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Some Biochemical Changes Associated with Taking Oral Contraceptive Pills Among Healthy Women in Gaza City

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بسم الله الرحمن الرحيم

" و قل اعملوا فسيرى الله عملكم و رسوله و المؤمنون "

صدق الله العظيم

Dedication

To the loveliest parents in the world who support me with all their hearts

To my brothers and sisters

To my husband for his tolerance circumstances

To my university which is working to improve the research

This study could not have been achieved without them

Islah M. Abu Hani

Declaration

I certify that this submission is my own research and that, to the best of my knowledge and belief, it contains material neither previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university of other institute, except where due acknowledgment has been made in the text.

Signed:

ISLAH M. ABU HANI

Date: November, 2010

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Some Biochemical Changes Associated with Taking Oral Contraceptive Pills Among Healthy Women in Gaza City

ABSTRACT

Aim: To evaluate some biochemical changes associated with oral contraceptive (OC) pills administration on healthy women in Gaza City (GC).

Methodology: The study design was a case control. The sample size was 80 healthy women aged 20-35 years from the Swidey Clinic who had taken OC pills for at least three continuous cycles. The control sample was healthy married women who were not going on OC before and match the cases in age and residence. The study questionnaire included issues about the following information: age, gender, weight, height, health history, blood pressure, Nature of menstrual cycle, bleeding, insomnia, pain in the stomach, difficulty in breath, pain in hands and feet, appetite and headaches.

Blood parameters analysis of the study population included complete blood count (CBC), C-reactive protein (CRP), high density lipoproteins cholesterol (HDL-C), low density lipoproteins cholesterol (LDL-C), total cholesterol (TC), and triacylglycerol (TAG). Leptin determination was carried out using a commercially available diagnostic system test kits. SPSS were used to analyze obtained data.

Results: The results showed statistically significant differences among study population with respect to regular of menstrual cycle (MC) ($\chi 2$=5.371, P= 0.024), increased appetite ($\chi 2$= 4.386, P= 0.002), increased headache ($\chi 2$= 6.82, P= 0.000), increased body mass index ($\chi 2$= 7.31, P= 0.015), cholesterol were significantly higher among the cases compared to control (179.1± 4.3mg/dl vs 157.5± 4.12mg/dl, and p=0.000), LDL-C were significantly higher among the cases compared to control (97.6±3.8mg/dl vs 86.2± 3.4 mg/dl, and p=0.002), increased C-reactive protein ($\chi 2$= 5.381, p= 0.034), there were a significant increase in the mean level of leptin among the cases compared to the controls (36.3± 2.3 ng/ml vs. 28.6± 2.1 ng/ml, and p= 0.003), and the levels of WBC, Gran and Mch were significantly higher among the cases (7.2±2.1 K/µL, 60.7±8.3 % and 26.9±2.2pg) compared to the controls (6.6±1.7 K/µL, 54.9±11.7 % and 25.7±3.4 pg), with p=0.001, p=0.000 and p=0.003, respectively. In contrast, the results

showed no statistically significant differences among the study population with respect to frequency of bleeding ($\chi 2$=0.192, P=0.135), frequency of insomnia ($\chi 2$=0.411, P=0.353), pain in stomach ($\chi 2$=0.386, P=0.183), difficulty in breathing($\chi 2$=0.497, P= 0.209), frequency of pain in hands and feet ($\chi 2$=0.631, P= 0.309), Systolic blood pressure (($\chi 2$=2.351, P=0.139), diastolic blood pressure ($\chi 2$=1.372, P=0.382), HDL-c (51.2±1.7mg/dl vs 47.6±1.3mg/dl and p=0.148), TAG (119.2± 6.0mg/dl vs 108.2± 8.6 mg/dl and p=0.218), the changes among the study population in Mid, RBc, Hb, Hct, Mcv, Mchc and PLt (7.8±8.7 %, 4.6±0.7M/µL,12.2±0.9g/dl, 38.0±2.4%, 80.8±11.9fl, 32.5±1.1g/dl and 279.3±87.6K/µL) compared to control were not significant (8.6±6.6 %, 4.6±0.47M/µL, 12.3±1.1g/dl, 37.5±3.7%, 80.6±8.5fl, 32.3±1.7g/dl and 267.8±74.0 K/µL), with p=0.352, p=0.394, p=0.387, p=0.571, p=0.415, p=0.272 and p=0.185, respectively.

Moreover, the results showed strong correlation between BMI and leptin (p= 0.000) among the study population.

Key words: Oral contraceptive, Healthy women, Biochemical parameters, Gaza City.

بعض التغيرات البيوكيميائية للنساء الأصحاء اللواتي يتناولن حبوب منع الحمل في مدينة غزة

مستخلص الرسالة

الهدف: لتقييم بعض التغيرات البيوكيميائية للنساء الأصحاء اللواتي يتناولن حبوب منع الحمل في مدينة غزة.

منهجية الدراسة: تقارن الدراسة بين مجموعة تجريبية من النساء الأصحاء عددهن 80 تتراوح أعمارهن ما بين (20-35 سنة) من عيادة السويدي و اللاتي يتناولن حبوب منع الحمل لمدة لا تقل عن ثلاث دورات شهرية، و عينة ضابطة من النساء المتزوجات اللواتي لا يتناولن حبوب منع الحمل و تشترك مع العينة المبحوثة في العمر و السكن، وتم الحصول على البيانات المستخدمة من خلال إستبانة تشمل على العمر ، الجنس ، الطول ، الوزن، مقدار الضغط ، طبيعة الدورة ، المعاناة من النزيف، المعاناة من الأرق، المعاناة من ضيق في التنفس، المعاناة من ألم في اليدين و الأقدام، المعاناة من فتح الشهية ، المعاناة من الصداع، و من خلال التحاليل الكيميائية للدم و تشمل الكولسترول، الكولسترول في الدهون منخفضة الكثافة، عالية الكثافة، الدهون الثلاثية، هرمون الليبتين، ومن ثم استخدام SPSS لعملية التحليل الإحصائي.

النتائج: أوضحت النتائج أن هناك فروقا ذات دلالة إحصائية بين المجموعة التجريبية والمجموعة الضابطة من حيث انتظام الدورة، زيادة فتح الشهية، الصداع، دليل الوزن، الكولسترول و الكولسترول منخفضة الكثافة، هرمون الليبتين، وبروتين الإلتهابات، وغير ذلك. على عكس ذلك أوضحت النتائج أنه لا يوجد فروقا ذات دلالة إحصائية بين المجموعتين من حيث النزيف، الأرق، ألم في المعدة، صعوبة في التنفس، ألم في اليدين و الأقدام، الضغط الانبساطي، الضغط الانقباضي، الدهون الثلاثية وغير ذلك.

وزيادة على ذلك بينت الدراسة أنه توجد بين أفراد المجموعة التجريبية علاقة قوية ما بين دليل الوزن ومستوى الليبتين في الدم.

الكلمات المفتاحية: حبوب منع الحمل، النساء الأصحاء، المعايير البيوكيميائية، مدينة غزة.

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LIST OF ABBREVIATIONS

BMI	Body Mass Index
BP	Blood Pressure
CBC	Complete blood count
COD	Cholesterol Oxidase
CRP	C- Reactive Protein
CVD	Cardiovascular disease
DSG	Desogestrel
DBP	Diastolic Blood Pressure
DSL	Diagnostic System Laboratories
EDTA	Ethylene Diamine Tetra Acetate
EE	Ethinyl Estradiol
ELISA	Enzyme- Linked immunosorbent assay
FSH	Follicle Stimulating Hormone
GC	Gaza City
GFR	Glomerular Filtration Rate
GnRH	Gonadotropin Releasing Hormone
Gran	Granulocytes
Hb	Hemoglobin
HCT	Hematocrit
HDL	High Density Lipoprotein
HRP	Horseradish Peroxidase
Jak	Janus-Activated Kinase
Lep	Leptin
Lymph	Lymphocytes
MAPK	Mitogen-activated protein kinases
MC	Menstural cycle
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MOH	Ministry Of Health
NPY	Neuropeptide Y
OCs	Oral Contraceptives
Ob	Obese
PLT	Platelet
RBC	Red Blood Cell
RDW	Red Cell Distribution Width
Stat	Signal Transducer and Activators of Transcription
SBP	Systolic Blood Pressure
TAG	Triacylglycerol
TMP	Tetramethylbenzidine
USA	United State of America
VTE	Venous Thromboembolism
WBC	White Blood Count
WHO	World Health Organization

Chapter 1

Introduction

1.1 Overview

Oral contraceptives (OCs) are the most popular type of birth control pills. The pills stop ovulation, preventing the ovaries from releasing eggs. they also thicken cervical mucus, making it harder for sperm to enter the uterus (Feminist Women's Health Center, 2008).

