (HDL-C) are important risk factors for cardiovascular disease in addition lipid disorders have been associated with risk of Venus thrombo-embolism (Ragoman *et al* ., 2016).

There are elevation in lipid profile, blood pressure and body mass index in women using combined oral contraceptive which are metabolic risk factors for the development of cardiovascular diseases (CVD). The women should be

screened for lipid profile and blood pressure before starting combined oral contraceptive and followed up regularly to prevent the risk of cardiovascular diseases (Mohammad *et al*., 2013).

1.2 Rationale

Combined oral contraceptive using lead to many side effect particulary headache, nausea, vomiting, loss of libido, breast enlargement, also may effect in menstrual cycle either bleeding or stopping, lactation is suppressed by combined oral contraceptive using. long term using of combined oral contraceptive lead to dyslipidemia,hypertension,cardiovascular disease, Venus thrombo embolism, breast cancer and cervical cancer . Insipid of these serious problems there is little information about combined oral contraceptive among women, and there is no awareness about there risks, so this study will insight light on lipid profile in women of reproductive age.

1.3 objectives

1.3.1 General objective

To evaluate lipid profile among women using combined oral contraceptive pills in Elgazera state.

1.3.2 Specific objectives

1- to measure lipid profile (plasma total cholesterol, HDL-C, LDL-C) among study group (case and control).

2- To measure weight, weight and calculate BMI

 3- To correlate between lipid profile and study variables (age ,BMI and duration of using combined oral contraceptive .

2. Literature Review

2.1 Contraception

Contraception means the ability to control fertility by reliable artificial methods has transformed both social and epidemiological aspects of human reproduction. men and women have used contraception, in one form or another, for thousands of years. There is no one method that will suit every one and individual will use different types of contraceptives at different stage of lives (Al-gazally *et al*., 2010).

2.1.1 Oral contraceptives

About one-third of all sexually active women in the United States use oral contraceptives, with over one half of young women 20 to 24 years old using these contraceptives (Bickmann *et al*., 2010) .

Oral contraceptives (OCs) are medications administered by mouth that prevent pregnancy primarily by inhibiting ovulation. They are one method of birth control and are of two main types i.e. the combined oral contraceptive pill (COC) containing both estrogen and progesterone and progestogen only pill (Osman *et al*. , 2017).

Combined oral contraceptives contain two hormones similar to the natural hormones in a woman's body-an estrogen and a progestin. Also called combined pills, combined oral contraceptives, oral contraceptives, the Pill, and birth control pills. Present-day combined oral contraceptives contain low doses of hormones. they are often called low-dose combined oral contraceptives. There are two types of pill packets, Some packets have 28 pills, These contain 21 "active" pills, which contain hormones, followed by 7 "reminder" pills of a different color that do not contain hormones. Other packets have only the 21 "active" pills (Hatcher *et al*., 2003).

Progestin-only contraceptives (progestin-only "minipill") act primarily by making the cervical mucus thick and relatively impermeable. ovulation continues normally in about 40% of patients using the progestin-only formulation. these oral contraceptives are of special usefulness in two clinical

situations: lactating women and women over 40, in the former group, the progestin effect coincides with the prolactin-induced suppression of ovulation; in the latter group, the inherent reduced fecundity adds to the progestin effect. there is no effect on the quality or quantity of breast milk or any evidence of short- or long term adverse effects on infants and the progestin-only pill may be started immediately after delivery in the breast feeding mother, the progestin-only pill is also a good choice for women in whom estrogen-containing formulations are contraindicated. because of the low dosages of progestin, the minipill must be taken at the same time each day, starting on the first day of menses. If a woman is more than 3 hours late in taking the minipill, a back-up contraceptive method should be used for 48 hours (Bichmann *et al* ., 2010).

2.1.2 Combined Oral Contraceptive Pill

Combined oral contraceptive pills are the most popular contraceptive method world-wide. 50-60 %of women of reproductive age world-wide use this method for birth control (Yasmin *et al*. , 2013).

2.1.3 Estrogen Component of Combination Oral Contraceptives

Estradiol is the most potent natural estrogen and is the major estrogen secreted by the ovaries. The major obstacle to the use of sex steroids for contraception was inactivity of the compounds when given orally. A major breakthrough occurred in 1938 when it was discovered that the addition of an ethinyl group at the 17 position made estradiol orally active. ethinyl estradiol is a very potent oral estrogen and is one of the two forms of estrogen in every oral contraceptive. The other estrogen is the 3-methyl ether of ethinyl estradiol, mestranol. Mestranol and ethinyl estradiol are different from natural estradiol and must be regarded as pharmacologic drugs. Animal studies have suggested that mestranol is weaker than ethinyl estradiol, because mestranol must first be converted to ethinyl estradiol in the body. Indeed, mestranol will not bind to the cellular estrogen receptor. Therefore, unconjugated ethinyl estradiol is the active estrogen in the blood for both mestranol and ethinyl estradiol (Seproff *et al., 2005*).

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2.1.4 Progestin Component of Combination Oral Contraceptives

The discovery of ethinyl substitution and oral potency led (at the end of the 1930s) to the preparation of ethisterone, an orally active derivative of testosterone. In 1951, it was demonstrated that removal of the 19-carbon from ethisterone to form norethindrone did not destroy the oral activity, and most importantly, it changed the major hormonal effect from that of an androgen to that of a progestational agent. Accordingly, the progestational derivatives of testosterone were designated as 19-nortestosterones (denoting the missing 19-carbon). The androgenic properties of these compounds, however, were not totally eliminated, and minimal anabolic and androgenic potential remains within the structure (Seproff *et al.* , 2005).

The dose of estrogen in the combined oral contraceptive pill varies from 15 to 50 μg. Only one50 μg pill is marketed in the UK and it contains mestranol. Most women now use the so-called low-dose pills containing 30–35 μg. Low-dose pills are potentially safer since the cardiovascular risks of the pill are mainly due to estrogens. Although the lowest-dose pill currently available (15 μg ethinylestradiol) has the same efficacy as 30 μg pills, cycle control is less effective and breakthrough bleeding more common. The progestogens used in currently available pills fall broadly into three groups, first and second generation progestins (e.g. norethindrone and levonorgestrel respectively) and the third generation series including gestodene, desogestrel and norgestimate.

Combined pills are available as monophasic preparations in which every pill in the packet contains the same dose of steroids and biphasic and triphasic preparations in which the dose of both steroids changes once or twice during the cycle. Phasic pills were introduced to reduce the total dose of progestogens and in the belief that a regimen which mimicked the normal cycle would produce better cycle control (Edmonds. 2007).

2.1.5 Efficiency of combined oral contraceptive

 Pregnancy rate with combined oral pill is 0.1 per 100 woman years, which is the lowest of all contraceptives in use today. During the first cycle of use, ovulation may not be suppressed and the patient is advised to use an additional

method to prevent pregnancy. Lately, starting the pill on the first day of the cycle has reduced the failure rate and the need to take the additional precaution in the first cycle. If she forgets to take a tablet, she should take two tablets the following day. If she forgets to take the tablet more than once in a cycle, she is no longer adequately protected and must use a barrier method during that cycle. The majority of failures with oral combined pills are due to the failure to take the pills regularly (Howkins and Bourne. 2015).

2.1.6 Mechanism of Action of combined oral contraceptive

The combination pill, consisting of estrogen and progestin components, is given daily for 3 of every 4 weeks. The combination pill prevents ovulation by inhibiting gonadotropin secretion via an effect on both pituitary and hypothalamic centers. The progestational agent in the pill primarily suppresses luteinizing hormone (LH) secretion (and thus prevents ovulation), while the estrogenic agent suppresses follicle-stimulating hormone (FSH) secretion (and thus prevents the selection and emergence of a dominant follicle). Therefore, the estrogenic component significantly contributes to the contraceptive efficacy. However, even if follicular growth and development were not sufficiently inhibited, the progestational component would prevent the surge-like release of LH necessary for ovulation. the estrogen in the pill serves two other purposes. It provides stability to the endometrium so that irregular shedding and unwanted breakthrough bleeding can be minimized; and the presence of estrogen is required to potentiate the action of the progestational agents. The latter function of estrogen has allowed reduction of the progestational dose in the pill. The mechanism for this action is probably estrogen's effect in increasing the concentration of intracellular progestational receptors. Therefore, a minimal pharmacologic level of estrogen is necessary to maintain the efficacy of the combination pill. because the effect of a progestational agent will always take precedence over estrogen (unless the dose of estrogen is increased many, many-fold), the endometrium, cervical mucus, and perhaps tubal function eflect progestational stimulation. The progestin in the combination pill produces an endometrium that is not receptive to ovum

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implantation, a decidualized bed with exhausted and atrophied glands; the cervical mucus becomes thick and impervious to sperm transport. It is possible that progestational influences on secretion and peristalsis within the fallopian tubes provide additional contraceptive effects (Seproff *et al*., 2005).

