Sudan University of Science and Technology

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Evaluation of Plasma Lipid Profile Among Women Using Combined Oral Contraceptive Pills in Gazira State.

تقييم مستوى الدهون في بلازما الدم لدى النساء اللائي يستخدمن حبوب منع الحمل المجتمعة عن طريق الفم في ولاية الجزيرة

A Dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science (Clinical Chemistry)

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الايـة

قَالَ تَعَـالَىٰ:

﴿ ٱللَّهُ لَآ إِلَٰهَ إِلَّا هُوَ ٱلۡحَيُّ ٱلۡقَيُّومُۚ لَا تَأۡخُذُهُۥ سِنَةٞ وَلَا نَوۡمٞۚ لَّهُۥ مَا فِي ٱلسَّمَٰوَٰتِ وَمَا فِي ٱلۡأَرۡضِۗ مَن ذَا ٱلَّذِي يَشۡفَعُ عِندَهُۥٓ إِلَّا بِإِذۡنِهِۦۚ يَعۡلَمُ مَا بَيۡنَ أَيۡدِيهِمۡ وَمَا خَلۡفَهُمۡۖ وَلَا يُحِيطُونَ بِشَيۡءٖ مِّنۡ عِلۡمِهِۦٓ إِلَّا بِمَا شَآءَۚ وَسِعَ كُرۡسِيُّهُ ٱلسَّمَٰوَٰتِ وَٱلۡأَرۡضَۖ وَلَا يَـُٔودُهُۥ حِفۡظُهُمَاۚ وَهُوَ ٱلۡعَلِيُّ ٱلۡعَظِيمُ ٢٥٥ ﴾

صدق الله العظيم (سورة البقرة)

الاية (255)

Dedication

To my Parents who always give me great love , patience , forgiveness, care and support all my life.

To my lovely sisters & brothers

To my husband for his encouragement and support& to My Son

To those whom I love more, and my Friends and all family members….

To my teachers ،who teach me everything.

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Firstly, I thank Allah for blessing my life, and helped me to start this work and supported my strength to complete this humanity work.

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Abstract

Background: Combined oral contraceptives pills are the most popular contraceptive method world-wide.50-60 % of women of reproductive age world-wide use this method for birth control. Dyslipidemia occurs in long tern oral contraceptives user women.

Objective: This study was aimed to evaluate lipid profile among women using combined oral contraceptive pills in Gazira state.

Materials and methods: This was cross sectional study concluded sample were collected using questionnaire the study conducted in Gazira state Total number of 40 women which using oral contraceptive conducted as case group and 40 of them not using oral contraceptive as control group ¸ fasting blood sample were collected for the estimation of lipid profile (cholesterol¸ triglycride ¸HDL¸LDL)the sample prepaired and spectrophotometrically measured ·

Result: analyzed result by using SPSS computer program version 22.0 with T test and P *value* was obtained (P *value* ≤ 0.05 was considered statistically significant. the mean of age in combined oral contraceptive group was (26.97± 6.21), the mean of height in combined oral contraceptive group was (160.2 ± 7.99), the mean of weight among case group was (88.8 ± 9.9) and mean of BMI was (34.3 ±3.32) among case group while (27.3 ± 8.1) among control group(P.value was (0.003)considered significant different,the mean of serum total cholesterol in combined oral contraceptive group was (224.2 ± 26.7 mg/dL) and (183.0 ± 19.2 mg/dL) among control group .P.value was (0.028) considered significant different ,the mean of serum triglyceride in combined oral contraceptive group was (141.0± 20.0 mg/dL) and (95.3 ± 15.1 mg/dl) among control (P.value was (0.001)considered significant different ,the mean of serum HDL-C in combined oral contraceptive group was (43.3± 10.4mg/dL) and (56.5 ± 8.5mg/dl) among control group(P.value was (0.004)considered significant different and the mean of serum LDL-C in combined oral contraceptive group was (87.6±20.9 mg/dL) and (66.5±6.2mg/dl) among control group(P.value was (0.001)considered significant different .Most of percentage presented that age group 20- 30 years among case population and control as , Majority of them used contraceptive during 3 years, Majority of them had used Familia contraceptive then few used Marvelon.

Conclusion: The study result concluded that women had received oral contraceptive had higher level of total plasma cholesterol, triglyceride, LDL-C while low level of HDL-C and insignificantly correlation between plasma total cholesterol and (age, body mass index, and duration of combined oral contraceptive use).

مستخلص الدراسة

خلفية:حبوب منع الحمل الفموية مجتمعة هي أشهر وسيلة لمنع الحمل على نطاق العالم. ويستخدم نحو 50-60 في المائة من النساء في سن الإنجاب على نطاق العالم هذه الطريقة لتحديد النسل ويحدث ارتفاع مستوى الدهون في النساء اللاتي يستخدمن وسائل منع الحمل الفموية لفترات طويلة.

الأهداف: وتهدف الدراسة هذه إلى تقييم صورة الدهون لدى النساء اللائي يستخدمن حبوب منع الحمل الفموية مجتمعة في ولاية الجزيره. **الادوات والطرق :** كانت هذه دراسه مقطعيه وتم جمع العينات باستخدام استبيان ؛اجريت الدراسه في ولاية الجزيره وقد تم الحصول على العدد الإجمالي للنساء اللائي يستخدمن وسائل منع الحمل الفموية كمجموعة حالة و 40 و 40 منهن لا يستخدمن وسائل منع الحمل الفموية كمجموعة تحكم ،تم جمع عينات الدم لقياس مستوى الدهون ,تم تحضير العيناتوقياسها باستخدام جهاز الضوء الطيفي.

النتائج: النتيجة المحللة باستخدام البرنامج الحاسوبي SPSS الإصدار (22-0 مع اختبار T وقيمة **p)** اعتبرت القيمة 0.05 $\geq$ P ذات دلالة إحصائية. وكان متوسط العمر في مجموعة منع الحمل الفموية مجتمعة هو (26.97 * 6.21) ، كان متوسط الارتفاع في مجموعة منع الحمل الفموية مجتمعة هو (160.2 * 7.99) ، كان متوسط الوزن بين فئات الحالات (88,8 + 9,9) ومعنى مؤشر كتلة الجسم كان (34,3 + 3,32) بين مجموعة الحالات بينما (27,3 = 8,1) فيما بين مجموعة التحكم (P.value) كان (0,003) يعتبر مختلفا بدرجة كبيرة ، كان متوسط الكوليسترول الكلي المصلي في مجموعة منع الحمل الفموية مجتمعة هو (224.2+ 26.7 مجم/ ديسيلتر) و (183.0+ 19.2 مجم/ ديسيلتر) بين مجموعة التحكم (0.028) تعتبر مختلفة بدرجة كبيرة ، كان متوسط ثلاثي الغليسيريد المصل في مجموعة موانع الحمل الفموية مجتمعة. (141.0 * 20.0 مجم/ديسيلتر) و (95.3 * 15.1 مجم/ديسيلتر) ضمن نطاق السيطرة. كان P.value (0.001)يعتبر مختلفا إلى حد كبير ، وكان متوسط HDL-C المصل في مجموعة منع الحمل الفموي مجتمعة. (43,3 +41.مجم/ديسيلتر) و (56,5 ، 8 ،5 مجم/ديسيلتر) بين مجموعة التحكم كان P.value (0.004)يعتبر مختلفا بشكل كبير وكان متوسط المصل LDL-C في مجموعة منع الحمل الفموي مجتمعة. (87.6 + 20.9 مجم/ديسيلتر) و (66.5 + 6.2 مجم/ديسيلتر) بين مجموعة التحكم (P.value) كانت تعتبر مختلفة بشكل كبير وتعرض معظم النسبة المئوية تلك الفئة العمرية 20- 30 سنوات، واستخدمت غالبيتهم وسائل منع الحمل خلال السنوات 3سنوات .

الخاتمة: وخلصت نتيجة الدراسة إلى أن النساء اللائي تلقين وسائل منع الحمل عن طريق الفم كان لديهن مستوى أعلى من الكوليسترول البلازمي الكلي ، وثلاثي الغليسيريدو,LDL-C , ومستوى اقل من ال HDL-C، والترابط غير ايجابي بين مجموع الكوليسترول البلازمي و (العمر ، ومؤشر كتلة الجسم ، ومدة الاستخدام المشترك لوسائل منع الحمل عن طريق الفم).

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List of Abbreviation

BMI	Body Mass Index
COCP	Combined oral contraceptive Pills
DVT	Deep vein thrombosis
FSH	Follicle Stimulating Hormone
HDL-C	High Densely Lipoprotein Cholesterol
LH	Luteinizing Hormone
LDL- C	Low Density lipoprotein Cholesterol
PE	Pulmonary embolism
VTE	Venous thromboembolism

Chapter One

Introduction

1.1 Introduction

Contraceptives are the only absolutely methods prevented unwanted pregnancy. They can be divided into mechanical, which includes condoms, diaphragms and intra-uterine devices (IUD), and chemical contraceptives, which include those taken internally and those applied externally. Oral contraceptives are biochemical compounds which contain sex hormones and can be divided into combined contraceptives which are mixture of a synthetic estrogen and progestin, the other type is containing only progestin and less effective than combined oral contraceptives, and their use is often restricted to women whose fertility is already reduced, such as older or lactating women.(Bankowski et al ., 2012)

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 et al ., 2012). 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 Problem statement

Lipid is essential for the synthesis of many hormones in human body and plays vital roles in many bioactivities and reactions. Despite the obvious importance of lipid, elevation over the normal levels can lead to drastic consequences, such as heart diseases .Many factors that increase or decrease its serum level affect lipid. Therefore, these factors can be harmful or beneficial accordingly. Pregnancy is one of the factors that can affect the physiological state of the body, thus it might have some sort of effect on lipid level. Determination of changes in lipid level at different stage of pregnancy gives clear picture to deal with pregnant women.