Obesity, breakthrough bleeding, nausea, high blood pressure (hypertension), high cholesterol, signs of a blood clot and bloating are some of the more commonly reported OC pills side effects (Bakir and Hilliquin, 1986). On the other hand, OC increases a woman's risk to cerebrovascular disease and cervical cancer. A woman taking the pill is 1.9 times more likely to die from cerebrovascular disease and 2.5 times more likely to die from cervical cancer (Life Site News, 1999). Oral contraceptives are taken by about 100 million women worldwide. Studies have shown that synthetic hormones used for OC greatly increased the risk of blood clotting. Clots typically form in the legs and can cause serious injury and death if they travel to the heart, lungs or brain (Baklinski, 2008).

High body weight could be also a side effect of OCs. Leptin's effects on body weight are mediated through effects on hypothalamic centers that control feeding behavior and hunger, body temperature and energy expenditure. It is a protein hormone with important effects in regulating body weight, metabolism and reproductive function. Leptin also acts as appetite suppressant. It stops eating too much as well as makes the body more active so it burns off more energy. The amount of leptin found in people increases as their body fat increases (Harrison, 2002).

1.2 Significance and general objectives

Although OC pills are usually used elsewhere since very long time, no studies were carried out about impact of OCs on healthy women in Gaza Strip. The general objective of this study, therefore, is to evaluate some biochemical changes associated with taking OC pills on healthy women in Gaza City.

1.3 Specific objectives

1- To define some side effects of OC pills administration.

2- To assess the effect of OC pills administration on serum leptin level as a marker of obesity.

3-To determine changes in serum level of cholesterol, HDL-C, LDL-C and TAG, C-reactive protein associated with OC pills administration.

4- To determine effect of OC pills on some blood parameters

5- To correlate body mass index with leptin level in women taking OCs.

Chapter 2

Literature Review

2.1 Types of oral contraceptives

2.1.1 Combination oral contraceptives

Combination OCs contain both estrogen and progesterone. Combination birth control pills may be monophasic, where each of the active pills contains the same amount of estrogen and progesteron, or biphasic, where the active pills contain varied amounts of hormones designed to be taken at specific times throughout the pill-taking schedule (National Women's Health Resource Center Inc, 2009). Synthetic hormones are used in OCs estrogens, such as, mestranol, ethinyl estradiol (EE). Progesterones used in OCs are synthetic progesterones, such as norethindrone, norgestrel, norethindrone acetate, ethynodiol diacetate, levonorgestrel, norgestimate, desogestrel, drospirenone. Different progesterons have different strengths and side effects (Watson Pharma Inc, 2006).

2.1.2 Progesterone-only oral contraceptives

This type of pill contains no estrogen, called the progesteron-only pill, or "mini-pill, it's for breastfeeding women because estrogen reduces milk production (National Women's Health Resource Center Inc, 2009). It also will be taken if women are hypertensive, or at risk for developing blood clots. Minipills are slightly less effective than regular pills and often cause irregular menstrual patterns. Minipills prevent pregnancies mainly by making the cervical mucus impermeable to sperm and by making it more difficult for an egg to attach to the uterus lining (Watson Pharma Inc, 2006).

Causes of bleeding

In 1982, more than 20% of women surveyed in a nationally representative sample had discontinued OCs on their own or at the recommendation of their physician due to bleeding or spotting.

Bleeding may be due to:

1) physiologic effects of OCs on the endometrium.

2) Oral contraceptive-related parameters, including dose and formulation.

3) patient behavior, including compliance, using concomitant medications, and smoking.

4) benign or malignant pathology (Goliath service, 2008).

2.1.3 Pharmacological classification

First generation OCs contain high dose (> 50 mg) of ethinyl estradiol (EE).

Second generation OCs contain levonorgestrel, norgestimate, norethindrone family and 30-35 mg EE .

Third generation OCs contain desogestrel or gestodene and <30 mg EE (Jessica and Lalley, 2000).

2.2 Mechanism of action of oral contraceptives

The pituitary gland produces two hormones called Follicle Stimulating Hormone (FSH) and Luteinizing Hormone(LH). These hormones stimulate the ovary to produce an egg each month. The ovary is the site of production of the woman's two central female hormones, estradiol (EST), a type of estrogen, and progesterone (PRO), a type of progestin. Oral contraceptive pills are a combination of synthetic estrogen and progesterone. They causes negative feedback decreases in the pulse frequency of gonadotropin-releasing hormone (GnRH) release by the hypothalamus, which decreasing the release of FSH and greatly decreases the release of LH by the anterior pituitary (Figure 1). These two hormones are needed for ovulation to occur, therefore, OCs suppress, but do not eliminate ovulation.

The progesterone component of the combination pill also causes the inner lining of the uterus to become thin and shriveled, unable to support implantation of the embryo.

Mucus cervix thickens under the influence of a progesterone impedes sperm migration (Lifeissues, 2000).

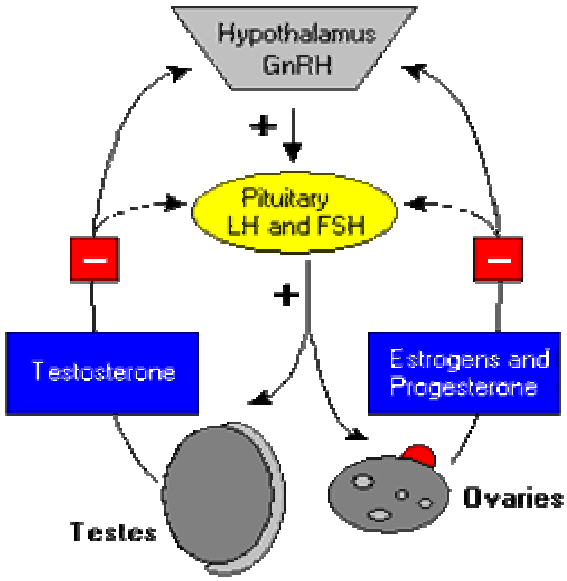

Figure 1: Gonadotropic hormones action and regulation (Bowen, 2004).

2.3 Side effects of oral contraceptives

2.3.1 Blood pressure: Women taking OCs could have an increase in both systolic blood pressure (SBp) and diastolic blood pressure (DBp). Early results from a controlled long-term prospective study have shown no significant change in mean systolic or diastolic pressures or mean weight in 31 women after four months on OCs. However, there was a significant change in Bp or weight over the short period of the experiment (Weir et al., 1969).

Calvin et al., (1969) examined the relation between use of OCs and Bp in 1,575 white working females aged 15 to 44 years. Of these, 31.5% were using OCs when their Bp was measured. Results analysis revealed slightly higher and statistically significant mean SBp and DBp in women using OCs, after correction for age, height, weight, and arm circumference. A sample of this population one year later, showed little tendency for Bp to be affected by OCs, except for a slight but significant fall in DBp among women who did not use these drugs.

Fisch et al., (1977) study on 13,358 women showed that OC use is associated with a slight but statistically significant (P <.05) rise in mean Bp. The age-adjusted proportion of OC users with a Bp over 140/90 mm Hg was about three times that of nonusers. Women continuing OC use had no appreciably greater change in Bp between users and nonusers.

Khaw and Peart, (1982) examined the relation between OCs and Bp in 461 women. The result showed that significantly higher mean systolic and diastolic blood pressures than those using non-hormonal contraception. There was a significant correlation of blood pressure with duration of current use of OCs but not with total duration of use. There was also a significant negative correlation of blood pressure with time since OCs were last taken, and women who had stopped using oral contraceptives over a month previously had similar blood pressures to those who had never taken them. In women taking OCs had either a history of hypertension in pregnancy or a family history of hypertension had significantly higher mean blood pressures than those who did not.

Wei , (1997) assessed whether the blood pressure is higher among women who take OCs than it is among those who do not. A cross-sectional survey of a stratified random sample of English adults (aged ≥ 16 years), sample of english adults, 3545 premenopausal women, of whom 892 were current users of OCs. Mean blood pressures adjusted for age were significantly higher among oral contraceptive users (125/70 mmHg) than they were among non-users (123/68 mmHg, P < 0.001 both for systolic and for diastolic blood pressures). These results remained unchanged after further adjustment for the body mass index, alcohol intake, physical activity and hypertension treatment. Blood pressure differences tended to be larger among older oral contraceptive users. Oral contraceptives containing progestogen only were not associated with higher blood pressures. Blood pressures should be screened before OCs are supplied and should be monitored regularly during oral contraceptive use.