2.1.7 Advantages of combined oral contraceptive

The combined oral contraceptives are very effective when used correctly, the use of combined oral contraceptive Increased sexual enjoyment because no need to worry about pregnancy, the monthly periods are regular; lighter monthly bleeding and fewer days of bleeding; milder and fewer menstrual cramps, so that can prevent or decrease iron deficiency anemia, combined oral contraceptives can be used at any age from adolescence to menopause, also can be used as an emergency contraceptive after unprotected sex, the women user can stop taking pills at any time and the fertility returns soon after stopping (Hatcher *et al*., 2003).

2.1.8 Side effect of combined oral contraceptive

Many side effect reported, particularly headache, weight gain and loss of ibido, are common among women not using combined oral contraceptives, those likely to be directly related to the contraceptive steroids include fluid retention, nausea and vomiting, and breast enlargement (Edmonds. 2007). Some time combined oral contraceptive cause intermenstrual spotting is common in the first 3 months of the start of the pills but it gradually disappears. heavy spotting can be stopped by increasing the dose for a few months, menstrual bleeding can become very scanty and occasionally a woman becomes amenorrhoeic causing undue fear of pregnancy. amenorrhoea of 6 months requires investigations; lactation is suppressed with combined pills. The combined pills are therefore contraindicated in a lactating mother (Howkins and Boure., 2015).

2.1.9 Benefits of combined oral-contraceptive use

2.1.9.1 Ovarian Cancer

The risk of ovarian cancer is reduced by at least half among women who use oral contraceptives, including those who use low estrogen formulations. The reduction in risk occurs after relatively short term use (5 years) and persists for 10 to 20 years after use has been discontinued. this benefit extends to women with a family history of ovarian cancer and women with a mutation in the BRCA1 or BRCA2 gene. The suggested mechanism for this effect is the suppression of ovulation (Petitti. 2003).

2.1.9.2 Acne

Randomized, double-blind, placebo-controlled trials show substantial reductions in the severity of acne among both patients given oral contraceptives and patients given placebo, but the patients who received oral contraceptives had greater improvement. Randomized trials comparing low-estrogen oral contraceptives including different progestins do not show consistent differences among formulations. Some formulations have been approved for a marketing claim regarding their beneficial effect on acne, but all low-dose combination oral contraceptives cause a similar decrease in the concentration of free testosterone, the presumed mechanism for the improvement of acne (Petitti. 2003).

2.1.9.3 Menstrual Disorders, Loss of Blood, and Anemia

One randomized, double-blind, placebo-controlled trial of low-dose oral contraceptives showed that their use reduces the severity of dysfunctional uterine bleeding. Oral contraceptive use decreases menstrual blood flow and is associated with a reduced prevalence of anemia and increased hemoglobin concentrations in anemic women (Petitti. 2003).

2.1.10 Risks of combined oral contraceptive

2.1.10.1 Dyslipidemia

Many authors of different countries reported dyslipidemia effects of long term use of oral contraceptives. They reported increased serum total cholesterol, LDL-C and triglyceride but decreased HDL-C (Yasmin *et al*., 2013).

2.1. 10. 2 Hypertension

The use of combined oral contraceptive increase in blood pressure is mostly due to estrogen whose mechanism involves the activation of the renin angiotensin aldosterone system, probably on sodium and water retention by the interaction with the mineralocorticoid receptor (Hyacinthe *etal*., 2017).

There was relationship between duration of oral contraceptive use and risk of hypertension, the risk of hypertension increased by 13%for every 5 years increment in oral contraceptive use (Zamane *et al*., 2017).

2.1.10.3 Cardiovascular diseases

There are elevation in lipid profile, B.P and BMI in women using combined oral contraceptive which are metabolic risk factors for the development of cardiovascular diseases (CVD), the women should be screened for lipid profile and blood pressure before starting combined oral contraceptive and followed up regularly to prevent the risk of cardiovascular diseases (Mohammed *et al*., 2013).

Long term use of low- dose oral contraceptive significantly increases the risk of both cardiovascular diseases, including a significant risk of vascular arterial complication with third generation oral contraceptive (Baillargeon *et al*., 2005).

2.1.10.4 Venous thromboembolism

Venous thromboembolism (VTE) is a term that includes deep vein thrombosis and pulmonary embolism. Deep vein thrombosis (DVT) is a blood clot that develops in a deep vein in the body—usually within muscles. (The opposite of a deep vein is a superficial vein—one that is close to the skin, or the surface). The most common place for a DVT to develop is in the veins of the legs or pelvis, but they can also develop in the arms, brain, or intestines Pulmonary embolism (PE) occurs when blood clots in the deep veins (DVT) break free, travel through the circulatory system to the lungs, and lodge in a main artery or arteries, blocking blood flow. This blockage can cause high blood pressure in the lungs. As a result, the heart pumps harder than usual, and may enlarge and eventually fail from being overwork (Gioramo *et al*., 2017).

Elevations in total cholesterol, low density lipoprotein (LDL-C) and triglycerides as well as depressions in high density lipoprotein (HDL-C) are important risk factors for cardiovascular disease in addition lipid disorders have been Associated with risk of Venus thromboembolism (Ragoman *et al*., 2016). There is a three to five fold increase in the risk of venous thromboembolism (VTE) associated with combined oral contraceptive use which is apparently independent of the dose of oestrogen, certainly if it is <50 μg. The risk is unaffected by age, smoking or duration of pill use, but is higher in obese women (BMI >25 kg/m2*)* and in women with a history of pregnancy-induced hypertension (PIH). Four studies published in 1995 and 1996 demonstrated a differential risk of VTE depending gestodene or desogestrel were shown to have a roughly two fold increased risk of VTE when compared with first or second generation combined pills (Edmonds. 2007).

2.1.10.5 Breast cancer

Use of the combined oral contraceptive was associated with increase in the risk of breast cancer. the increased risk persists for 10 years after stopping the pill. The relative risk for current users was 1. 24; for 1–4 years after a small stopping, 1.16; and for 5–9 years after stopping, 1.07. After 10 years the relative risk was the same as that of non-users. although the relative risk was higher for women who started the pill at a young age, there was little added effect from the duration of use, dose or type of hormone. ever-users were significantly less likely (RR 0.88) to have metastatic disease even if they had stopped the pill more than 10 years earlier (Edmonds. 2007).

2.1.10.6 Cervical cancer

Significant increase risk of cervical cancer has been reported in association with combined oral contraceptive use of more than 5years .the risk seem to be related to the presence of high-risk human papillumavirus (Brynhilelsen. 2014).

2.2 Lipids

Lipids are present in humans, animals, plants and micro-organisms to some extent. Animal fat, egg yolk, butter and cheese are lipids of animal origin, vegetable or cooking oils are lipids are plant origin (Rao. 2006).

Lipids are defined as organic compounds that are poorly soluble in water but miscible in organic solvent (Crook . 2012).

2.2.1 Biomedical Important of lipid

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, that are related more by their physical than by their chemical properties. They have the common property of being are relatively insoluble in water and also soluble in non polar solvents such as ether and chloroform. they are important dietary constituents not only because of the high energy value of fats, but also because essential fatty acids and fat soluble vitamins and other lipophilic micronutrients are contained in the fat of natural foods. Dietary supplementation with long chain v3 fatty acids is believed to have beneficial effects in a number of chronic diseases, including cardiovascular disease, rheumatoid arthritis and dementia. Fat is stored in adipose tissue, where it also serves as a thermal insulator in the subcutaneous tissues and around certain organs. non polar lipids act as electrical insulators, allowing rapid propagation of depolarization waves along myelinated nerves. Lipids are transported in the blood combined with proteins in lipoprotein particles . Lipids have essential roles in nutrition and health and knowledge of lipid biochemistry is necessary for the understanding of many important biomedical conditions (Ferrier. 2014).

2.2.2 Classification of lipid

2.2.2.1 Simple lipids

Include fats and waxes which are esters of fatty acids with various alcohols:

Fats are Esters of fatty acids with glycerol. Oils are fats in the liquid state,

Waxes are Esters of fatty acids with higher molecular weight monohydric alcohols (Rodwell *et al*., 2010).

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2.2.2.2 Complex lipids

Are esters of fatty acids containing groups In addition to an alcohol and one or more fatty acids they can be divided into three groups: Phospholipids are Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. they frequently have nitrogen-containing bases (e.g., Choline) and other substituents. In many phospholipids the alcohol is glycerol (glycerophospholipids), but in sphingophospholipids it is sphingosine, which contains an amino group, other group Glycolipids (glycosphingolipids): are lipids containing a fatty acid, sphingosine, and carbohydrate, other complex lipids: Lipids such as sulfolipids and amino lipids. lipoproteins may also be placed in this category (Devlin. 2002).

2.2.2.3 Precursor and derived lipids

These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, ketene bodies, hydrocarbons, lipid-soluble vitamins and micronutrients, and hormones. because they are uncharged, acylglycerols (glycosides), cholesterol, and cholesteryl esters are termed neutral lipids (Rodwell *et al*., 2010).