1.3 Rationale

Combined oral contraceptive using lead to many side effects particularly headache, nausea, vomiting, loss of libido, breast enlargement, also may affect 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 these risks, so this study will insight light on lipid profile in women of reproductive age who used oral contraceptive pills

1.4. Objectives

1.4.1. General objective

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

1.4.2. Specific objectives

- To measure and compare lipid profile (plasma total cholesterol, triglyceride, HDL-C, LDL-C) among study groups.
- To correlate between lipid profile and study variables (age, BMI and duration of using combined oral contraceptive.

Chapter Two

2. Literature Review

2.1Contraception

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 cause slight increases in some procoagulant factor and reduce the levels of some natural anticoagulant in particular anti thrombin and proteins. then effect is more marked with third- generation pills (containing degestrol or gestodene) other with second- generation pills (containing levonorgestrel) (WHO.2004)

Most of the commonly used COCPs are ‘low dose’ and contain ethinyloestradiol in a dose of 15–35 μg COC formulations also contain one of manydifferent synthetic progestogens. The most widely available COCs in the publicsector contain the

progestogenslevonorgestrel (LNG) or norethisterone (NET)which is also known as norethindrone. (WHO.2004). Some newer pills contain oestradiolvalerate or oestradiol hemihydrate, which is more similar in structure to the 'naturally occurring' oestradiol, but confers no other proven benefits. Most 'traditional' preparations contain 21 pills followed by a 7-day pill-free interval (or 7 placebo tablets in place of a 7-daypill-free interval). Some preparations contain 24 days of pills with a shorter pill-free interval (Sharon.2017).

Three types of preparation are currently available:- Monophasic combination tablets, contain a single synthetic estrogen /progestogen dose combination and are taken daily (from a 'blister' pack)starting on the first or fifth day of menstruation, for 21 days followed by a 7-dayinterval of dummy (placebo) tablets (or pill-free days) during which the sudden removal of progestogen initiates (the latter may be psychologically reassuring to6some women 'withdrawal menstruation, that pregnancy has indeed been prevented); the cycle is then repeated. It is essential that the tablets are taken at the same time each day, as their effectiveness can fall if they are taken late or missed (i.e. taken more than 12hours late).Effectiveness can also be reduced if they are taken in combination with other drugs that influence estrogen/progestogen metabolism.(Andrew *et al.*,2005)-Biphasic or triphasic combination tablets contain estrogen/progestogen combination doses that vary throughout the cycle; (this may be more suitable for some women, than the monophasic preparations) The tablets are started on day 1or day 5 of a period and must be taken in the correct order to be effective (1daily for 21 days, then 7 tablet-free [or placebo] days). (Andrew *et al.*,2005).

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*).

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 progestationalderivatives of testosterone was 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 progestogens used incurrently available pills fall broadly into three groups, first and secondgenerationprogestins (e.g. norethindrone and levonorgestrel respectively) andthe third generation series including gestodene, desogestrel and norgestimate.Combined pills are available as monophasic preparations in which every pill inthe 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 et al. 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. 2011).

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 surgelike 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 eflectprogestational stimulation. The progestin in the combination pill produces an endometrium that is not receptive to ovum 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 effects reported, particularly headache and weight gain are common among women not using combined oral contraceptives, thoselikely to be directly related to the contraceptive steroids include fluid retention,nausea and vomiting, and breast enlargement (Hatcher *et al*., 2003). Sometimecombined oral contraceptive cause intermenstrual spotting is common in thefirst 3 months of the

start of the pills but it gradually disappears. heavyspottingcan be stopped byincreasing the dose for a few months, menstrual bleeding canbecome very scanty and occasionally a woman becomes amenorrhoeiccausingundue fear of pregnancy. (Howkins and Boure., 2011).

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. (Petitti et al. 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 et al. 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 (Gioramo*et al*., 2017). 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. (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. (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 papillomavirus (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 (Crook. 2012). 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 et al. 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(Ferrier. 2014).

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 (Ferrier. 2014).

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 (Brynhilelsen.2014).

2.2.2.4. Cholesterol:

Cholesterol, from the Ancient Greek chole- (bile) and stereos (solid) followed by the chemical suffix -ol for an alcohol, is an organic molecule. It is a sterol (or modified steroid).and an essential structural component of animal cell membranes that is required to establish proper membrane permeability and fluidity. Cholesterol is thus considered within the class of lipid molecules. (Brynhilelsen.2014).

2.2.2.4.1Clinical significance of cholesterol

2.2.2.4.1.1 Hypercholesterolemia:

According to the lipid hypothesis, abnormal cholesterol levels(hypercholesterolemia) — actually higher concentrations of LDL particles and lower concentrations of functional HDL particles — are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis).

2.2.2.4.1.2Triglyceride:

A triglyceride (TG, triacylglycerol, TAG, or triacylglyceride) is an ester derived fromglycerol and three fatty acids. As a blood lipid, it helps enable the bidirectional

transference of adipose fat and blood glucose from the liver. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so.Saturated compounds are "saturated" with hydrogen. (Nelson; 2014).

2.2.2.4.7. Lipoprotein

A lipoproteinis a biochemical assembly that contains both proteins and lipids. The lipids or their derivatives may be covalently or non-covalently bound to the proteins.

Many enzymes, transporters, structural proteins, antigens, adhesins and toxins are lipoproteins. Examples include the high density (HDL) and low density (LDL) lipoproteins which enable fats to be carried in the blood stream, the transmembrane proteins of the mitochondrion and the chloroplast, and bacterial lipoproteins function

of lipoprotein particles is to transport water-insoluble lipids (fats) and cholesterol around the body in the blood (Gorge N;2014).

2.2.2.4.8. Pregnancy and body Mass index (BMI):-

BMI is the relationship between height and weight and Is used to determine whether

we are underweight, just right, Overweight or obese. BMI is recorded in pregnancy notes and is a useful measurement for pregnancy (Nelson**.**2014).

-BMI less than 19.8= Underweight.

-BMI 19.9 - 25.9= healthy weight

-BMI 26 - 29.9= mildly overweight

-BMI 30 - 35= moderately overweight

-BMI over 35= overweight

2.2.2.4.8.1. Weight gain

The amount of weight a woman may gain in pregnancy can vary a great deal. Only some of it is due to increased body fat – the baby, placenta, amniotic fluid and increases in maternal blood and fluid retention all contribute. However, must be encouraged to keep weight gain to the Institute of Medicine (IOM) guidelines (Nelson. 2014)

-BMI of less than 19.8=12.5 - 18kg (28 - 40lbs)

-BMI 19.9 - 25.9=11.5 - 16kg (25 - 35lbs)

-BMI 26 - 29 =7 - 11.5kg (15 - 25lbs)

-BMI 30 or more=6kgs (15lbs) or less(Nelson; 2014)

Chapter Three

3-Materials and methods

3.1 Materials

3.1.1 Study design

Descriptive cross-sectional study.

3.1.2 Study area

This study was conducted in Gazira State –Alkamleen city .

3.1.3 Study Duration

The study was carried during period from August 2019 to Septemper 2020

3.1.4 Study population:

This study was included 40 women were using oral contraceptive and 40 women not using contraceptive

3.1.5 Inclusion criteria

women in reproductive age using oral contraceptive more than one year continuously.

3.1.6 Exclusion criteria

Women with dyslipidemia , hypertension , diabetes mellitus, cardiovascular disease ¸ hypothyroidism ¸ nephritic syndrome and infertile women.

3.1.7 Ethical consideration

Ethical clearance was obtained from the research committee of Medical Laboratory college of the Sudan University of Science and Technology .

3.2 Methods:

3.2.1 Data collection:

Data were collected using a structure interviewing questionnaire, carefully constructed to collect and maintain all valuable information concern each case examined. Demographic data were collected from every sample investigated.

3.2.2 Reagent preparation, storage and stability:

Biosystem lipid profile reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles. Once opened vial is stable for 3 months at 2-8°C

3.2.3 Instruments:

-Automatic micropipette. -Centrifuge. -Spectrophotometer.

3.2.4 Specimen collection and preservation:

Fasting blood sample was collected into lithium heparin container then plasma separated into plain container immediately centrifuge at 3000 rpm for 5 minutes for investigation of Cholesterol, Triglyceride, HDL and LDL and stored at -210C until used.

3.2.5 Cholesterol estimation:

3.2.5. .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.

3.2.5.2 Procedure of cholesterol estimation:

cholesterol reagent were Brought in room temperature, pipette 1ml 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.