Bertschi et al., (2003) assessed the systemic and renal hemodynamic and tubular responses to salt in 27 young healthy women taking OCs containing monophasic combination of 30g EE and 150g desogestrel for >6 months. All women were assigned at random to receive a low (40 mmol/day) or a high (250 mmol/day) sodium diet for 1 week on two consecutive menstrual cycles during the active OC phase. At

the end of each diet period, 24-hour Bp, renal hemodynamics, sodium handling, and hormonal profile were measured, The use of OCs is not associated with an increase Bp response to salt, however, OCs affect the renal hemodynamic response to salt, a high salt intake leading to an increase in glomerular filtration rate (GFR) and filtration fraction. This effect is possibly mediated by the estrogen-induced activation of the renin-angiotensin system. Oral contraceptives also appear to increase the tubular responsiveness to changes in sodium intake. This indicated that synthetic sex steroids have a significant impact on renal function in women.

2.3.2. Change of lipid profiles

Doar et al., (1969) studied the effects of OCs on fasting serum lipid levels in two groups of women. One hundred and twenty-eight subjects (group A) were tested before and during therapy; 52 subjects (group B) were tested initially during therapy and again after this had been discontinued. In both groups OC therapy was associated with significantly raised mean serum triacylglycerol (TAG) and total cholesterol (TC) levels. A significant elevation of the mean fasting serum TAG level was also found in a group of 19 women receiving low-dose glucocorticoid therapy, though the percentage increase (16%) was less than that in the women receiving OCs (49%).

Narboni et al., (1978) investigated a systematic treatment for patients on OCs in whom hyperlipidism is induced by the OCs. Thirty six women aged 20-40 years who used OCs containing 50 mcg of EE and Norgestrel were included in the study. Nine of them women had elevated TAG levels, 5 had elevated TC levels, and 22 had both. The average level of TAG was 1.56 and of TC was 2.88 g/l. When the EE dosage of the 1st group was reduced to 30 mcg, the average TAG level fell from 1.44 to l g/l. In a 2nd group, in which either lipid levels were very high, the use of combined OCs was discontinued; the average level of TAG declined from 1.64 to 0.95g/l.

Hennekens et al., (1979) measured fasting plasma TAG, plasma TC and HDL-C cholesterol levels for 190 white women, ages 21--39 years, who used OCs. The mean level of fasting TAG was higher among current OC users (95 mg/100 ml) than among nonusers (73 mg/100 ml) ($p = 0.002$). After adjustment for the possible confounding effects of age, weight, current cigarette smoking and fasting glucose level, current OC users still had a mean plasma TAG level 19 mg/100 ml higher than that of nonusers (p

= 0.007). Current OC users also appeared to have higher levels of TC. There was a nonsignificant inverse relationship of OC use with HDL cholesterol levels.

Parks et al., (1989) studied the effect of two different combination OCs steroid preparations containing equivalent amounts of estrogen but different progesterone components on plasma lipids and lipoproteins. For 2 years, one group (n= 23) received 75 micrograms norgestrel and 7.5 micrograms EE daily, while another group (n = 25) received 150 micrograms ethynodiol diacetate and 7.5 micrograms EE daily. The control group n = 24 received no treatment. The result of the study showed that the two oral contraceptive groups had higher TC and TAG concentrations compared to control group.

Willy et al., (1990) studied the effects of OCs on serum lipids and blood pressure were among young women in the Netherlands. Fifty-three participants, ages 14 to 24 years. They continued OCs use for at least 2 subsequent years. From 53 age-matched control subjects, who did not use OCs, data were obtained for the same follow-up period. Women using oral contraceptives showed a significantly greater rise in serum total cholesterol levels than did the reference subjects (14 mg/100 ml/2 year vs 4 mg/100 ml/2 year; 95% confidence interval of the difference was 0.1 to 19.6). The increase in systolic blood pressure (4.7 mm Hg/2 year vs 2.1 mm Hg/2 year; 95% confidence interval of the difference was −1.8 to 6.9) did not differ between the groups. These findings suggest that OCs use may be associated with an enhanced rise in total cholesterol during adolescence.

Emokpae et al., (2010) studied the effect of duration of oral contraceptive use on lipid and lipoproteins in Nigerian women. One hundred and twenty women (mean age 24.1±5 years) were used who were on biphasic lofeminal tablets for a period ranging from 1- 48 months. Fifty age matched women with regular menstruation with no history of hormonal use within the last six months before the investigation were used as controls. Statistically significant increases were observed for TAG, LDL-c, and VLDL-c with the duration of oral contraceptive use.

2.3.3 Inflammatory diseases

C-reactive protein (CRP) is produced by the liver. The level of CRP rises when there is inflammation throughout the body (Ridker et al., 2007). High or increasing amount of CRP blood suggests that there is an acute infection or inflammation. CRP levels can be elevated in the later stages of pregnancy as well as with use of birth control pills or hormone replacement therapy (i.e., estrogen). Higher levels of CRP have also been observed in the obese individual (American Association for Clinical Chemistry, 2009).

Swan, (1991) provided the data on hospital admission and death rates in relation to the use of OCs in a large suburban population which has been followed up for 10 years. The incidence of hospital admission for inflammatory disease was significantly increased among women under age 40 who had ever used OCs, and the systems affected include the respiratory, digestive, urogenital, and musculoskelatal. The relative risk for such a hospital admission is 2.8 in past OC users and 1.8 in current OC users. This effect is considerably reduced in older users. A systemic increase in the risks of inflammatory disease resulting from OC use is consistent with a modification of the immune system. The author also observed an increase in white blood cell count among OC users.

Dreon et al., (2003) found that plasma CRP levels were two times higher among OC users than among non-users (2.0 ± 0.2 versus 0.9 ± 0.3 mg/l, $p < 0.0001$) independent of diet. The results suggested that estrogenic hormones significantly affect pro-inflammatory pathways.

Wanpen Vongpatanasin et al., (2003) demonstrated that OCs caused a sustained increase in CRP, implicating a pro-inflammatory effect. Because CRP is synthesized in the liver, they hypothesized that estrogen-induced CRP elevation is related to first-pass hepatic metabolism.

Cauci et al., (2008) observed an increase in low-grade inflammatory status measured by high-sensitivity CRP concentrations in response to OCs use. Alteration of

inflammatory status in OC users could affect the risk of venous thromboembolism, cardiovascular disease, and other oral contraceptive-associated adverse conditions in young women.

Life Site News (2008) concluded that the OCs elevates the levels of CRP in women, which raises the risk of cardiovascular disease. In comparing healthy women who used the pill with a group that didn't, the study found that pill-users were more than four times more likely to have high levels of CRP in their bodies which leading to a high risk of cardiovascular disease.

2.3.4 Cardiovascular diseases

There is an association between OCs use and cardiovascular disease (CVD). It is thought that the hormones contained in the OCs formulas create an environment which affects the clotting cascade by altering clotting factors. Blood clots form, dislodge, and block arteries and veins of the heart or the brain, interrupting the blood supply and causing a heart attack or stroke. Estrogen affects events in the arteries and veins. Progesterones affect events that take place in the arteries only. The levels of clotting factors, such as fibrinogen, and cholesterol in the blood have been related to the risk of CV events. Oral contraceptives users had more fibrinogen, factor VIIc and factor XII (Hageman factor) than nonusers. Furthermore, the more estrogen contained in the OCs formula, the more the clotting factors are increased, it is highly likely that the same mechanism is at work in women taking OCs. Kelleher, (1998) reported that women who are taking OCs are at risk for CV events through changes in the clotting process, leading to thrombosis. Women who use birth control pills are at a slightly increased risk of having a blood clot in the legs or lungs. Studies consistently showed that the risk of venous thromboembolism is two to six times higher in OCs users than in nonusers. The risk of blood clots is highest in women with clotting disorders or who have previously had a deep venous thrombosis or pulmonary embolism. Other risk factors of CVD include: obesity, older age, having several family members who've had blood clots before old age, air travel, and having to lie or sit for a prolonged period (Birth Control Pills Advantages and Disadvantages, 2007).

However, the chances of OCs contributing to a heart attack are small except with smoking. Studies have shown that smoking dramatically increases the risk of heart attack in women administration Ocs aged 35 years or older (Women's Health, 2004).