2.2.3 Cholesterol

Cholesterol is a waxy substance made by animal liver and also supplied in diet through animal products such as meats, poultry, fish and dairy products. cholesterol is needed in the body to insulate nerves, make cell membranes and produce certain hormones, and it is an important lipid in some membranes.

however, the body makes enough cholesterol, so any dietary cholesterol isn't needed (Hongbao and Shieh., 2006).

 Cholesterol is synthesized by virtually all tissues in humans, although liver, intestine, adrenal cortex, and reproductive tissues, including ovaries, testes, and placenta (Ferrier. 2014).

2.2.3.1 Type of cholesterol

Cholesterol can be good or bad. HDL-C is called "good cholesterol" that is good for the cardiovascular system and LDL-C is called "bad cholesterol" that is bad for the cardiovascular system. These are the form in which cholesterol travels in the blood. LDL-C have little protein and high levels of cholesterol

and HDL-C has a lot of protein and very little cholesterol. LDL-C is the main source of artery clogging plaque. HDL-C actually works to clear cholesterol from the blood   (Hongbao and Shieh., 2006).

2.2.3.2 Function of cholesterol

Cholesterol, the characteristic steroid alcohol of animal tissues, performs a number of essential functions in the body. For example, cholesterol is a structural component of all cell membranes, modulating their fluidity, and, in specialized tissues, cholesterol is precursor of bile acids, steroid hormones, and vitamin D. It is, therefore, critically important that the cells of the body be assured an appropriate supply of cholesterol. To meet this need, a complex series of transport, biosynthetic, and regulatory mechanisms has evolved. The liver plays a central role in the regulation of the body's cholesterol homeostasis. For example, cholesterol enters the liver's cholesterol pool from a number of sources including dietary cholesterol as well as that synthesized de novo by extra hepatic tissues and by the liver itself. cholesterol is eliminated from the liver as unmodified cholesterol in the bile, or it can be converted to bile salts that are secreted into the intestinal lumen. It can also serve as a component of plasma lipoproteins that carry lipids to the peripheral tissues. In humans, the balance between cholesterol influx and efflux is not precise, resulting in a gradual deposition of cholesterol in the tissues, particularly in the endothelial linings of blood vessels. This is a potentially life-threatening occurrence when the lipid deposition leads to plaque formation, causing the narrowing of blood vessels (atherosclerosis) and increased risk of cardiovascular disease (Devlin. 2002).

2.2.4 Triglycerides

As can be inferred from the name, triglycerides contain three fatty acid molecules attached to one molecule of glycerol by ester bonds. Each fatty acid in the triglyceride molecule can potentially be different in structure, thus producing many possible structural forms of triglycerides. Triglycerides containing saturated fatty acids, which do not have bends in their structure, pack together more closely and tend to be solid at room temperature. In

contrast, triglycerides, containing cis unsaturated fatty acids, typically form oils at room temperature. most triglycerides from plant sources, such as corn, sunflower seeds, and safflower seeds, are rich in polyunsaturated fatty acids and are oils, whereas triglycerides from animal sources contain mostly saturated fatty acids and are usually solid at room temperature. As can be seen by inspecting the structure of triglycerides, there are no charged groups or polar hydrophilic groups, making it very hydrophobic and virtually water insoluble. because it has no charge, triglyceride is classified as a neutral lipid (Bishop *et al*., 2010).

2.2.5 Lipoproteins

Lipoprotein is soluble complex of proteins (apolipoprotein) and lipids that transport lipid in the circulation of all vertebrates and even insects. Lipoproteins are synthesized in the liver, in the intestine, arise from metabolic changes of precursor lipoprotein, or are assembled At cell membranes from cellular lipid and exogenous lipoprotein or apolipoprotein In the circulation lipoproteins are highly dynamic they under go enzymatic reactions on their lipid components, facilitated and spontaneous lipid transfers of soluble apolipoproteins, and conformational changes of apolipoproteins in response to the compositional changes (Vance. 2002).

2.2.6 Classification of Lipoproteins

Based on their density, the lipoproteins of blood plasma are classified into 4 classes. The four classes of lipoproteins can be separated by ultra centrifugation. density of a lipoprotein is inversely related to the lipid content. the greater the lipid content, lower is the density. different classes of lipoproteins based on the density are: chylomicrons, very low density lipoproteins, low density lipoproteins and high density lipoproteins (Rao. 2006).

2.2.6.1 Chylomicron

Chylomicrons are the largest of the lipoproteins and the least dense because of their rich triacylglycerol content. They are synthesized from dietary lipids (the "exogenous" lipoprotein pathway) within the epithelial cells of the small

intestine and then secreted into the lymphatic vessels draining the gut, they enter the bloodstream, the major apoproteins of chylomicrons are apoB-48, apoCII, and apoE. The apoCII activates lipoprotein lipase (LPL), an enzyme that projects into the lumen of capillaries in adipose tissue, cardiac muscle, skeletal muscle, and the acinar cells of mammary tissue. this activation allows LPL to hydrolyze the chylomicrons, leading to the release of free fatty acids derived from core triacylglycerides of the lipoprotein into these target cells. The muscle cells then oxidize the fatty acids as fuel while the adipocytes and mammary cells store them as triacylglycerols (fat) or, in the case of the lactating breast, use them for milk formation. The partially hydrolyzed chylomicrons remaining in the bloodstream (the chylomicron remnants), now partly depleted of their core triacylglycerols, retain their apoE and apoB48 proteins. Receptors in the plasma membranes of the liver cells bind to apoE on the surface of these remnants, allowing them to be taken up by the liver through a process of receptor-mediated endocytosis (Smith *et al*., 2005).

2.2.6.2 Very Low Density Lipoproteins

VLDL is produced by the liver and contains apo B-100, apo E, and apo Cs; like chylomicrons, they are also rich in triglycerides.18, 19 they are the major carriers of endogenous (hepatic-derived) triglycerides and transfer triglycerides from the liver to peripheral tissue. like chylomicrons, they also reflect light and account for most of the turbidity observed in fasting hyper lipidemic plasma specimens, although they do not form a creamy top layer like chylomicrons, because they are smaller and less buoyant . excess dietary intake of carbohydrate, saturated fatty acids, and tarn's fatty acids enhances the hepatic synthesis of triglycerides, which in turn increases VLDL production (Bishop *et al*., 2010).

2.2.6.3 Low-Density Lipoproteins

LDL primarily contains apo B-100 and is more cholesterol rich than other apo B–containing lipoproteins. they form as a consequence of the lipolysis of VLDL. LDL is readily taken up by cells via the LDL receptor in the liver and peripheral cells. In addition, because LDL particles are significantly smaller

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than VLDL particles and chylomicrons, they can infiltrate into the extra cellular space of the vessel wall, where they can be oxidized and taken up by macrophages through various scavenger receptors. Macrophages that take up too much lipid become filled with intracellular lipid drops and turn into foam cells, which are the predominant cell type of fatty streaks, an early precursor of atherosclerotic plaques (Rao. 2006).

2.2.6.4 High-Density lipoproteins

HDL, the smallest and most dense lipoprotein particle, is synthesized by both the liver and intestine. HDL can exist as either disk-shaped particles or, more commonly, spherical particles. discoidal HDL typically contains two molecules of apo A-I, which form a ring around a central lipid bilayer of phospholipid and cholesterol. discoidal HDL is believed to represent nascent or newly secreted HDL and is the most active form in removing excess cholesterol from peripheral cells. The ability of HDL to remove cholesterol from cells, called reverse cholesterol transport, is one of the main mechanisms proposed to explain the anti atherogenic property of HDL. When discoidal HDL has acquired additional lipid, cholesterol esters and triglycerides form a core region between its phospholipid bilayer, which transforms discoidal HDL into spherical HDL. HDL is highly heterogeneous separable into as many as 13 or 14 different sub fractions. There are two major types of spherical HDL based on density differences: HDL2 and HDL3. HDL2 particles are larger in size and richer in lipid than HDL3 and may reflect better efficiency in delivering lipids to the liver (Bishop *et al*., 2010).

2.2.7 Association between lipid profile and combined oral contraceptive:

Combined oral contraceptive pills adversely affect the lipid profile in females of child bearing age. It is suggested that lipid profile should be estimated before and during the course of combined oral contraceptives. their study showed significant increase of serum total cholesterol and triglycerides in subjects using combined oral contraceptives as compared to control. HDL-C was insignificant decrease in combined oral contraceptive users as compared to controls; whereas LDL-C was significant decrease in combined oral

contraceptive users as compared to non users in the present study (Faryal *et al.*, 2012).

Other study showed significant increase of total cholesterol, triglycerides, VLDL and LDL-C concentration and significant decrease of HDL-C concentration in sera of women who used combined oral contraceptive when compared with those of the control group. Also this study showed significant increase of total cholesterol, triglycerides, VLDL and LDL-C concentration, and significant decrease in HDL-C concentration in sera of women who used combined oral contraceptive when compared with women who used Intra uterine device group. None significant decrease of total cholesterol, HDL-C, triglycerides, VLDL and LDL-C concentration in sera of women who used Intra uterine device when compared with those of the control group (AL-Gazally et al., 2010).