3.2.6 HDL-C estimation

3.2.6.1 Principle of HDL-C estimation

HDL-Cholestrolis obtained trough selective precipitation of LDL and VLDL lipoproteins thus HDL lipoprotein remains in solution.HDLCholesterol **in** supernatant is treated as sample for Cholesterol assay 0

3.2.6.2 Procedure of HDL-C estimation

Firstly prepaire precipitation 200 µL of sample was added and 500 µL of precipitant in test tube mix and allow standing for 10 minutes at room tempreture centerfuge for 10 minutes at 4000 rpm separate for the clear supernatant within two hour and determine the Cholestrol content by the CHOD-PAP method pipette 1ml of cholesterol reagent in to labeled test tubes (blank, and sample), then 100µL was add from sample to sample tube and 100µL from Distilled Water to Blank tube, mixed thoroughly and incubated the tubes for 10 minutes at room temperature and measure the absorbance of specimen against reagent blank within 30 minutes calculation.

Concentration of HDLCholesterol (mg/dl) in supernatant = Absorbance of specimen * 325

3.2.7 LDL-C estimation

3.2.7.1 Principle of LDL –C estimation ,

LDL in the sample precipitate with polyvinylsulphate their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centerfugation

3.2.7. 2 Procedure of LDL-C estimation :

firistly prepair precipitant pipette into labelledcenterfuge tube 200 µL of sample and 200 µL of reagent A (Cholestrol LDL kit)was added mix thoroughly and stand for 15 minutes at room tempreture ¸ centerfuge at 4000 rpm for 15minutes ¸ collect the supernatant ¸pipette into labelled test tube (Blank ¸Standard ¸Sample)then 20µL was add from sample supernatant to sample tube and 20µL from Distilled Water to Blank tube and 20µL of standard to standard tube , mixedthoroughly and incubated the tubes for 10 minutes at room temperature andmeasure theabsorbance of specimen against reagent blank at 500 nm within 30 minutes

3·2.8 principle of Triglyceride Estimation

Triglycerides oxidase/ peroxidase method:

•The Principle

Triglycerides in the sample 0riginates, by means of the coupled chemical reactions, producing colored complex that can be measured by spectrophotometer

•Procedure:

Firstly reagents were brought to room temperature, and then in three [blank , stander , sample] labeled test tubes 1 ml of reagent [A] was added , and 10 µl of

cholesterol standard in standard tube was added ,and 10 µl of the sample in sample tube.

All three tubes were mixed Incubated at room temperature for 15 minutes.

- The absorbance (A) of sample and standard weremeasured at 500nm against reagent blank
- Concentration(conc) of standard = 200mg/ dl
- Calculation by equation : concentration of test =

$$\frac{\text{(A) Of test} \times \text{(conc) of std}}{\text{(A) Of std}}$$

- Normal range:

Up to 200 ml/dl

3.3 BMI calculation

BMI was calculated as body weight (Kilogram) divided by height squired (meters).

3.4 Quality control

Control sera were run with samples to ensure quality control.

3.5 Statistics analysis:

Collected data were analyzed using the application of statistical package for social science (SPSS) version 22, by using independent T test p value less than 0.05 was considered significant for the difference between variables, pearson's correlation was applied to correlate between study variables.

Chapter Four

4. Results

This study was conducted on 40 women using oral contraceptive and 40 women not using contraceptive at Gazira state.The results obtained after conducting the appropriate test were as following:

the mean of age in combined oral contraceptive group was (26.97± 6.21), the mean of height in combined oral contraceptive group was (160.2 ± 7.99), the mean of weight among case group was (88.8 ± 9.9) and mean of BMI was (34.3 ±3.32) among case group while (27.3 ± 8.1) among control group as showed in Table (4.2).

the mean of serum total cholesterol in combined oral contraceptive group was (224.2 ± 26.7 mg/dL)and (183.0 ± 19.2 mg/dL) among control group ,the mean of serum triglyceride in combined oral contraceptive group was (141.0± 20mg/dL)and **(**95.3 ± 15.1 mg/dl) among control group ,the mean of serum HDL-C in combined oral contraceptive group was (43.3± 10.4mg/dL) and (56.5 ± 8.5mg/dl) among control group and the mean of serum LDL-C in combined oral contraceptive group was (87.6±20.9mg/dL) and (66.5±6.2mg/dl) among control group as showed in Table (4.4).

Most of percentage presented that age group 20- 30 years among case population and control as in Table (4.1),Majority of them used contraceptive during 3 years Showed in Table (4.3-),Majority of them had used Familia contraceptive then few used Marvelon.Table (4.5).

Figure (4.1) represented insignificant correlation between cholesterol level (mg/dl) and age (year) **P¸v= 0.071, r = 0.288**

Figure (4.2) represented insignificant correlation between Triglycride level (mg/dl) and age (year) **P˛v= 0.203, r = 0.206**

Figure (4.3) represented no correlation between HDL-C level (mg/dl) and age (year) **P˛v= 0.676, r = 0.068**

Figure (4.4) represented no correlation between LDL-C level (mg/dl) and age (year) **P˛v= 0.202, r = 0.206**

Figure (4.5) represented no correlation between cholesterol level (mg/dl) and BMI

Figure (4.6) represented no correlation between Triglycride level (mg/dl) and BMI

Figure (4.7) represented no correlation between HDL-C level (mg/dl) and BMI

Figure (4.8) represented no correlation between LDL-C level (mg/dl) and BMI

Figure (4.9) represented no correlation between cholesterol level (mg/dl) and Duration

Figure (4.10) represented no correlation between Triglycride level (mg/dl) and Duration

Figure (4.11) represented no correlation between HDL-C level (mg/dl) and Duration

Figure (4.12) represented nos correlation between LDL-C level (mg/dl) and Duration

Table (4.1): Age distribution among case group and control group.

Age group	Study population		Total
	Case	Control	
less than 20 years	8	2	10
20-30 years	19	23	42
more than 30 years	13	15	28
Total	40	40	80

Table (4.2): Comparison between mean of age and BMI among Sudanese women using oral contraceptive and control group.

	Case	control	P.value
Age	26.97± 6.21	28.62 ± 5.29	0.127
Height	160.2 ± 7.99	158.8 ± 13.1	0.151
Weight	88.8 ± 9.9	67.1 ± 14.1	0.01
BMI	34.3 ±3.32	27.3 ± 8.1	0.003

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

-P value considered significant ≤0.0**5**

Table (4.3): distribution of duration /years of using contraceptive among Sudanese women using oral contraceptive.

Duration /years	Frequency	Percent
One year	4	10.0
2 years	9	22.5
3 years	20	50.0
4 years	5	12.5
5 years	2	5.0
Total	40	100.0

Table (4.4): Comparison between mean of Cholesterol, Triglyceride, HDL and LDL among Sudanese women using oral contraceptive and control group.

	Case	control	P.value
Cholesterol	224.2 ± 26.7	183.0 ± 19.2	0.028
Triglyceride	141.0 ±20.0	95.3 ± 15.1	0.001
HDL	43.3 ± 10.4	56.5 ± 8.5	0.004
LDL	87.6±20.9	66.5±6.2	0.001

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

-P value considered significant ≤0.05

Table (4.5): distribution of Type of contraceptive among Sudanese women using oral contraceptive.

Type of contraceptive	Frequency	Percent
Marvelon	11	27.5
Familia	29	72.5
Total	40	100