2.3.5 Obesity

Body mass index is a common measure expressing the relationship (or ratio) of weight-to-height. It is a mathematical formula in which a person's body weight in kilograms is divided by the square of his or her height in meters i.e. $wt/(ht)^{2.}$. The BMI is more highly correlated with body fat than any other indicator of height and weight. Individuals with a BMI of 25 to 29.9 are considered overweight, while individuals with a BMI of 30 or more are considered obese (National Research Council, 1989).

Obesity is an excessively high amount of body fat or adipose tissue in relation to lean body mass. The amount of body fat (or adiposity) includes concern for both the distribution of fat throughout the body and the size of the adipose tissue deposits (Stunkard and Wadden, 1993).

Hirschberg et al., (1996) investigated the role of the "satiety peptide" cholecystokinin and some other gastrointestinal hormones among 10 young healthy women taking OCs. A standardized meal test was used for recordings of appetite and gastrointestinal hormone response before and after 5 months of treatment with a monophasic combined OCs. Oral contraceptivs caused a suppression of basal levels of serum cholecystokinin, which was correlated to an increase in body fat. Serum levels of gastrin and insulin were also unchanged, whereas TAG and postprandial glucose levels were elevated. Therefore reduced cholecystokinin levels may be related to mild impairment of glucose tolerance and promote body fat storage during OCs.

A combination of medical disorders of OCs includes: diabetes mellitus type 2, high blood pressure, high blood cholesterol, and high triglyceride levels (Grundy, 2004).

Due to OCs, the level of metabolism alters. This is because both the estrogen and progesterone present in these pills can cause fluid retention, a temporary effect that often begins in the first month as a result of an increase in sodium. The cause of weight gain (increase of hips, breast, or thigh) is due to estrogen. Oral contraceptives

contain progesterone which cause increase in appetite and permanent weight gain (Crystal, 2005).

Obesity increases the risk of many physical and mental conditions. These comorbidities are most commonly shown in metabolic syndrome, Certain medications may cause weight gain or changes in body composition, these include: insulin, steroids, some forms of hormonal contraception and so on (Haslam and James., 2005).

Coronary artery disease, fatty liver, gall stones, sleep apnea, arthritis, and cancer may shorten the lifespan (Ogden et al., 2007).

Syed et al., (2008) studied the CVD risk factors in users of second generation contraceptives by measure changes in BMI, blood pressure and electrocardiogram. Sixty four women volunteered for this study (age range 20-35 years). Results showed that women aged less than 30 years and using OCs for more than three years had a tendency to gain weight and increase in systolic and diastolic blood pressures. World Health Organization (2009) reported that obesity and overweight pose a major risk for chronic diseases, including type 2 diabetes, cardiovascular disease, hypertension and stroke, and certain forms of cancer.

2.3.6 Change in leptin level

Leptin (Greek leptos meaning thin) is a 16 KDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. It is one of the most important adipose derived hormones (Brennan and Mantzoros, 2006). The Ob (Lep) gene (Ob for obese, Lep for leptin) is located on chromosome 7 in humans (GreGreen et al., 1995). In addition to white adipose tissue, leptin can be produced by brown adipose tissue, placenta (syncytiotrophoblasts), ovaries, skeletal muscle, stomach (lower part of fundic glands), mammary epithelial cells, bone marrow, pituitary and liver (Margetic et al., 2002).

Leptin interacts with different types of receptors (Ob-Ra-Ob-Rf, or LepRa-LepRf) which in turn are encoded by a single gene, LEPR (Wang, 1996). Ob-Rb is the only receptor isoform that can signal intracellularly via the Jak (Janus-Activated Kinase) - Stat (Signal Transducer and Activators of Transcription) and MAPK (Mitogen-activated protein kinases) signal transduction pathways and is present in hypothalamic

nuclei (Malendowicz et al., 2006). It is unknown whether leptin can cross the blood-brain barrier to access receptor neurons, because the blood-brain barrier is somewhat absent in the area of the median eminence, close to where the Neuropeptide Y (NPY) neurons of the arcuate nucleus. It is generally thought that leptin might enter the brain at the choroid plexus, where there is intense expression of a form of leptin receptor molecule that could act as a transport mechanism.

Once leptin has bound to the Ob-Rb receptor, it activates the stat3, which is phosphorylated and travels to the nucleus to, presumably, effect changes in gene expression. One of the main effects on gene expression is the down-regulation of the expression of endocannabinoids, responsible for increasing appetite. There are other intracellular pathways activated by leptin, but less is known about how they function in this system. In response to leptin, receptor neurons have been shown to remodel themselves, changing the number and types of synapses that fire onto them (Margetic et al., 2002).

Rechberger et al., (1999) investigated serum leptin concentrations in women taking OCs containing the same OCs. Thirty women taken 20 microg of EE and 150 microg of desogestrel, second group of 30 women received 30 microg of EE and 150 microg of desogestrel. Serum leptin concentrations after the first day of the cycle prior to the onset of therapy as well as after the 3rd and 6th treated cycles were measured. In both groups a positive correlation between serum leptin and BMI was found ($r=0.56$; $P<0.001$ and $r=0.67$; $P<0.001$). The initial serum leptin concentration in the first group was 7.62+/-8.46 ng/ml. This value was not statistically different from values after 3 months (9.31 +/-8.23 ng/ml) and after 6 months (10.53+/-8.03 ng/ml) of treatment. Very similar results were found in patients of second group: 8.81+/-6.56 ng/ml initially; 11.62+/-11.16 ng/ml at 3 months, and 10.38+/-7.32 ng/ml at 6 months.

2.3.7 Other side effects

Some other important OCs side effects have been also reported by different sources (Women's Health, 2004; Birth Control Pills Advantages and Disadvantages, 2007; National Women's Health Resource Center Inc, 2009) which are:

- Migraines and stroke: Women who take OCs and have a history of migraines have an increased risk of stroke.
- Headaches: Headaches may start in women who have not previously had headaches.
- Depression: Depression (sometimes severe) and other mood changes may occur.
- Nausea and vomiting.
- Bleeding or spotting: Spotting or bleeding between menstrual periods is very common in the first cycle of pills.
- Chloasma (spotty darkening of the skin on the face): Darkening of the skin on the upper lip, under the eyes, or on the forehead (chloasma).
- Diarrhea or vomiting: Anything that makes the pill go through your system too fast can make the pill not work as well because it was not absorbed or, worse, if it is lost in the vomit
- Worsen severe diabetes: The estrogen in OCs may increase glucose levels and decrease the body's insulin response, while the progesterone in the pills may encourage overproduction of insulin.
- Possible acceleration of gallbladder disease. Estrogen may cause bile to become oversaturated with cholesterol, which can lead to gallstones.
- No decreased risk of sexually transmitted infections. Oral contraceptives do not protect against sexually transmitted diseases.
- Cancer of the breast or reproductive tract, liver problems or cancer, hyperbilirubinemia.

Chapter 3

Materials and Methods

3.1 Study design: The study design was a case control.

3.2 Target population: The target population is healthy women aged 20-35years in Gaza City.

3.3 Setting of the study: The present study was carried out on women attending the Swidey family control clinic in Gaza City.

3.4 Sample size: The experimental sample size was 80 healthy married women aged 20-35 years from the Swidey Clinic who had taken OC pills for at least three continuous cycles. The control sample was healthy married women who were not going on OC before and match the experimental sample in age and residence.

3.5 Tools of the study: The questionnaire included issues about the following information: age, gender, weight, height, health history, blood pressure, nature of menstrual cycle, bleeding, insomnia, pain in the stomach, difficulty in breath, pain in hands and feet, appetite and headaches.

3.6 Exclusion and inclusion criteria

3.6.1 Exclusion criteria

1- Nursing mother
2- Going on regime
3- Going on exercise
4- Diabetic woman
5- Any woman taking OC less than three frequent menstrual cycle (MC).

3.6.2 Inclusion criteria

1- Married woman aged 20-35 years who are living in Gaza City.
2- Any woman going on OC three cycles or more.

3.7 Blood sampling and processing: About 6 ml fasting blood were collected from each women in the study sample. About 4 ml of the blood were collected in a tube without anticoagulation. Then, serum samples were be obtained by centrifugation at 3000 rpm for 20 min. The separated serum was be divided into two plastic tubes. One sample was stored at 2-5˚C for no more than 24 hours prior to blood parameters analysis, and the other was stored at -70˚C for leptin determination. The remainder quantity of the blood (2ml) was placed into EDTA tube to perform CBC test, using Cell-Dyn 1800.