Other study was noted that the mean of serum cholesterol level of women using combined oral contraceptive was increased with p-value of high significance (0.0001) , HDL-C was not changed significantly (p> 0.83), while the mean of LDL-C and VLDL was increased with significant p-value of (0.002) and(0.0001) respectively ,the BMI in the women using combined oral contraceptive was found to be significantly high (p<0.0004) when compared with control of their respective age group (Mohammed *et al.*, 2013).

Other study showed Serum total cholesterol and LDL-C levels were significantly higher (P<0.001) and triglycerides non-significantly increased (p>0.05) but serum HDL-C level was significantly decreased (p<0.001) in oral contraceptives users' women than control group (Yasmin *et al.*, 2013).

Other study showed the concentrations of total lipids in all lipoprotein subclasses were increased; the strongest associations were for the high-density lipoprotein (HDL-C) subclasses and for the smallest very-low-density lipoprotein (VLDL) subclasses. Only slight increases were observed for low-density lipoprotein (LDL-C) subclasses (Wang *et al.*, 2016).

3. Materials and methods

3.1 Study design

Descriptive cross sectional case control study.

3.2 Study area

This study was conducted in Alwaha Village.

3.3 Study population:

This study was included 50 women were using combined oral contraceptive (more than one year continuously) and 50 women were non using combined oral contraceptive as control group during September 2017 to March 2018.

3.4 Inclusion criteria

Group (1): women in reproductive age using combined oral contraceptive more than one year continuously.

Group (2): women non using combined oral contraceptive from the same age as control group.

3. 5 Exclusion criteria

Women with dyslipidemia, hypertension, diabetes mellitus, cardiovascular disease and infertile women.

3.6 Ethical consideration

Ethical clearance was obtained from the research committee of college of post graduate studies of the Sudan University for Science and Technology.

3.7 Sampling technique

Random blood sample was collected into lithium heparin container and then plasma separated into plain container for measurement of plasma total cholesterol, HDL-C, and LDL-C by using full automated analyzer (DIRUI.CS.T 240).

3.8 Cholesterol estimation:

3.8 .1 Principle of test

Free and esterfied cholesterol in the sample originates, by mean of the coupled reactions described below, a colored complex that can be measured by spectrophotometry.

Cholesterol ester +H2O → cholesterol +fatty acid.

Cholesterol+½ O2 +H2O→ cholesetnone +H2O.

2H2O+4-Aminoantipyrine +phenol → Quinoneimine +4H2O.

(Allain C.C *et al*., 1974).

3.8.2 Procedure of cholesterol estimation:

Brought cholesterol reagent in room temperature, pipette 1mLl in to labeled test tubes (blank, standard and sample), then 10μL was add from cholesterol standard to standard tube and 10μL from sample to sample tube, mixed thoroughly and incubated the tubes for 10 minutes at room temperature and measured the absorbance (A) of the standard and sample at 500 nm against the blank .the colour is stable for at least 2hours.

3.9 HDL-C estimation

3.9.1 Principle of HDL-C estimation

The liquid auto HDL cholesterol assay is a homogenous method for directly measuring serum HDL-C levels without the for any off-line pretreatment or centrifugation steps. the method is in a two reagent format. The first reagent contains ǫ-cyclodextran and dextran sulphate to stabilize LDL-C, VLDL-C, and chylomacrons. the second reagent contains PEG modified enzymes that selectively react with the cholesterol present in the HDL-C particles. consequently, only the HDL cholesterol is subject to cholesterol measurement. (Gotto A.M *et al*., 1988).

3.9.2 Procedure of HDL-C estimation

Pipetted 300μL from reagent 1 in test tube then 4 μL was add from sample incubated for 5 minutes in room temperature then add 100 μL from reagent 2, incubated 5 minutes in room temperature and measured the absorbance at 700 nm.

3.10 LDL-C estimation

3.10.1 Principle of LDL –C estimation

The auto LDL cholesterol reagent is a two- part, liquid stable method for directly measuring LDL-C levels in serum or plasma. The method depends on the properties of unique detergent which eliminates the need for any off-line

pretreatment or centrifugation steps .this detergent (reagent1) solublizes only the non LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in non color formation .A second detergent (reagent2) solubilizes the remaining LDL-C particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of coupler produces color that is proportional to the amount of LDL cholesterol present in the sample (Gotto A.M *etal* ., 1988).

3.10. 2 Procedure of LDL-C estimation

Pipetted 300μL from reagent 1 in test tube then 3 μL was add from sample incubate 5 minutes in room temperature then add 100 μ from reagent 2, incubate 5 minutes in room temperature and measured the absorbance at 660 nm

3.11 BMI calculation

BMI   was calculated as body weigh (Kilogram) divided by height squired (meters) (Nuttall., 2015).

3.12 Quality control

Control sera were run with samples to ensure quality control.

3.13 Statistics analysis:

Collected data were analyzed using the application of statistical package for social science (SPSS) version 16, by using independent T test p value less than 0.05 was considered significant for the difference between variables, person's correlation was applied to correlate between study variables.

4-Results

This study was included (100) women, (50) women were using combined oral contraceptive more than one year and (50) women were non using combined oral contraceptive served as control group, the mean of age in combined oral contraceptive was (31.1±5.7 mg/dL) which was insignificantly decreased compare to control group (31.4±5.6 mg/dL) ,p value was (0.75), while the mean of BMI in combined oral contraceptive was(31.5±2.8 mg/dL) which was significantly increased compare to control group ,p value (0.00*) as it shows in table (4.1) . The mean of plasma total cholesterol in combined oral contraceptive group was (182± 24.8 mg/dL) which was significantly increased compared to control group (140.2± 24.4 mg/dL), p value was (0.00), as it shows in table (4.2), statically significant positive correlation between total plasma cholesterol and age (r= 0.48), p value (0.00*) , significant positive correlation between total plasma cholesterol and BMI (r= 0.35), p value (0.013) , and significant positive correlation between total plasma cholesterol and duration of contraceptive (r =0.51), p value (0.00) as it shows in figure (4.1a),(4.2 a),(4.3 a), the mean of serum HDL-C in combined oral contraceptive group was (51.9± 11.7 mg/dL) which was insignificantly decreased compared to control group (54.1±9.4 mg/dL) , p value was (0.29), there was insignificant negative correlation between HDL-C and age (r 0.19-),p value (0.18), insignificant positive correlation between HDL-C and BMI (r 0.15) ,p value (0.31) , also there was insignificant negative correlation between HDL-C and duration of contraceptive (r =0.08-), p value (0.60) the mean of LDL-C in combined oral contraceptive was (96.7± 24.4) mg/dL which was insignificantly increased compared to control group (91.8± 15.8) mg/dL, p value (0.23), insignificant positive correlation between LDL-C and age (r =0.19) ,p value (0.181),and insignificant positive correlation between LDL-C and BMI (r =0.16) ,p value (0.27), also insignificant positive correlation between LDL-C and duration of contraceptive using (r0.23) , p value (0.10) .

Table (4.1) Comparison between means of age/year, body mass index among Sudanese women using combined oral contraceptive to control group.

Variables	Case X±SD	Control X±SD	P-value
Age /years	31.1±5.7	31.4±5.6	0.75
BMI	31.5±2.8	28.1±2.5	0.00*

-independent T test was used to compare between two means.

 -P value considered significant ≤0.

Table (4.2) Comparison between means of plasma total cholesterol, plasma HDL-C, plasma LDL-C among Sudanese women using combined oral Contraceptive to control group.

| variables	Case X±SD	Control X ±SD	P value
Plasma cholesterol (mg/dL)	182.±24.8	140.±24.4	0.00*
Plasma HDL-C (mg/dL)	51.9±11.7	54.1± 9.4	0.29
Plasma LDL-C (mg/dL)	96.7±24.4	91.8±15.8	0.23

-independent T test was used to compare between two means.