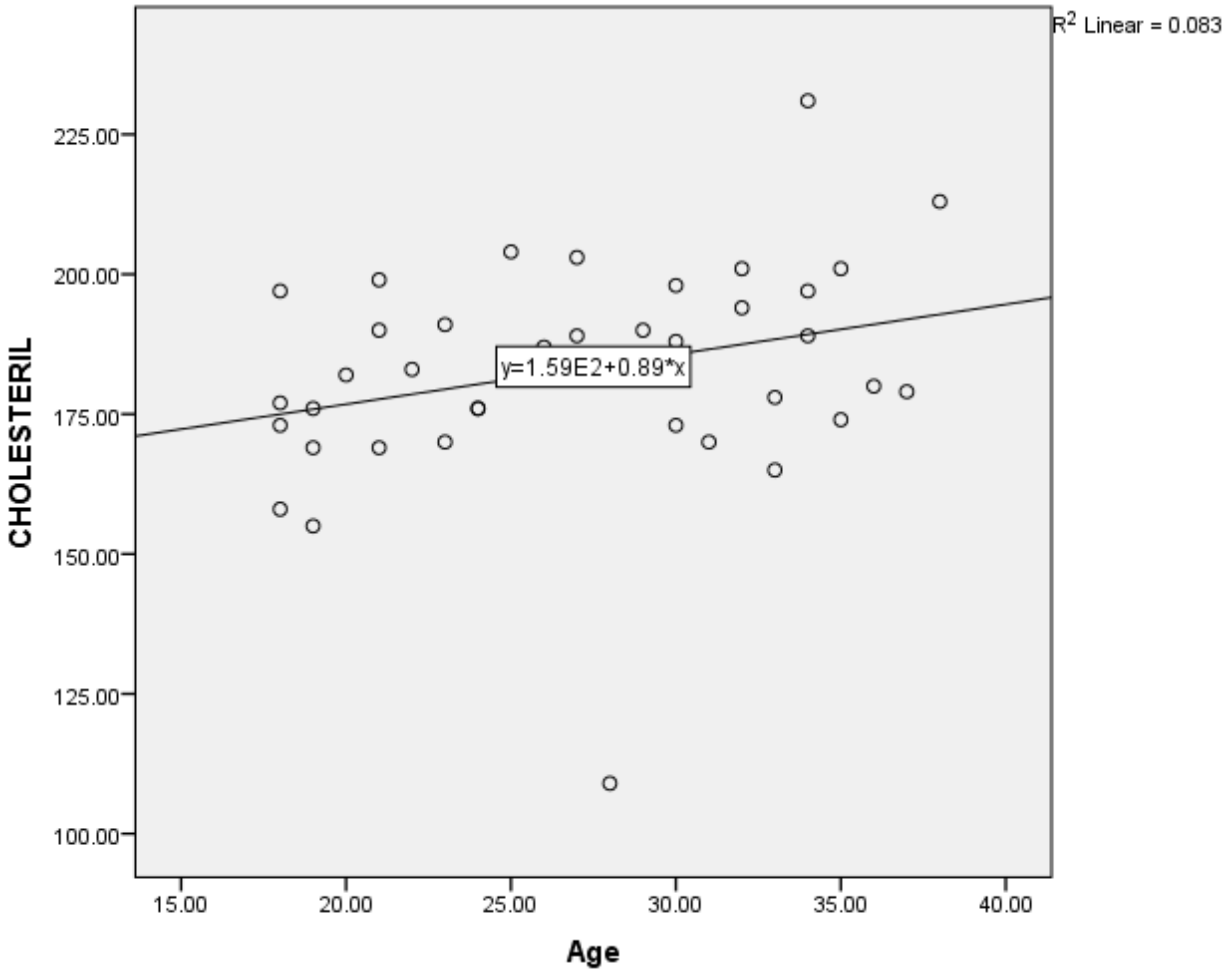

Figure (4.1): scatter plot between Cholesterol level and age, P = 0.071, r = 0.288

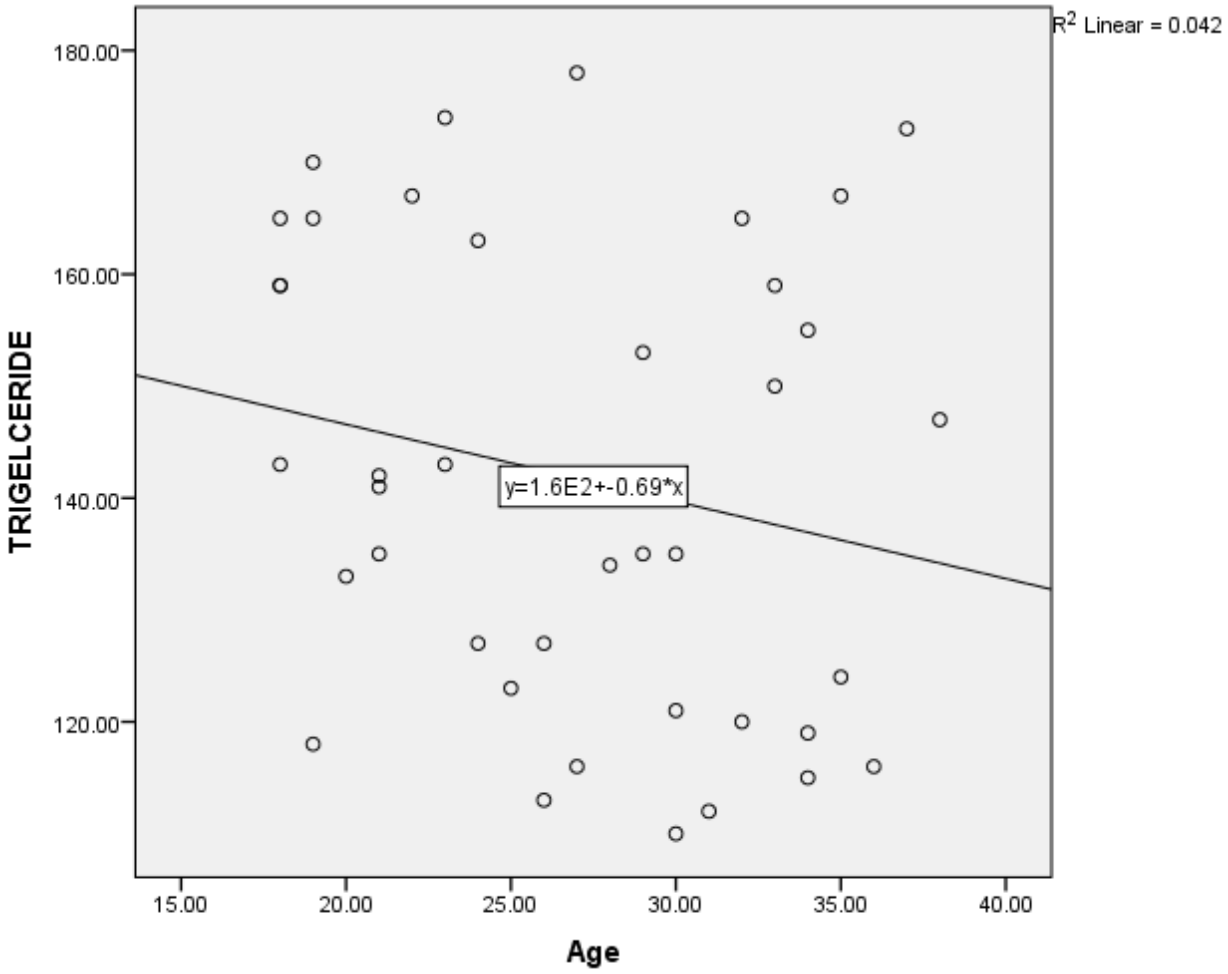

Figure (4.2): scatter plot between Triglyceride level and age, P = 0.203, r = -0.206