3.8 Blood parameters analysis: Blood parameters analysis including CBC, CRP, HDL-C, LDL-C, cholesterol and TAG were carried out using a commercially available diagnostic system test kits (Friedewald et al., 1972; Deeg and Ziegenhorn, 1983).

Calculation of colorimetric tests for CBC, CRP, cholesterol, TAG were performed by the autoanalyzer automatically according to beer's law after calibration and adjustment of the photometers against water blank using a specific program of every test inserted to the instrument.

The concentration of colorimetric test = $\frac{A_{Test} \times C_{CALS}}{A_{CALS}}$

Serum high density lipoprotein cholesterol (HDL-C) was determined spectrophotometrically and then low density lipoprotein cholesterol (LDL-C) value were determined, using specific formula (shown in page 19).

3.8.1 Determination of serum cholesterol

Serum cholesterol was determined by cholesterol oxidase (COD)/POD method (Meiatlini et al., 1978) using BioSystems kit, Spain.

Principle

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured photometrically.

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol Esterase}}$ Cholesterol + Fatty acid

Cholesterol + 1/2 O_2 + H_2O $\xrightarrow{\text{COD}}$ Cholestenone + H_2O_2

2 H_2O_2 + 4-Aminoantipyrine + Phenol $\xrightarrow{\text{POD}}$ Quinoneimine + 4 H_2O

Reagents

Reagent	Component	Concentration
Reagent 1	Pipes	35 mmol/L
	Sodium cholate	0.5 mmol/L
	Phenol	28 mmol/L
	Cholesterol esterase	> 0. 2 U/mL
	COD	> 0.1 U/mL
	POD	> 0.8 U/mL
	4-ammoantipyrine	0.5 mmol/L
	pH	7.0

Procedure

Half ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	140
Serum volume (µl)	2
Calibrator 1 (mg/dl)	0.0
Calibrator 2 CALS (mg/dl)	236
Incubation time (s)	240
Wavelength (nm)	510
Calibrator type	Linear
Measurement Type	End point

Calculation:

The concentration of cholesterol test = $\frac{\text{(A) Test x (C) Standard}}{\text{(A) Standard}}$

Reference value:

Cholesterol < 200 mg/dl

3.8.2 Determination of serum triacylglycerol

Serum triacylglycerol was determined by Glycerol phosphate oxidase/peroxidase method (Bucolo and David,1973). using BioSystems kit, Spain.

Principle

Triacylglycerol in the sample were determined by means of the coupled reactions described below and colored complex formed was measured photometrically

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol - 3 - P} + \text{ADP}$$

$$\text{Glycerol - 3 -P} + O_2 \xrightarrow{\text{G-3-P-oxidase}} \text{Dihydroxyacetone - P} + H_2O$$

$$2H_2O_2 + \text{4- Aminoantipyrine} + \text{4-Chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_20$$

Reagents

Reagent	Component	Concentration
Reagent 1	Pipes	45 mmol/L
	Magnesium chloride	5 mmol/L
	4-chlorophenol	6 mmol/L
	Lipase	> 100 U/mL
	Glycerol kinase	> 1.5 U/mL
	Glycerol-3-phosphate oxidase	> 4 U/mL
	Peroxidase	> 0.8 U/mL
	A-aminoantipyrine	0.75 mmol/L
	ATP	0.9 mmol/L
	pH	7.0

Procedure

Half ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	140
Serum volume (µl)	2
Calibrator 1 (mg/dl)	0.0
Calibrator 2 CALS (mg/dl)	157
Incubation time (s)	240
Wavelength (nm)	510
Calibrator type	Linear
Measurement Type	End point

Calculation:

The concentration of Triacylglycerol test = $\frac{\text{(A) Test x (C) Standard}}{\text{(A) Standard}}$

Reference value:

Triacylglycerol < 150 mg/dl

3.8.3 Determination of serum high density lipoproteins

HDL-C was determined by precipitating method (Grove, 1979) using Lab kit, Spain.

Principle

The VLDL and LDL-C from serum or plasma were precipitated by phosphotungstate in the presence of magnesium ions. After removed by centrifugation the clear supernatant contained high density lipoproteins (HDL-C) and used for its determination.

Reagents

Reagent	Component	Concentration
Reagent 1	Phosphotungstic acid Magnesium chloride	14 mmol/L 2 mmol/L

Procedure

1. Centrifuge tube 25 µl of HDL-C reagent and 250 µl serum, were mixed well and were allowed to stand for 10 minutes at room temperature.

2. The mixture were centrifuged at 4000 rpm for 10 minutes then the supernatant and test HDL-C were collected.

3. Centrifuge tube 1 ml cholesterol reagent and 10 µl of the supernatant, were mixed

well and were allowed to stand for 10 minutes at room temperature.

4. The Unicam spectrophotometer United Kingdom, was Seted, at 505 nm and adjust it to zero with blank reagent. The absorbance (A) of the test was readed, and standard against reagent blank.

Calculation:

$$\text{HDL Concentration} = \frac{\text{(A) Test} \times \text{(C) Standard}}{\text{(A) Standard}}$$

Reference value:

HDL-C 40-60 mg/dl

3.8.4 Determination of serum low density lipoproteins

They were calculated using the empirical relationship (Friedewald, 1972)

Principle

The ultracentrifugal measurement of LDL-C was time consuming and expensive and requires special equipment. For this reason, LDL-C was most commonly estimated from quantitative measurements of total and HDL-cholesterol and plasma triacylglycerol (TAG) using the empirical relationship of Friedewald.

The Equation:

$$\text{LDL-C} = \text{Total Cholesterol} - \text{HDL-C} - \text{TAG}/5$$

Reference value:

LDL-C <130 mg/dl

3.8.5 Determination of serum C- reactive protein

Principle

CRP latex was used for determination of CRP in human serum (Generic assay Gmbh, 2009).

The CRP reagent kit was based on an immunological reaction between CRP antisera bound to biological inert latex particle and CRP in the test specimen. When serum containing greater than 0.8 mg/dL CRP was mixed with the latex reagent , visible agglutation occurs.

Materials required

Parameter
Timer
Test tubes and rack
Serological pipettes
High intensity light
Glycine saline buffer (alternatively PBS)
Rocking shaker (optional)

Assay procedure

Qualitative evaluation

1. All reagents and samples were allowed to reach room temperature prior to testing then they were shaked well before use.
2. One drop (appr 40 µl) of the positive control (P) was placed on field no.1 of the agglutination slide.
3. One drop (appr 40 µl) of the negative control (N) was placed on field no.2 of the agglutination slide.

4. 40 µl of each undiluted patient sample was placed to the following field on the agglutination slide were used different serological pipettes.
5. Gently resuspend the CRP latex reagent (A) and a 1 drop (40 µl) was dded to each test field.
6. Mixed well with used separate stirring sticks.
7. The slide was rocked for 2 minutes by hand or used a rocking shaker (80-100rpm).
8. Immediately under direct light was readed.

Evaluation of results

Positive

A positive reaction was indicated by any observable agglutination in the reaction mixture. The specimen reaction was compared to the CRP negative control.

Negative

A negative reaction was indicated by a uniform milky suspension with no agglutination as observed with the CRP negative control.

3.8.6 Determination of serum leptin

Determination of human serum leptin level was carried out by competitive enzyme immunoassay (Diagnostic System Laboratories (DSL). USA) technique.

Principle

The DSL-10-23100 ACTIVE Human Leptin ELISA is an enzymatically amplified "two-step" sandwich-type immunoassay. In the assay, standards, controls and unknown serum or plasma samples were incubated in microtitration wells, which have been coated with anti-human leptin antibody. After incubation and washing, the wells were treated with another anti-human leptin detection antibody labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells were incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured was directly proportional to the concentration of

human leptin present. A set of human leptin standards was used to plot a standard curve of absorbance versus human leptin concentration from which the human leptin concentrations in the sample was calculated.

The assay procedure sheets are available with the kit, the application of assay procedure mentioned below.

Assay procedure

All specimens and reagents were allowed to reach room temperature (~25°C) and mix thoroughly by gentle inversion before use. Standards, controls and samples should be assayed in duplicate.

1. The microtitration strips were marked to be used.

2.Twenty five microlitters of the standards, controls and samples were pipeted into the appropriate wells.

3. One hundred microlitters of the assay buffer E were added to each well using a semi-automatic dispenser.

4. Incubated the wells, shaked at a fast speed (500-700 rpm) on an orbital microplate shaker, at room temperature (~25 °C) for 2 hours.

5. Aspirated and washed each well 5 times with the wash solution using an automatic microplate washer. Bloted dry by inverting plate on absorbent material.