- P value considered significant ≤0.05.
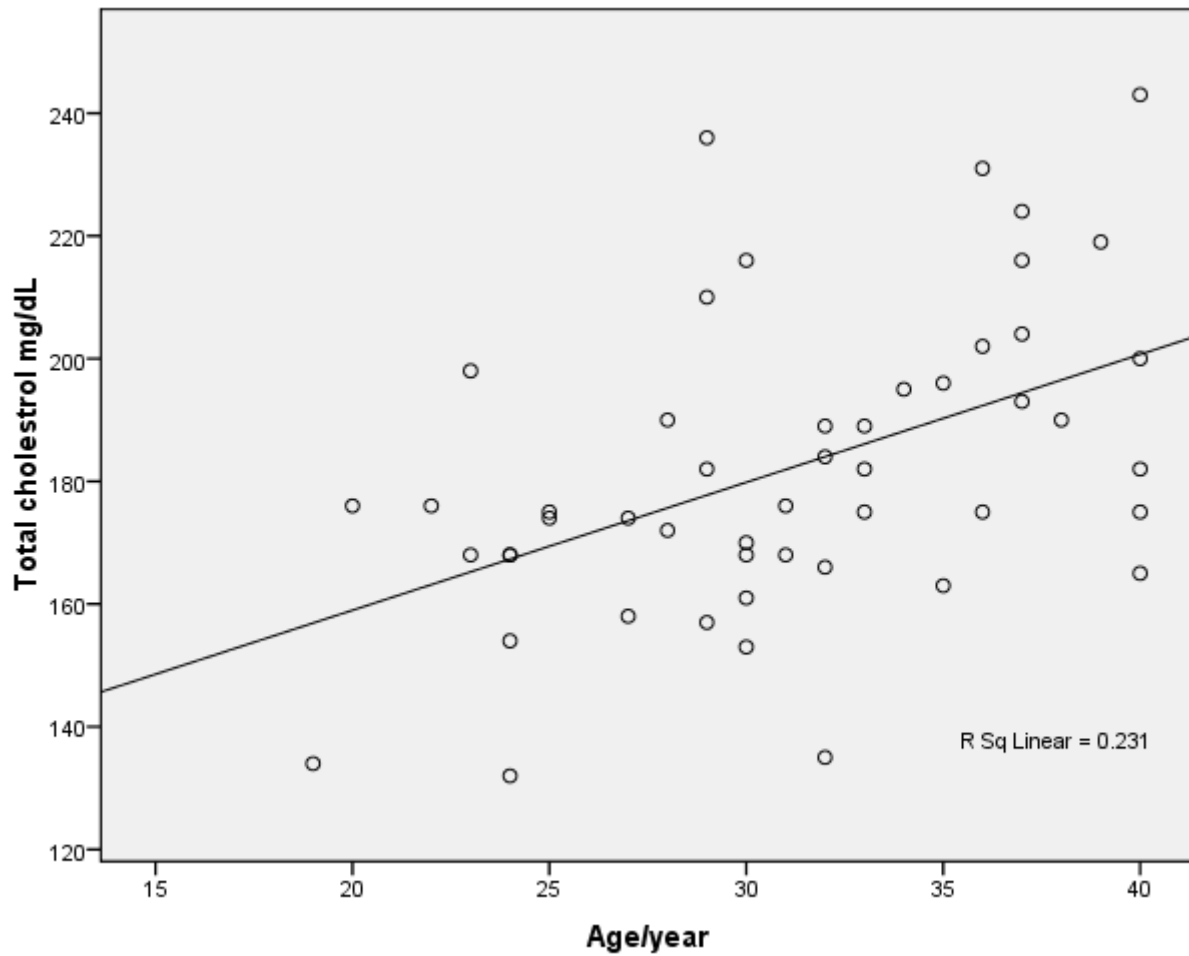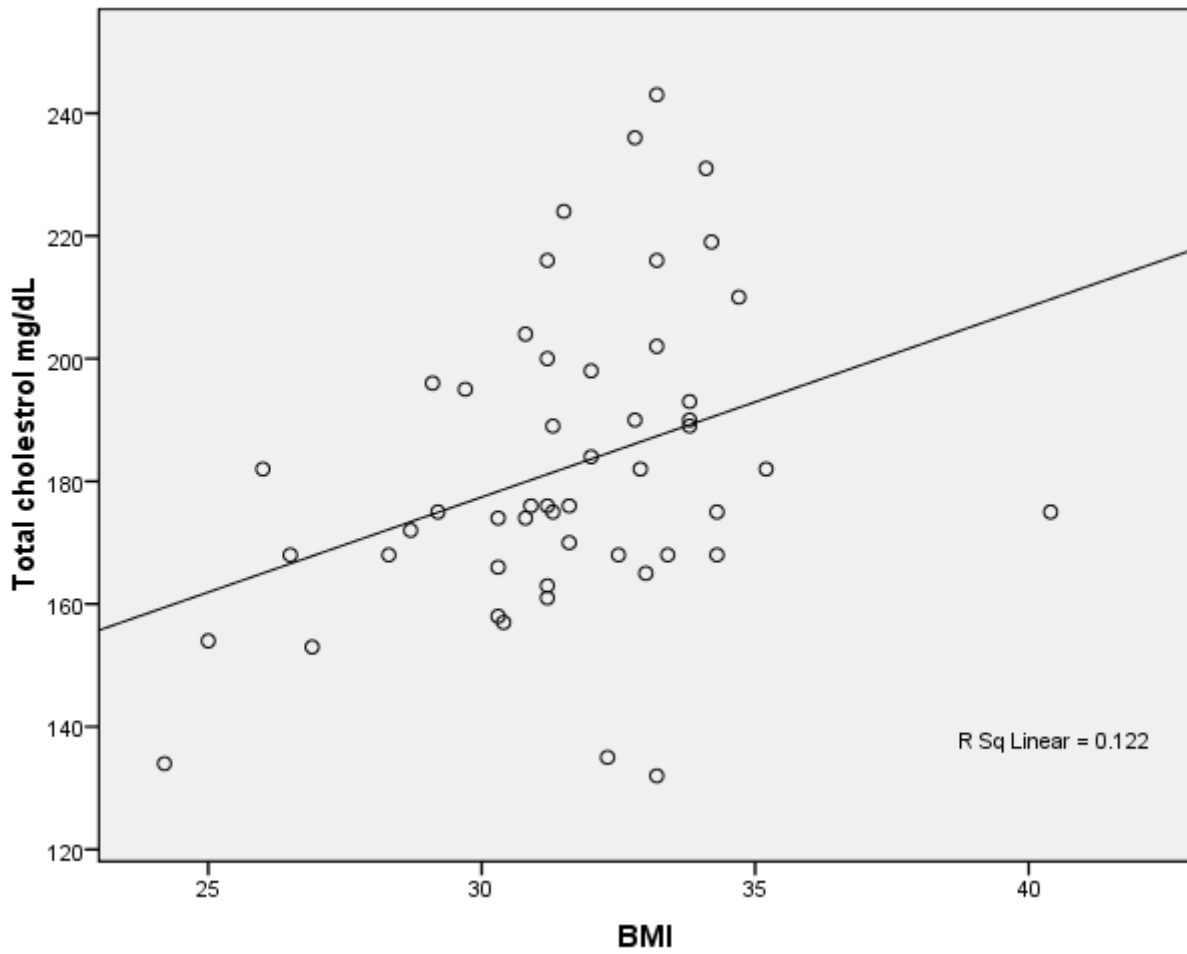
Figure (4.1.a): scatter plot between age/year and plasma total cholesterol, significant positive correlation, P = 0.00, r= 0.48.
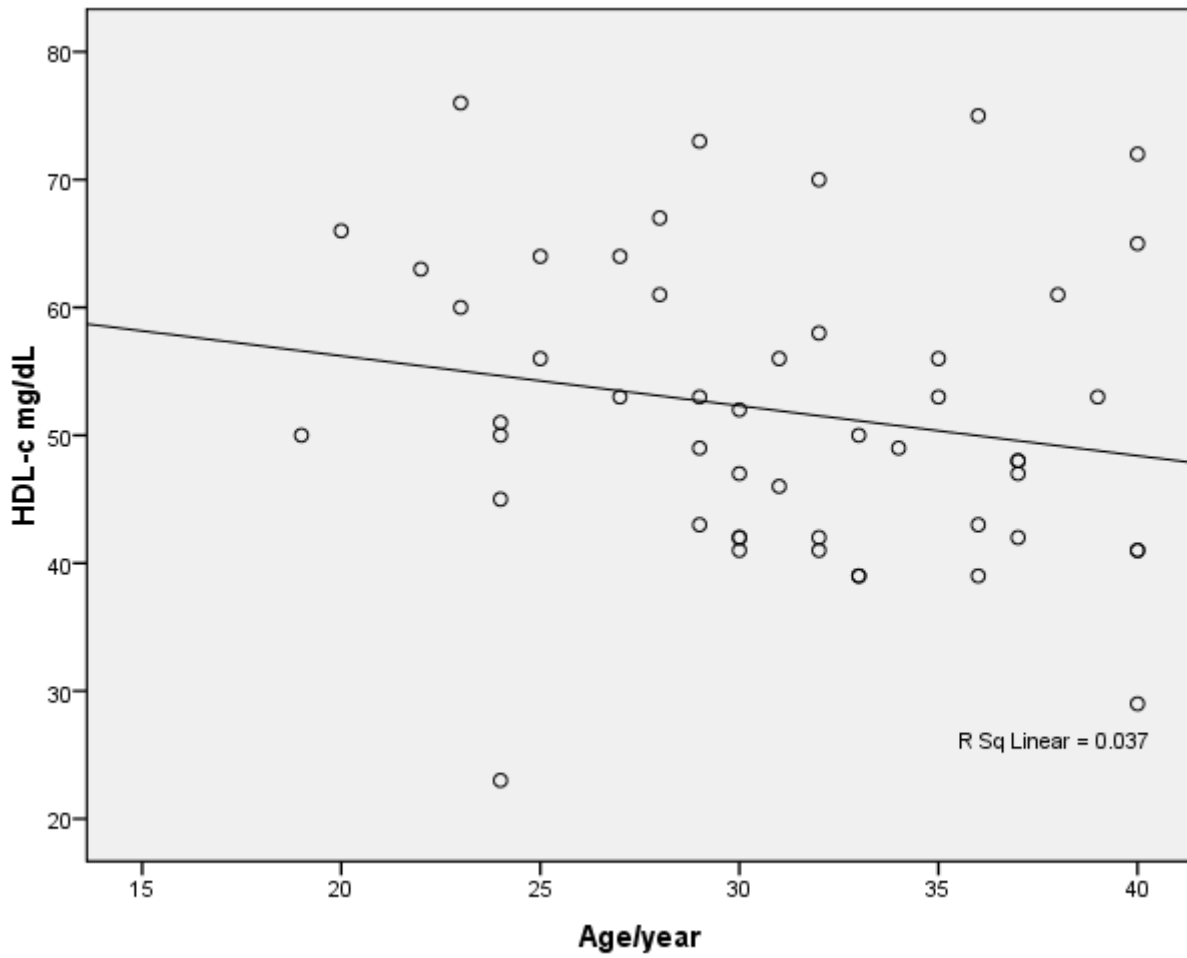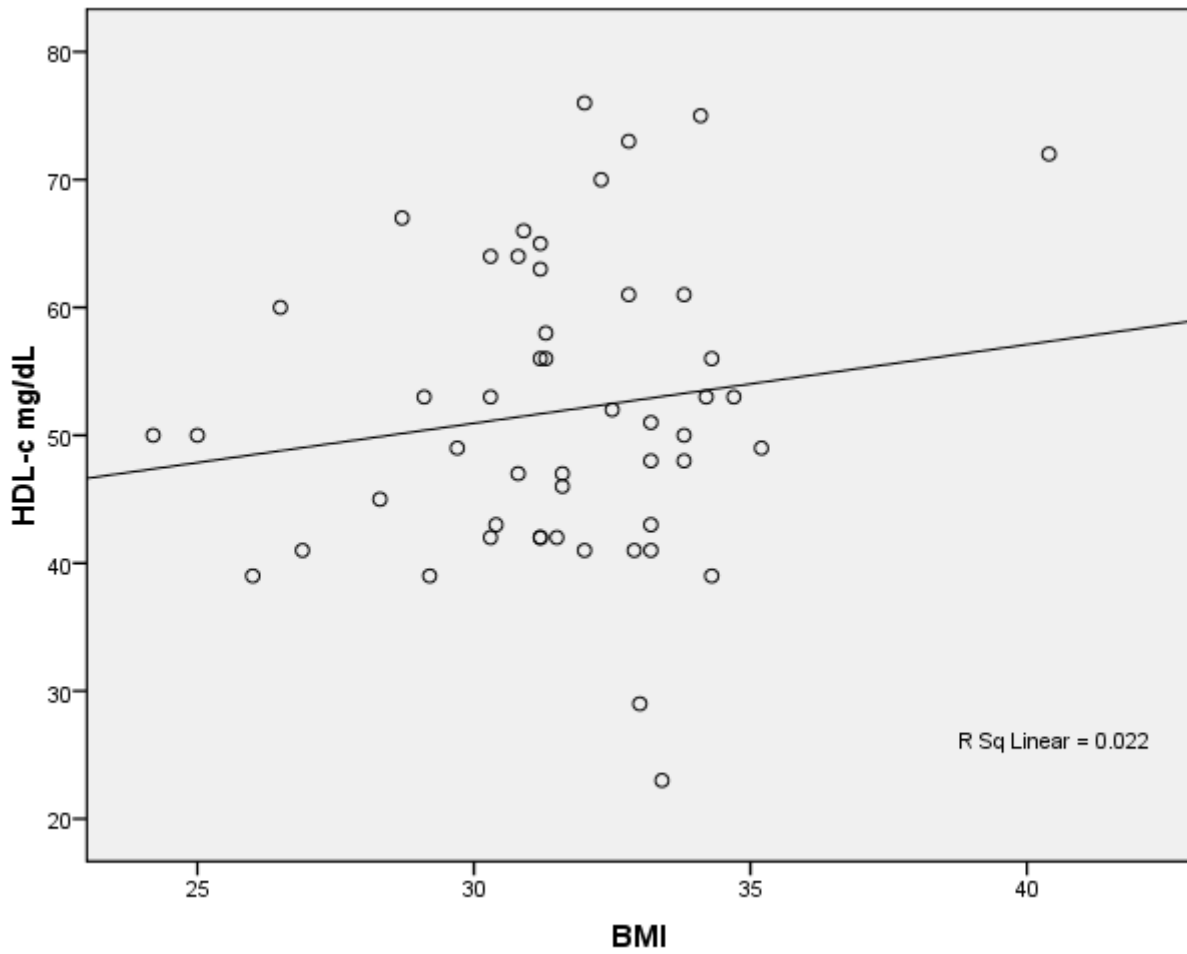