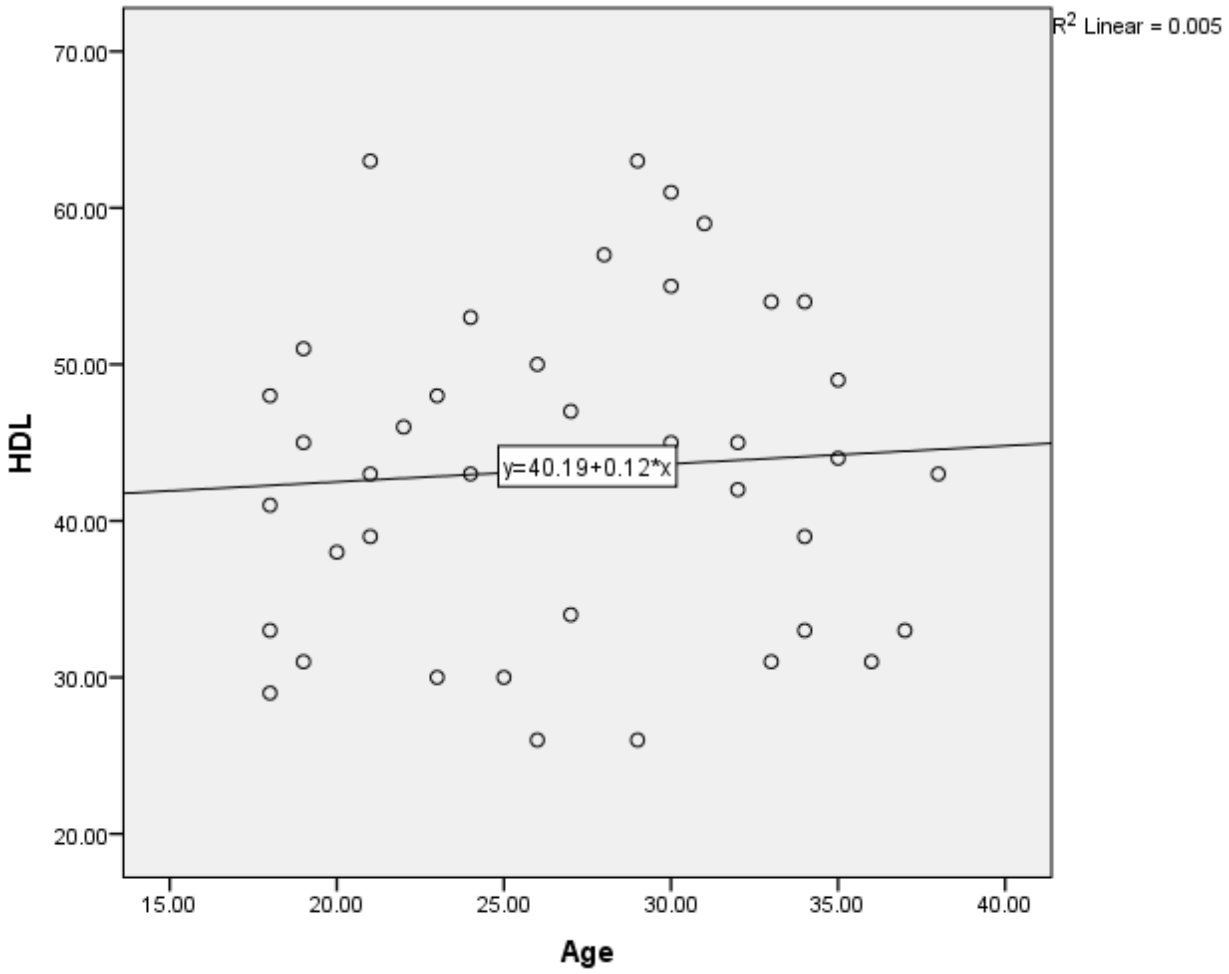

Figure (4.3): scatter plot between HDL level and age, P = 0.676, r = 0.068

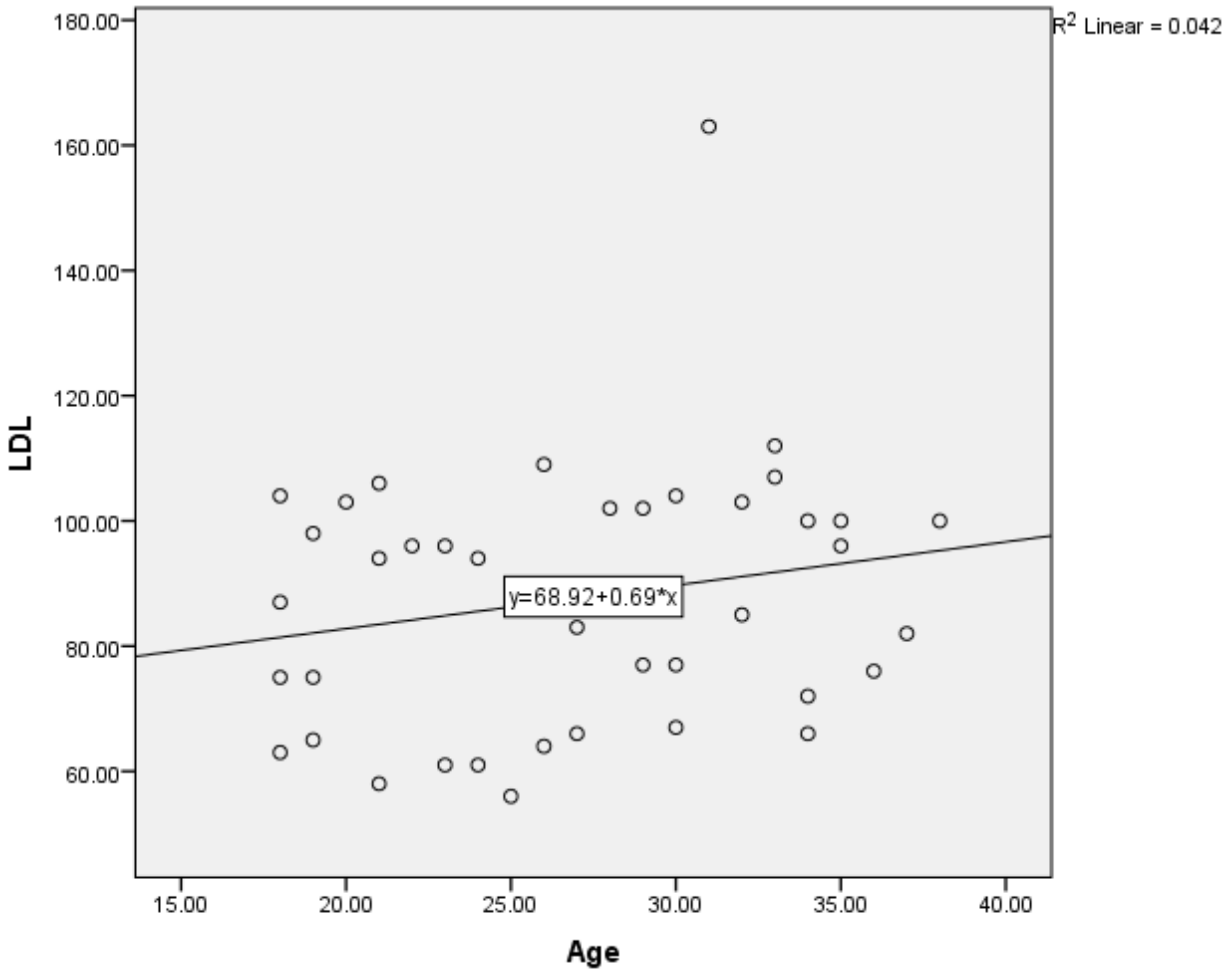

Figure (4.4): scatter plot between LDL and age,P = 0.202, r = 0.206

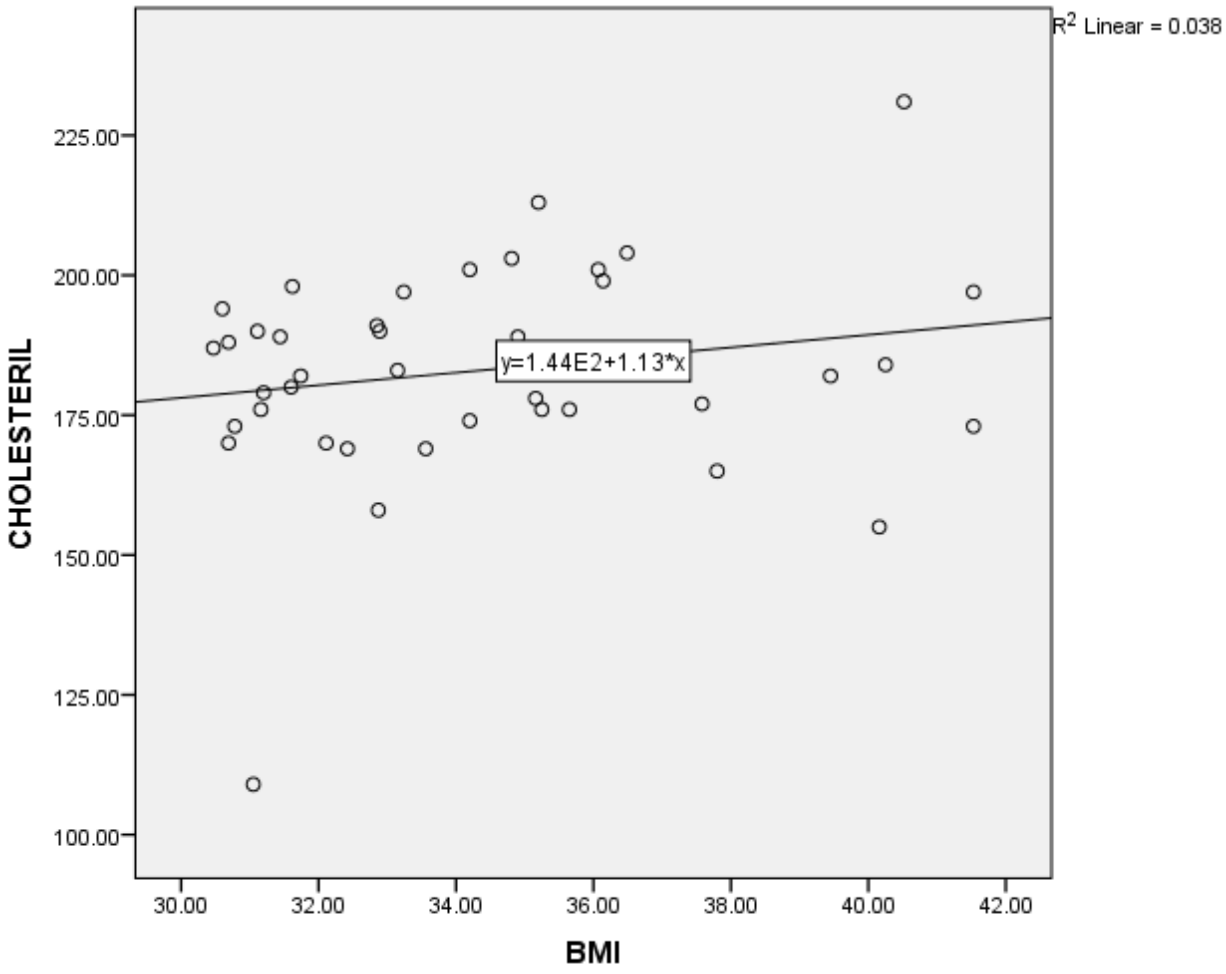

Figure (4.5): scatter plot between Cholesterol level and BMI, P = 0.228, r = 0.195