6. The antibody-enzyme conjugate solution was prepared by diluting the antibody-enzyme conjugate concentrate in the assay buffer.

7. One hundred microlitters of the antibody-enzyme conjugate solution was added to each well using a semi-automatic dispenser.

8. The wells were incubated, shacked at a fast speed (500-700 rpm) on an orbital microplate shaker, at room temperature (~25 °C) for 1 hour.

9. Aspirated and washed each well 5 times with the wash solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.

10. One hundred microlitters of the TMB chromogen solution was added to each well using a semi-automatic dispenser.

11. Incubated the wells, shaked at a fast speed (500-700 rpm) on an orbital microplate shaker, at room temperature (~25°C) for 10 minutes. Avoid exposure to direct sunlight.

12. One hundred microlitters of the stopping solution (0.2M sulfuric acid) was added to each well using a semi-automatic dispenser.

13. The absorbance of the solution in the wells was read within 30 minutes, using a microplate reader set to 450 nm.

Calculation

A. The mean absorbance for each standard, control and samples were calculated.

B. Plot the log of the human leptin concentrations in ng/mL along the x-axis versus the mean absorbance readings for each of the standards along the y-axis versus, using a linear curve-fit. Alternatively, the data can be plotted linear vs. linear and a smoothed spine curve-fit can be used.

C. Determine the human leptin concentrations of the controls and samples from the standard curve by matching their mean absorbance readings with the corresponding human leptin concentrations.

3.9 Pilot study

Pilot study on 10 women who had gone on OCs regimen for at least three cycles was carried out prior the beginning of data collection in order to test the validity and reliability of the questionnaire used. Comprehensive revision to it was made and modified as necessary.

3.10 Statistical analysis

The obtained data were analyzed by using a software statistical package for the social science (SPSS version 13). Frequency and descriptive analyses were used to describe the data. Chi square and Pearson correlation coefficient were used to test the relationship between the variables. Student t-test was also used to differentiate

between two numerical data. Any difference or correlation was considered significant if p value less than 5 %.

Chapter 4

Results

The present study was a case control and included 160 women (80 controls and 80 cases). The average age ± SD of the control women was 29.8± 4.8 years whereas that of the cases was 29.3± 4.6 years. The control women also matched the cases in the residence.

4.1 Side effects of oral contraceptives

Table1 shows that 96.3% of the cases had regular MC, whereas 3.8% of them had irregular one. On the other hand, 83.8% of the controls had regular MC, and 16.3% of them had irregular one. The difference among both groups with respect to nature of MC was statistically significantly (χ^2=5.371, P= 0.024).

Table 1: Nature of menstrual cycle of the study population (n=160)

Parameter	Case **(n=80)**		control **(n=80)**	
	Regular	**Irregular**	**Regular**	**Irregular**
Frequency	77	3	67	13
Percentage	96.3	3.8	83.8	16.3
χ^2	5.371			
P value	0.024			

Table 2 shows that 95.0% of the cases and 100% of the controls did not suffer from bleeding. The difference in frequency of bleeding among both groups was not statistically significantly (χ^2=0.192, P=0.135).

Table 2: Frequency of bleeding among the study population (n=160)

Parameter	Case **(n=80)**			Control **(n=80)**		
	Yes	**No**	**Sometimes**	**Yes**	**No**	**Sometimes**
Frequency	0	76	4	0	80	0
Percentage	0	95.0	5	0	100	0
χ^2	0.192					
P value	0.135					

Table 3 shows that 6.3% of the cases sometimes suffer from insomnia, and 5.0% of them suffered from it. On the other hand, 2.5% of the controls frequently or sometimes suffered from insomnia. The difference in insomnia level among the study population was not statistically significant (χ^2=0.411, P= 0.353).

Table 3: Frequency of insomnia among the study population (n=160)

Parameter	Case **(n=80)**			Control **(n=80)**		
	Yes	**No**	**Sometimes**	**Yes**	**No**	**Sometimes**
Frequency	4	71	5	2	76	2
Percentage	5.0	88.7	6.3	2.5	95.0	2.5
χ^2	0.411					
P value	0.353					

Table 4 points out that 90.0% and 93.8% of the cases and the controls did not suffer from pain in the stomach, respectively (χ^2=0.386, P= 0.183).

Table 4: Frequency of pain in the stomach among the study population (n=160)

Parameter	Case (n=80)			Control (n=80)		
	Yes	**No**	**Sometimes**	**Yes**	**No**	**Sometimes**
Frequency	3	72	5	2	75	3
Percentage	3.8	90.0	6.3	2.5	93.8	3.8
χ^2	0.386					
P value	0.183					

Table 5 illustrates that 91.3% and 95.0 % of the cases and the controls did not suffer from difficulty in breathing, respectively (χ^2=0.497, P= 0.209).

Table 5: Frequency of difficulty in breathing among the study population (n=160)

Parameter	Case (n=80)			Control (n=80)		
	Yes	**No**	**Sometimes**	**Yes**	**No**	**Sometimes**
Frequency	3	73	4	1	76	3
Percentage	3.8	91.3	5.0	1.3	95.0	3.8
χ^2	0.497					
P value	0.209					

Table 6 points out that 78.8% and 90.0 % of the cases and the controls, respectively did not suffer from pain in hands and feet (χ^2=0.631, P= 0.309).

Table 6: Frequency of pain in hands and feet among the study population (n=160)

Parameter	Case (n=80)			Control (n=80)		
	Yes	**No**	**Sometimes**	**Yes**	**No**	**Sometimes**
Frequency	11	63	6	6	72	2
Percentage	13.8	78.8	7.5	7.5	90.0	2.5
χ^2	0.631					
P value	0.309					

Table 7 shows that 16.3 % of the cases had an increase in their appetite compared to 2.5 % of controls. The difference among the two group was statistically significant (χ^2= 4.386, P= 0.002), indicating that Ocs increase appetites.

Table 7: Frequency of increasing of appetite among the study population (n=160)

Parameter	Case (n=80)			Controls(n=80)		
	Yes	**No**	**Sometimes**	**Yes**	**No**	**Sometimes**
Frequency	13	59	8	2	75	3
Percentage	16.3	73.8	10.0	2.5	93.8	3.8
χ^2	4.386					
P value	0.002					

Table 8 illustrates that 23.8 % of the cases suffered from headaches versus 10.0 % of the controls. The difference among the two groups was statistically significant (χ^2= 6.82, P= 0.000), indicating that OCs contributes to headaches.

Table 8: Frequency of headache among the study population (n=160)

Parameter	Case (n=80)			Control (n=80)		
	Yes	**No**	**Sometimes**	**Yes**	**No**	**Sometimes**
Frequency	19	49	12	8	68	4
Percentage	23.8	61.3	15.0	10.0	85	5.0
χ^2	6.82					
P value	0.000					

The body mass index values of the cases and the controls are illustrated in Table 9. The percent of normal, overweight and obese of the cases were 22.5, 37.5 and 40.0% whereas among the controls the percentage were 13.8, 62.5 and 22.5% (χ2= 7.31, P= 0.015).

Table 9: Body mass index of the study population (n=160)

BMI	Case n (%)	Control n (%)	P value
Normal	18 (22.5)	12 (13.8)	
Overweight	30 (37.5)	50 (62.5)	χ^2 =7.31
Obese	32 (40.0)	18 (22.5)	**P** =0.015
Total	**n**=80	**N**=80	

People with BMI=18.5–24.9 were considered to have normal weight, people with BMI=25.0–29.9 were classified overweight, people with BMI≥30.0 were considered obese (WHO, 2000).

The systolic blood pressure (SBp) among the cases and the controls are illustrated in Table 10. The percent of cases with normal, low and high Bp were 53.8, 0.0 and 46.3% whereas the percentage among the controls were 51.3, 2.6 and 46.3%, respectively. The differences between the groups were not statistically significant (χ^2= 2.351, P= 0.139).

Table 10: Systolic blood pressures among the study population (n=160)

systolic blood pressures (mmHg)	Case n (%)	Control n (%)	P value
Normal blood pressure	43 (53.8)	41 (51.3)	χ^2 =2.351 **P** =0.139
Low Blood pressure	0 (0.0)	2 (2.5)	
High Blood pressure	37 (46.3)	37 (46.3)	
Total	**N**=80	**N**=80	

People with systolic blood pressure (SBp)= 110-130 mmHg were considered to have normal Bp, People with SBp > 130 mmHg considered to have high Bp, People with SBp < 110 mmHg considered to have low Bp (Disabled World, 2008).