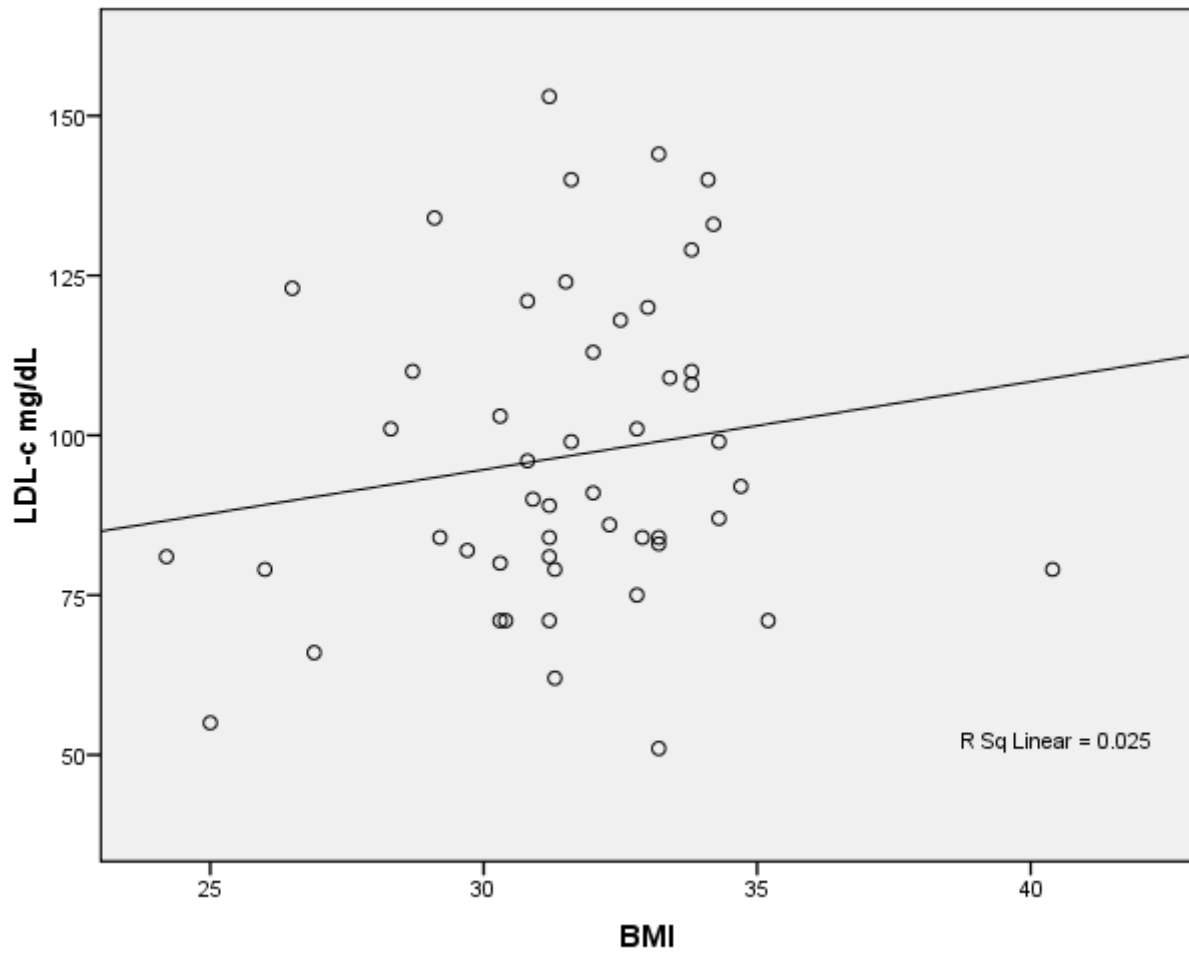**Figure (4.1.b): scatter plot between age/year and plasma HDL-c insignificant negative correlation, P = 0.18, r= -0.19.**
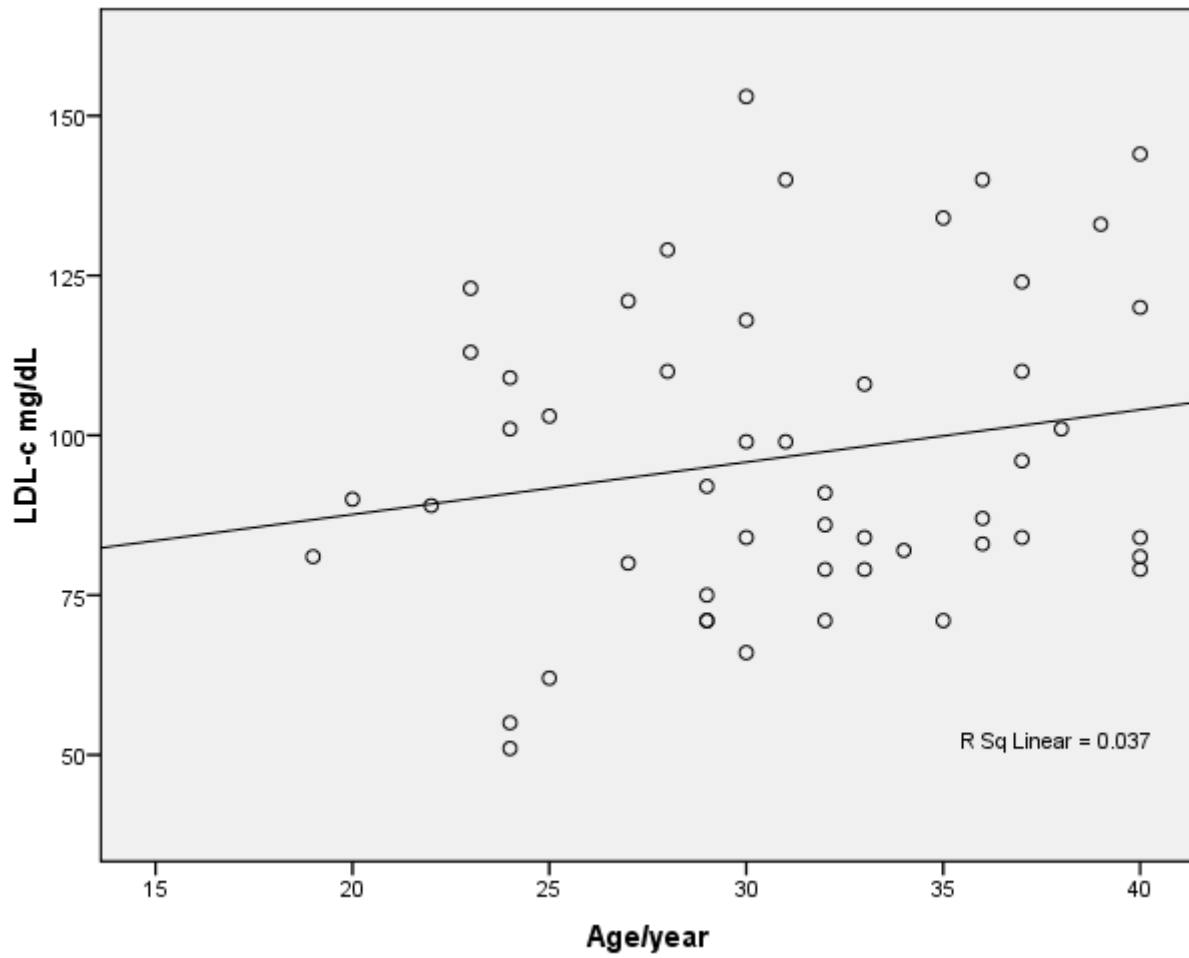
Figure (4.1.c): scatter plot between age/year and plasma LDL-c,
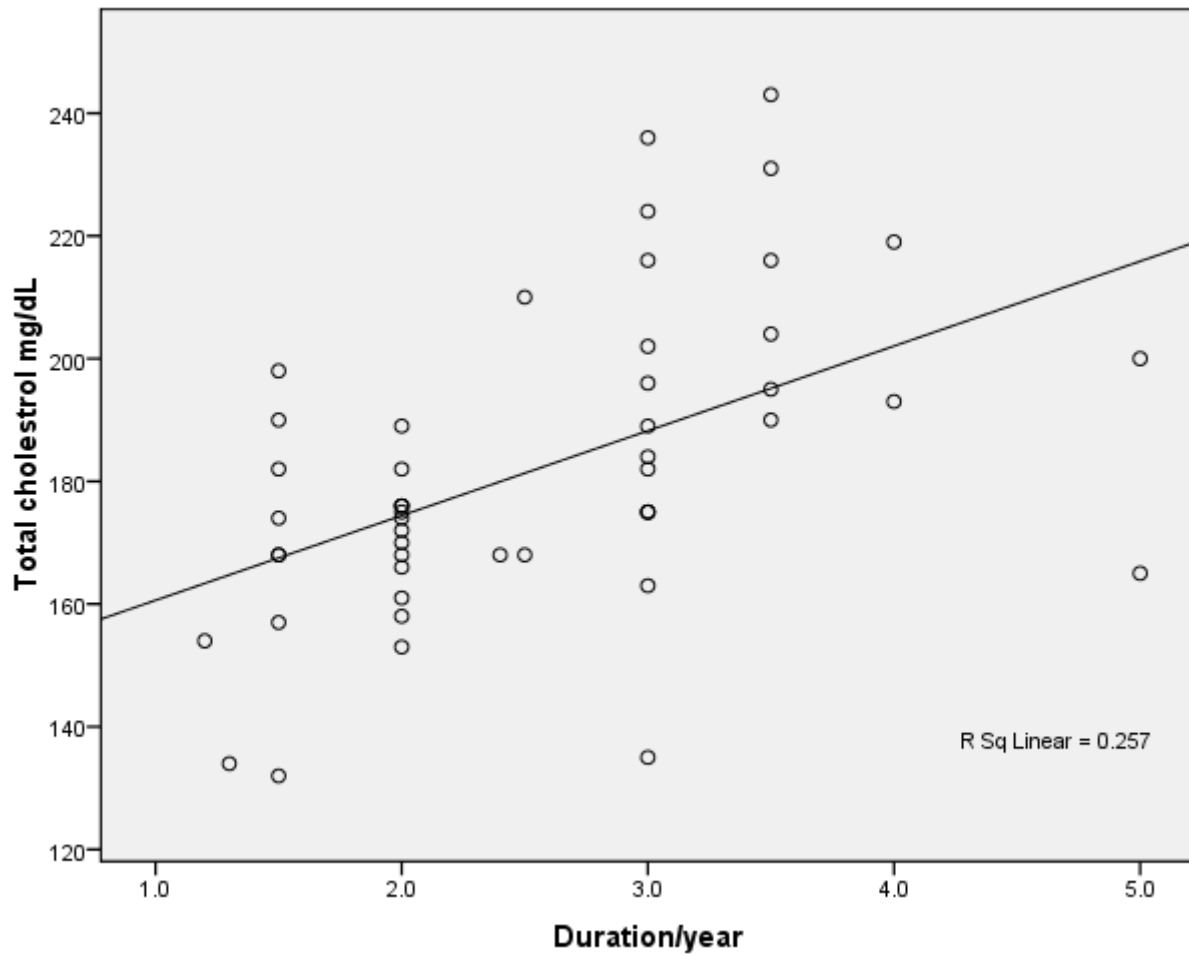**Insignificant positive correlation, P = 0.18, r = 0.19.**