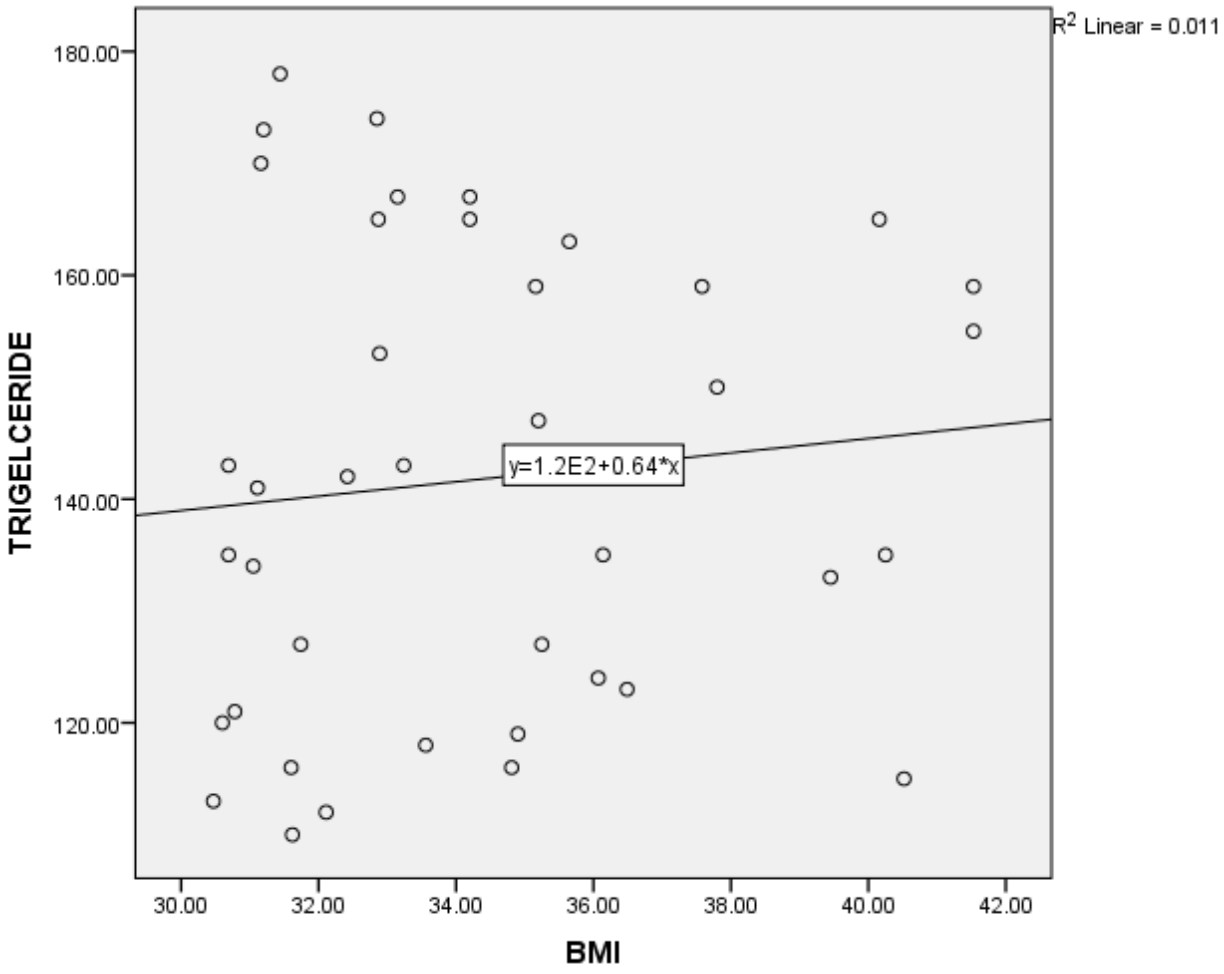

Figure (4.6): scatter plot between Triglyceride level and BMI, P = 0.527, r = 0.103