The diastolic blood pressures (DBp) among cases and the controls are illustrated in Table 11. The percent of cases with normal, low and high DBp were 88.8, 5.5 and 6.3 whereas the percentages among the controls were 88.8, 6.3 and 5.1, respectively (χ^2= 1.372, P= 0.382).

Table 11: Diastolic blood pressure among the study population (n=160)

Diastolic blood pressures (mmHg)	Case n (%)	Control n (%)	P value
Normal blood pressure	71 (88.8)	71 (88.8)	χ^2 =1.372 **P** =0.382
Low Blood pressure	4 (5.5)	5 (6.3)	
High Blood pressure	5 (6.3)	4 (5.1)	

People with diastolic blood pressure (DBp)= 75-85 mmHg were considered to have normal Bp, People with DBp > 85 mmHg considered to have high Bp, People with SBp < 75 mmHg considered to have low Bp (Disabled World, 2008).

4.2 Some biochemical parameters and oral contraceptives

4.2.1 C- reactive protein

Table 12 shows that 13.8% of the cases had positive CRP test compared to 5.0% of the controls. The difference among the two groups was statistically significant (χ^2= 5.381, p= 0.034).

Table 12: C-reactive protein test among the study population

Parameter	Case (n=80)		Control (n=80)	
	Positive	**Negative**	**Positive**	**Negative**
Frequency	11	69	4	67
Percentage	13.8	86.3	5.0	95.0
χ^2	5.381			
P value	0.034			

4.2.2 Lipid profile

As depicted from Table 13, the average levels ± SE of serum cholesterol, and LDL-C were significantly higher among the cases (179.1± 4.3, and 97.6±4.0 mg/dl) compared to the controls (157.5± 4.1 and 86.2± 3.4 mg/dl). The differences among the study population with respect to cholesterol level were very significant (p= 0.000 and p= 0.002, respectively). The mean values ± SE of HDL-C and TAG slightly increased among the cases (51.2±1.7 and 119.2± 6.0 mg/dl) compared to the controls (47.6±1.3 and 108.2± 8.6 mg/dl). These changes in HDL-c and TAG among the study population were not significant (p=0.148 and p=0.218, respectively).

Table 13: Lipid profile among the study population (n=160)

Lipid Profile (mg/dl)	Case (n=80)		Control (n=80)		T	P value
	Mean	S.E	Mean	S.E		
Cholesterol	179.1	4.3	157.5	4.1	3.647	0.000
HDL-C	51.2	1.7	47.6	1.3	1.700	0.148
LDL-C	97.6	3.8	86.2	3.4	2.238	0.002
Triglycerides	119.2	6.0	108.2	8.6	1.047	0.218

S.E: standard error, HDL-c: high density lipoprotein cholesterol, LDL-c: low density lipoprotein cholesterol. Reference range: cholesterol<200 mg/dl, LDL-C<130 mg/dl, triglyceride 90-150 mg/dl (Mark Cichocki, 2007), HDL-C=40-60 mg/dl (Richard and Fogoros, 2009).

4.2.3 Leptin analysis

Table 14 shows average ± SE serum leptin level among the study population. There was a significant increase in the mean level of leptin among the cases compared to the controls (36.3± 2.3 ng/ml vs. 28.6± 2.1 ng/ml, and p= 0.003).

Table 14: Serum leptin level among the study population (n=160)

Parameter (units)	Case (n=80)		Control (n=80)		T	P value
	Mean	S.E	Mean	S.E		
Leptin (ng/ml)	36.3	2.3	28.6	2.1	2.459	0.003

S.E: standard error, T: t-test.

4.2.4 Lipid profile, BMI and leptin

Table 15 shows a positive correlation between leptin level and BMI. This correlation was statistically significant ((r=0.445, p=0.000). On the other hand no significant corrrelations were observed between leptin and cholesterol or HDL-C or LDL-C or TAG (p=0.843, p=0.300, p= 0.252 and p=0.397, respectively).

Table 15: The correlation between leptin and lipid profile of the group study

Parameter	Leptin	
	Pearson correlation (r)	**P-value**
Cholesterol (mg/dl)	-0.023	0.843
HDL-C (mg/dl)	0.177	0.300
LDL-C (mg/dl)	-0.129	0.252
Triacylglycerol (mg/dl)	0.096	0.397
BMI	0.445	0.000

4.3 Complete blood count analysis

The tested CBC parameters are illustrated in the table 16. The average ± SE levels of WBC, Gran and Mch were significantly higher among the cases (7.2±2.1 K/µL, 60.7±8.3 % and 26.9±2.2pg) compared to the controls (6.6±1.7 K/µL, 54.9±11.7 % and 25.7±3.433 pg) with p=0.001, p=0.000 and p=0.003, respectively. The mean ± SE of Lymph and RDW were decreased among the cases (32.4±7.3% and 13.4±1.2%) compared to the controls (36.3±7.8% and 14.2±1.5%). These changes were also highly significant (p=0.002 and p=0.001). However, the changes among the study population in Mid, RBc, Hb, Hct, Mcv, Mchc and PLt were not significant.

Table 16: Screening of blood count parameters among the study population (n=160)

Blood parameter	Case		Control		T	P value
	Mean	SD	Mean	SD		
WBC (K/µL)	7.2	2.1	6.6	1.7	2.065	0.001
Lymph (%)	32.4	7.3	36.3	7.8	3.233	0.002
Mid (%)	7.8	8.7	8.6	6.6	0.659	0.352
Gran (%)	60.7	8.3	54.9	11.7	3.580	0.000
RBC (M/µL)	4.6	0.7	4.6	0.4	0.558	0.394
Hb (g/dL)	12.2	0.9	12.3	1.1	0.457	0.387
Hct (%)	38.0	2.49	37.5	3.7	0.967	0.571
MCV (fL)	80.8	12.0	80.6	8.5	0.094	0.415
MCH (pg)	26.9	2.29	25.7	3.4	2.542	0.003
MCHC (g/dl)	32.5	1.1	32.3	1.7	0.852	0.272
RDW (%)	13.4	1.2	14.2	1.5	3.681	0.001
PLt (K/µL)	279.3	87.6	267.8	74.0	0.899	0.185

WBC: White blood count; Lymph: Lymphocytes; Gran: granulocytes, types of white blood cells; MID cells include less frequently occurring and rare cells correlating to monocytes, basophils: blasts and other precursor white cells; RBC: Red blood cells; Hb. The hemoglobin; HCT: Hematocrit MCV: mean cell volume; MCH: mean cell hemoglobin; and MCHC: mean cell hemoglobin concentration; RDW. Red cell distribution width; PLt: The platelet count.

Chapter 5

Discussion

Oral contraceptives provide highly reliable contraceptive protection. Even though imperfect use (skipping an occasional pill) is considered, the OCs are still very effective in preventing pregnancy. This case control study is the first one to determine some risk factors of having OCs among healthy women in Gaza City.

5.1 Oral contraceptives and nature of menstrual cycle

The study showed that OCs increased regularity of MC. This finding was very consistent with other studies which showed that OCs are helpful for women periods that come too often or too late. Menstrual periods tend to be lighter and shorter with OCs intake and the timing of a period can be controlled (Women's Health, 2009). Regular MC and less blood loss is helpful in preventing anemia.

5.2 Oral contraceptives and an increase of appetite

The results showed that continuous OCs intake as anabolic hormone lead to an increase in appetite where there was significant difference among both study groups with respect to this parameter and high BMI. Thus, an increase in appetite led to an increase in body weight. It was shown that both estrogen and progesterone present in OC pills can cause fluids and salt (sodium) retention (Crystal, 2005).

5.3 Oral contraceptives and headache

The findings showed that there was significant difference with respect to feeling of headache among both study groups. Thus, frequent use of steroid hormones could cause headaches. The exact relationship between OCs and headache is unclear (Hormonal Headaches, 2010). This finding was consistent with other studies which showed that most women who were went on OCs suffer from migraines headaches (Wilson, 2006).

In addition, this finding was in agreement with other studies showed that OCs caused headache activity among most women during early cycles of OCs use and disappeared with continued use (Loder et al., 2005).

5.4 Oral contraceptives and body weight

The results showed that significant difference among both study groups with respect to BMI. Body mass index is an indirect method to determine body fat that accumulates in the body if energy input was higher than energy output. These findings were very consistent with the finding that OCs intake increased an appetite and abnormal high concentrations of serum lipids among women (Reubinoff et al., 1995). A survey of women in the United Kingdom reported that nearly three in four believed that weight gain was associated with oral contraceptive use. A study of US adolescents found that, overall, 45% were very concerned about the potential for weight gain while taking OCs (86% among suburban teens). A similar survey of Canadian teens reported more than half had heard that OC use causes weight gain. (The Contraception Report, 2001).Weight gain might be also related to a reduction in physical activity. Progesterone, however, in the OCs causes an increase in an appetite, and fat deposits. Nevertheless, obese women should look for another method of contraceptive.