Figure (4.2.a): scatter plot between BMI and plasma Total cholesterol, significant positive correlation, P = 0.013, r = 0.35.

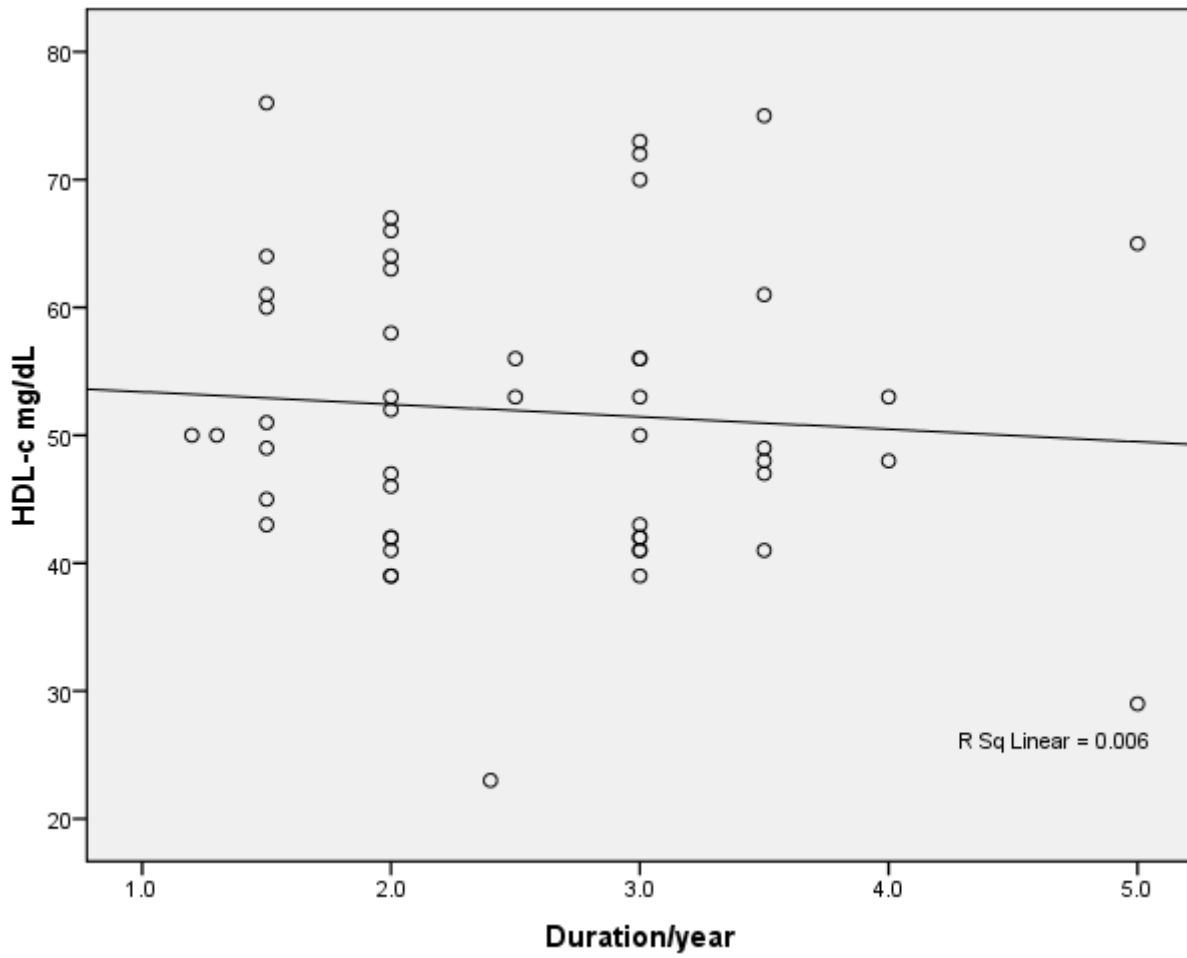**Figure (4.2.b): scatter plot between BMI and plasma HDL-c, Insignificant positive correlation, P = 0.31, r = 0.15.**