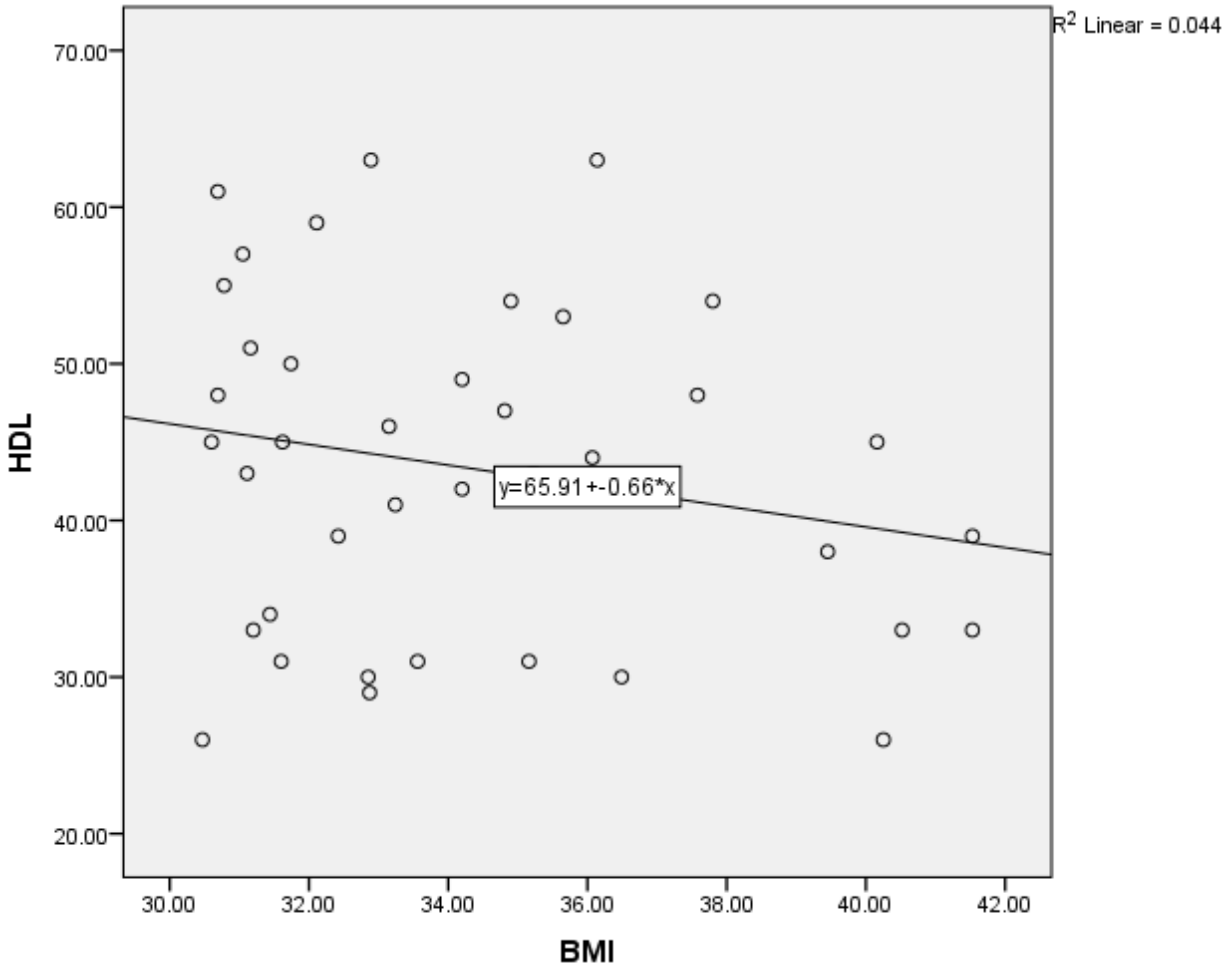

Figure (4.7): scatter plot between HDL and BMI, P = 0.195, r = - 0.209

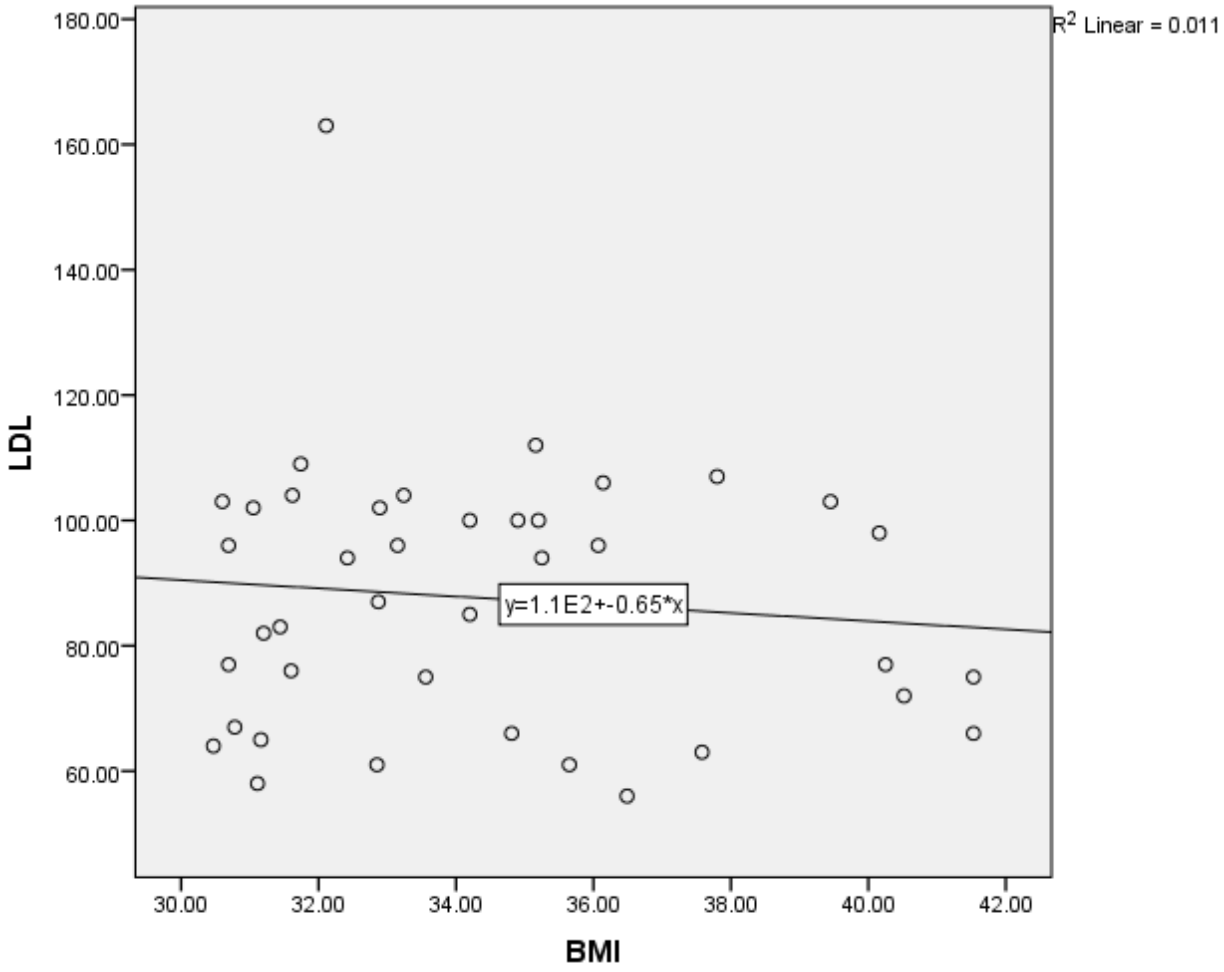

Figure (4.8): scatter plot between LDL and BMI, P = 0.522, r = -0.104

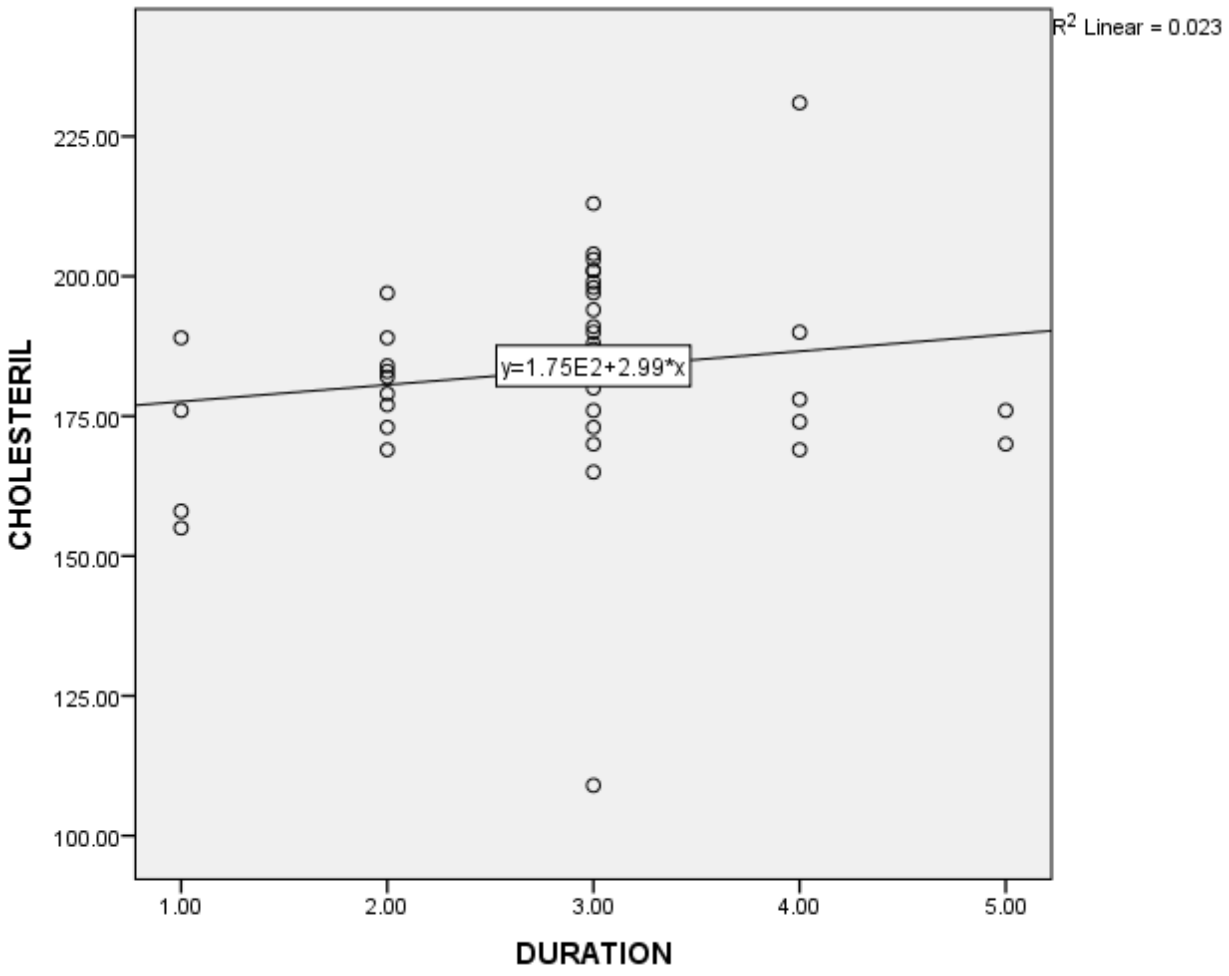

Figure (4.9): scatter plot between Cholesterol level and Duration /years, P = 0.354, r = 0.150

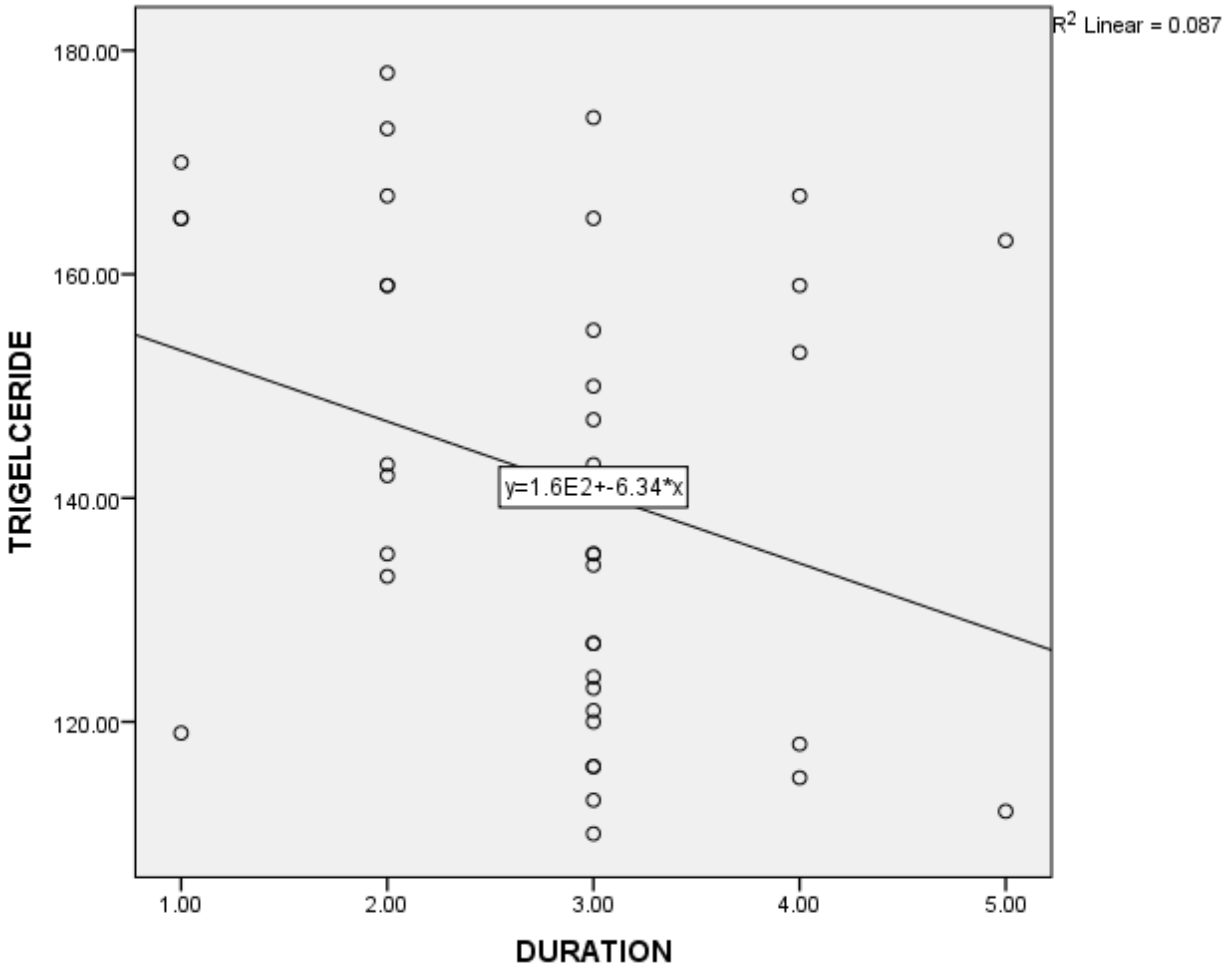

Figure (4.10): scatter plot between triglyceride and Duration /years, P = 0.065, -r = 0.294

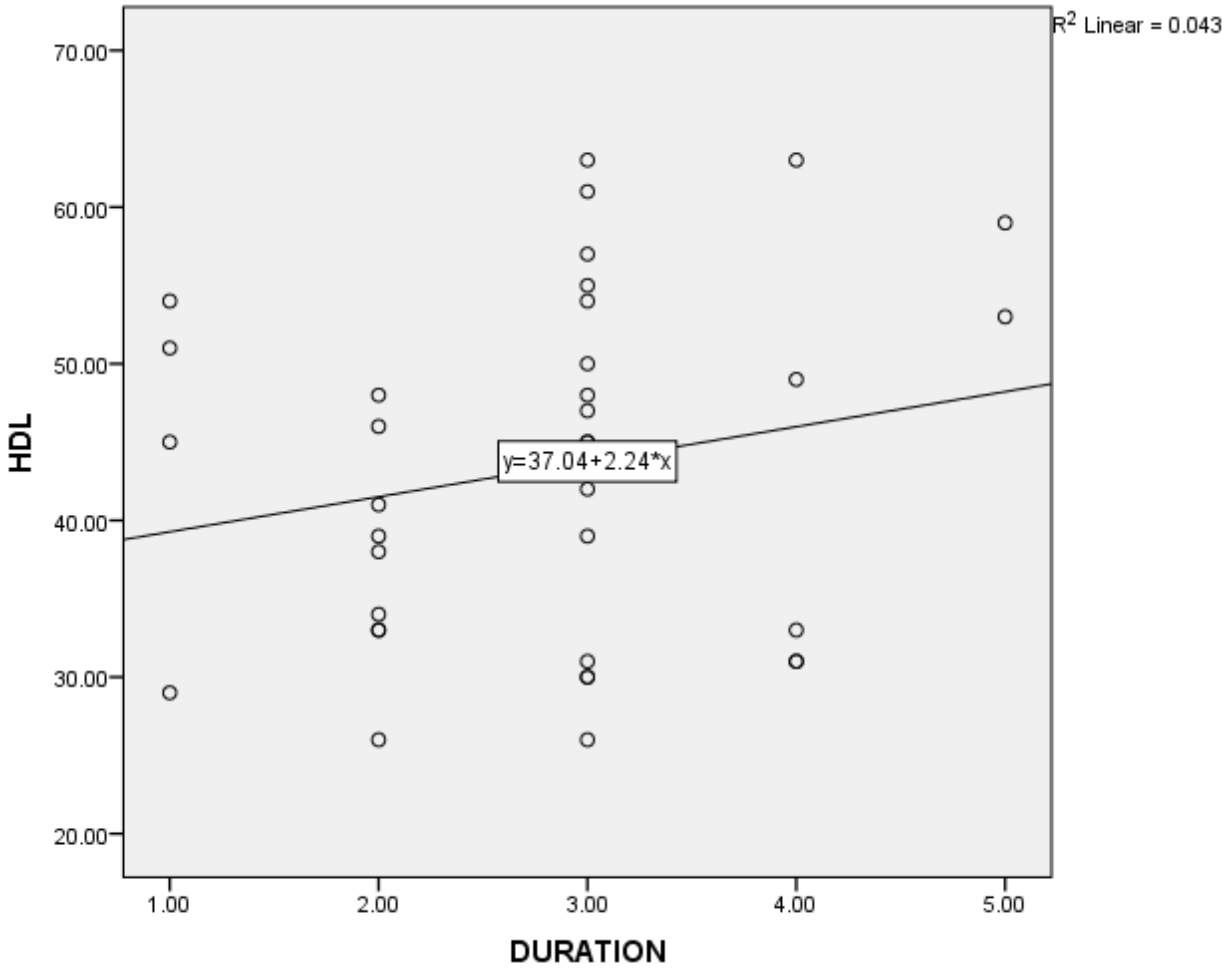

Figure (4.11): scatter plot between HDL and Duration /years, P = 0.208, r = 0.201

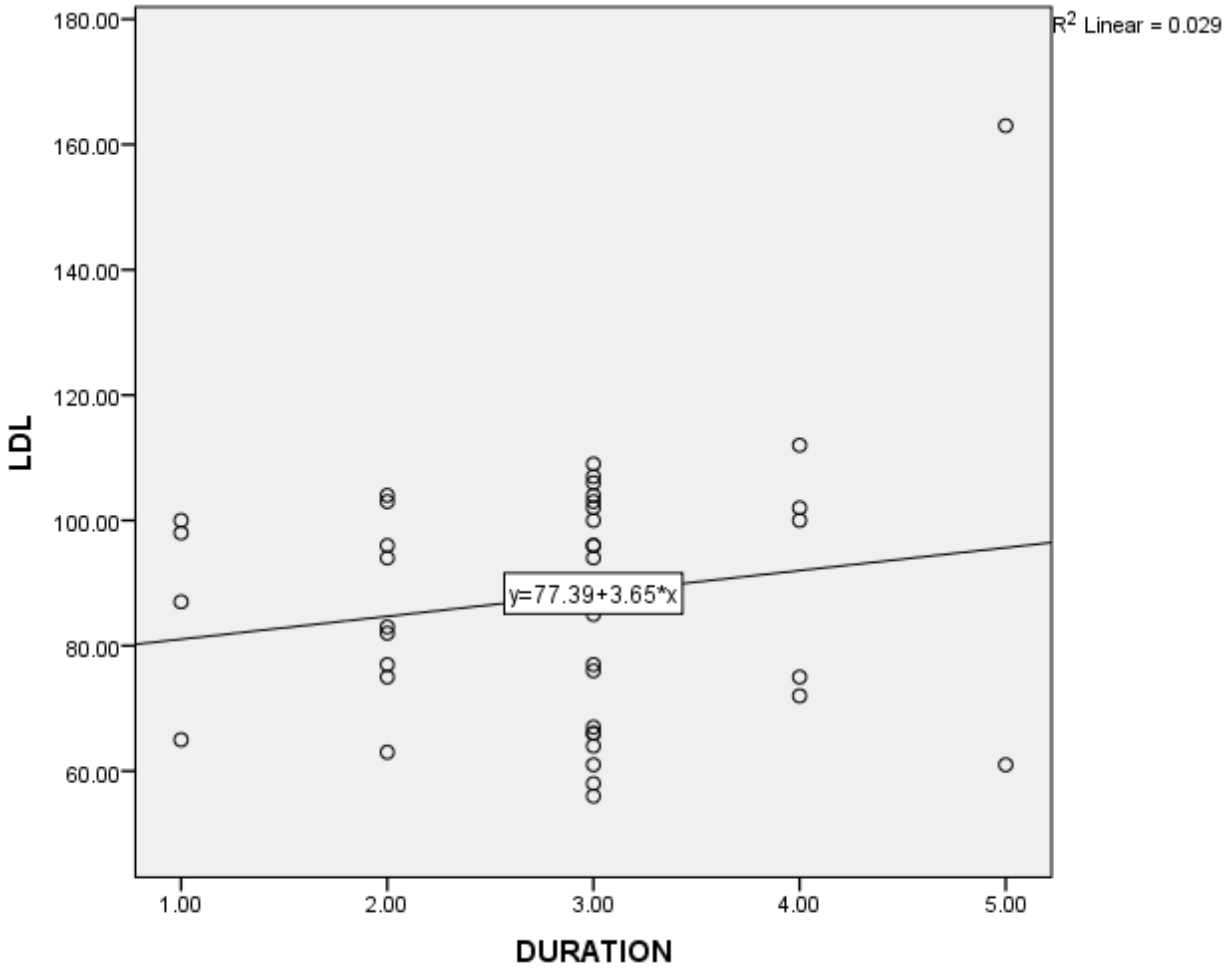

Figure (4.12): scatter plot between LDL level and Duration /years, P = 0.298, r = 0.169

Chapter Five

Discussion, conclusion and recommendation

5.1Discussion

The hormonal components of oral contraceptives exert major effects on plasma lipoprotein metabolism. Estrogens may increase production of plasma triglycerides, leading to increase levels of very low-density lipoproteins; however, these may also reduce levels of cholesterol enriched and potentially atherogenic intermediate- and low-density lipoproteins. Progesterone increase LDL absorption and decrease HDL absorption by increasing absorption of apo-B and decrease absorption of apo-A (Junge *et al.*,2011).

In the present study showed significant increase in body mass index (BMI) and plasma total cholesterol in female using 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) and another agreement to study conducted on the Nigerian women using contraceptive showed increased level of cholesterol with $p<0.001$(Obisesan *et al.*,2012).Significantly increased of serum total cholesterol in contraceptive user women might be due to impaired lipoprotein metabolism. oral contraceptive increase apolipoprotein B-100 synthesis and thus increased LDL-C. low density lipoprotein in 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).

In our study the levels of triglycerides were found significantly elevated (p=0.001) in Oral Contraceptives users Therefore, by causing an increase in TG levels, Oral Contraceptives use may be worsening cardiovascular risk., Increased serum triglyceride might be due production and transport of VLDL that endogenously synthesized triglycerides in the blood. Progestin components of oral pill increased hepatic lipase enzyme activity as a result decreased serum HDL level. lipoprotein metabolism, our finding agreement to study conducted by (Minozzi *et al.*,2011).

In current study there was significant different of HDL-C, this result is an disagreement with previous study showed insignificant decrease of HDL-C concentration (Faryal *et al.,* 2012) but was agree with previous study showed significant differnts 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 significant 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 concluded that women had received oral contraceptive had higher level of total plasma cholesterol, triglyceride, LDL-C while low level of HDL-C and no correlation between plasma total cholesterol and (age, body mass index, and duration of combined oral contraceptive use).

5.3 Recommendation

This study it recommended that:

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

- Lipid profile must be evaluated during using oral contraceptives to decrease possibility getting dyslipidemia.

-increase awareness of women about contraception

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

-Further large-scale multicenter study should be performed to avoid the selection bias of the study.

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