Figure (4.2.c): scatter plot between BMI and plasma LDL-c, insignificant positive correlation, P = 0.27, r = 0.16.

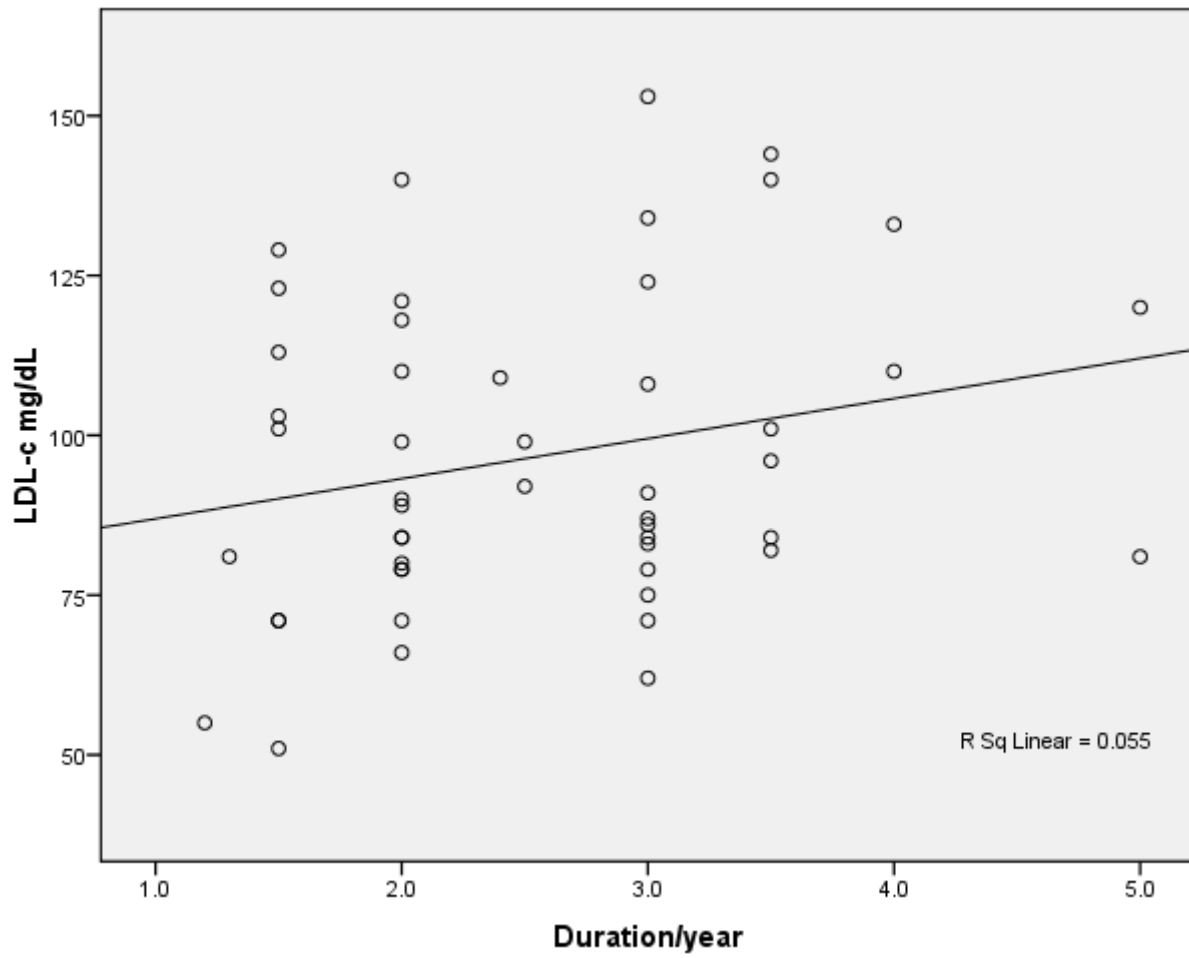**Figure (4.3.a): scatter plot between duration/year and plasma Total cholesterol significant positive correlation, P = 0.00, r = 0.51.**

Figure (4.3.b): scatter plot between duration/year and plasma HDL-c, Insignificant negative correlation, P = 0.60, r = -0.08.

Figure (4.3.c): scatter plot between duration/year and plasma LDL-c, Insignificant positive correlation, P = 0.10, r = 0.24.

5 Discussions, Conclusion and Recommendation

5.1 Discussion

A combined oral contraceptive pill adversely affects of lipid profile in females of child bearing age. It is suggested that lipid profile should be estimated during the course of combined oral contraceptives (Faryal *et al*., 2012).

This study was showed significant increase in body mass index and plasma total cholesterol in female using combined oral contraceptive compared to control group this result is an agreement with previous study showed significant increase in body mass index, total cholesterol (Mohammed *et al*., 2013).Significantly increased of serum total cholesterol in contraceptive user women might be due to impaired lipoprotein metabolism. combined oral contraceptive increase apolipoprotein B-100 synthesis and thus increased LDL-C. low density lipoprotein in combined oral contraceptives user's women higher might be due to increase lipoprotein synthesis rather than impaired lipolytic catabolism, in association with accumulation of cholesterols as result increased LDL-C. Progestin components of oral pill increased hepatic lipase enzyme activity as a result decreased serum HDL-C level (Yasmin *et al*. 2013). Cholesterol follow in two different path way in the body: a path from the liver to peripheral cell, whose marker are LDL-C, VLDL-C cholesterols and apoprotein B, other path return of excess cholesterol from the tissue to liver marker by HDL cholesterol and plasma apoprotein A, the imbalance between two path lead to excess amount of cholesterol in the body (Rao., 2000). there was insignificant decrease of HDL-C, this result is an agreement with previous study showed insignificant decrease of HDL-C concentration (Faryal *et al*., 2012).and disagree with previous study showed significant decrease of HDL-C (Al-gazally *et al*., 2012) might be due to habits and life style which are different from communities this study was showed insignificant increase of LDL-C, the result is an agreement with study showed slightly increased in LDL-C concentration (Wang et al., 2016).

5.2 Conclusion

The study result concludes that women had received combined oral contraceptive had higher level of total plasma cholesterol ,LDL-C and lower level of HDL-C and significantly positive correlation between plasma total cholesterol and (age, body mass index, and duration of combined oral contraceptive use).

5.3Recommendation

From the finding of this study it recommended that:

1- the women in reproductive age should be reduce body mass index and check routinely before and during using of combined oral contraceptive

2- Lipid profile must be evaluated during using combined oral contraceptives to decrease possibility getting dyslipiemia.

3-increase Awareness of women about contraception

4- finding other Alternative method of contraception to avoid effect of hormones.

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