5.5 Oral contraceptives and lipid profile

The results showed that there were significant differences among both study groups with respect to total cholesterol and LDL-c levels in the blood. However, a sgnificance difference among both groups with respect to TAG level in the blood was not observed. This finding about the effect of OCs on cholesterol have been reported before (Parks et al., 1989). Thus, women who are going on OCs regime are more vulnerable to suffer from cardiovascular diseases such as heart attack, stroke, atherosclerosis etc. (Birth Control Pills Advantages and Disadvantages, 2007). However other studies emphasized that the chances of OCs contributing to a heart attack are small unless you smoke (Women's Health, 2004). Doar et al., (1969) reported that OCs were associated with significantly raised mean serum TAG and TC levels. In addition, Hennekens et al., (1979) reported that, the mean level of fasting TAG was higher among current OC users than among

nonusers. After adjustment for the possible confounding effects of age, weight, current cigarette smoking and fasting glucose level, current OC users still had higher TAG blood level than that of nonusers.

Moreover, Parks et al., (1989) observed that OC pills increased TC and TAG concentrations in the blood. Statistically significant increases were observed for TAG, LDL-c, and VLDL-c with the duration of OCs use (Emokpae et al., 2010).

5.6 Oral contraceptive and blood pressure:

The study showed that readings of SBp and DBp remained within the normal range among OCs women. It seemed that an increase in LDLs level among the study group could not lead to precipitation of cholesterol on artery walls. Early results from a controlled long-term prospective study have shown no significant change in mean systolic or diastolic pressures or mean weight in women after four months on OCs. However, there was a significant change in Bp over the short period of the experiment (Weir et al., 1969).In contrast to the present finding, Calvin et al., (1969) revealed slightly higher and statistically significant mean SBp and DBp in women using OCs, after correction for age, height, weight, and arm circumference. One year later, they showed little tendency for Bp to be affected by OCs. Moreover, Fisch et al., (1977) study showed rise in mean Bp. The age-adjusted proportion of OCs users with a Bp over 140/90 mm Hg was about three times that of nonusers. This effect is possibly mediated by the estrogen-induced activation of the renin-angiotensin system. Oral contraceptives also appear to increase the tubular responsiveness to changes in sodium intake. This indicated that synthetic sex steroids have a significant impact on renal function in women (Bertschi et al., 2003). These results should not prevent continuous monitoring of BP for several months after the woman start taking OCs.

5.7 Oral contraceptives and serum leptin level

The results showed that there were significant difference among both study groups with respect to leptin. Body fat is responsible for release of leptin. Positive correlation between serum leptin and BMI was also found Thus, intake increased an appetite and thus, body

fat and leptin level. In contrast to out results, statistical analysis of other study, did not reveal any significant difference at each investigated time point in either study group with respect to serum leptin or BMI (Rechberger et al., 1999). This contradiction between both studies could be attributed to type of OCs pills or sample size used.

5.8 Oral contraceptives and C- reactive protein

C-reactive protein (CRP) increases when blood vessels become inflamed. According to the present results, there was significant difference among both study groups with respect of CRP. It seemed that OCs affected liver functions in such a way which leaded to an increase in C-reactive protein blood level. This finding was consistent with recent cross-sectional survey which demonstrated that CRP levels are significantly increased among OC users versus non-users (Krupa., 2003). Other studies showed that more than 50 % of apparently healthy women taking OCs had CRP levels >3 mg/l (Rietzschel et al., 2007). These findings indicated that OC pills could cause high-risk of inflammation and thus, could activate immune response.

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5.9 Oral contraceptives and complete blood count

5.9.1 Oral contraceptives and white blood cells

The results showed significant increase in WBC of cases compared to controls. This increase reflected that OCs could cause some type of internal infection among the women. Moreover, the increase in Gran (%) among the study group could reflect this type of infection to be bacterial. In contrast, the case group showed a significant decrease in lymphocytes and this contradiction requires further investigation.

This finding was consistent with an other study which showed that smoking and OCs use are factors that alter WBC count among healthy women. However, a total leukocyte count greater than 10,000/cu mm was found in 44% of obese, heavily smoking women who took OCs as compared to 2% of women without these attributes (Fisch and Freedan, 1975).

5.9.2 Oral contraceptive and mean cell hemoglobin

The finding showed that there was significant differences among study groups with respect to Mch and RDW. An increase in MCH could reflect anemia of chronic diseases. In contrast, the slight decrease in RDW could reflect change in internal iron level among the cases that also requires further investigation.

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

- Frequent use of OCs could cause regulation of menstrual cycle, increase appetite, feeling of headaches as well as increases in BMI, cholesterol, LDL-C, and Leptin.
- Frequent use of OCs could not affect bleeding, frequency of insomnia, stomachache, difficulty in breathing, frequency of pain in hands and feet, SBp, DBp, HDL-c , and TAG.
- There was strong correlation between BMI and leptin among the case study .
- Frequent use of OCs could cause significant increase in WBC, Gran, and MCH among the study population.

6.2 Recommendations

- The risk factors of OCs intake such as increase in appetite, body fat composition, blood fats and modifying immune system, should be taken in consideration and required integrated relevant interventions.
- More awareness sessions on health eating pattern for mothers who are going on OCs should be implemented in schools, audio-vision systems, and local organizations
- Monitoring body weight as well as biochemical examinations must be carried out monthly for every OCs woman.
- More deep and screening research studies about the effect of OCs intake on some other anabolic steroid hormones and heat generating hormones such as thyroid ones could be addressed and conducted by higher level of responsibility from Ministry of health (MOH) in cooperation with universities in Gaza strip.
- More research could be conducted on long term consequences of OC use.

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Annex 1

الجـــــــــامعة الإسلامية – غزة كلية العلوم
The Islamic University of Gaza
مدير برنامج ماجستير العلوم الحياتية

التاريخ/ 2009/4/12م...

الأخ/ د. محمد المقادمة مدير برنامج الصحة في وكالة الغوث حفظه الله ،،،

السلام عليكم ورحمة الله وبركاته ،،،

الموضوع / تسهيل مهمة باحثة

تشهد إدارة ماجستير العلوم الحياتية بالجامعة الإسلامية أن الطالبة: *إصلاح محمد أبوهاني* طالبة في ماجستير العلوم الحياتية تخصص – *تحاليل طبية* تقوم بإجراء البحث النهائي في برنامج الماجستير والذي بعنوان:

"بعض التغيرات البيوكيميائية للنساء اللواتي يتناولن حبوب منع الحمل في مدينة غزة"

الباحثة بحاجة لأخذ عينات دم من قسم تنظيم الأسرة في سويدي النصر لكي تتمكن من استكمال دراستها.

لذا نرجو من سيادتكم مساعدة الطالبة

ولكم منا جزيل الشكر والتقدير ،،،

مدير برنامج ماجستير العلوم الحياتية
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Annex 2

بسم الله الرحمن الرحيم

الأخوات العزيزات السلام عليكم ورحمة الله و بركاته

يهدف البحث إلى معرفة تأثير حبوب منع الحمل على صحة المرأة في مدينة غزة ، وعليه يتطلب البحث الإجابة على بعض الأسئلة القصيرة الموضحة في الإستبانة التالية ، و أخذ عينة دم بسيطة.

1. تاريخ سحب العينة			
2. الاسم			
3. العمر			
4. رقم الهاتف			
5. هل أنتِ مرضعة؟	نعم ( )	لا ( )	
6.هل أنتِ مصابة بمرض السكري	نعم ( )	لا ( )	
7. هل تستعملين رجيم غذائي	نعم ( )	لا ( )	
8. هل تمارسين الرياضة	نعم ( )	لا ( )	
9. هل تتناولين حبوب منع الحمل	نعم ( )	لا ( )	
10. منذ متى تتناولين حبوب منع الحمل			
11. الطول	..... سم		
12. الوزن	..... كيلو جرام		
13. مقدار ضغط الدم	.....		
14. دليل الوزن	.....		
15.طبيعة الدورة	منتظمة ( )	غير منتظمة ( )	
16. هل تعاني من نزيف	نعم ( )	لا ( )	أحيانا ( )
17.هل تعاني من الأرق	نعم ( )	لا ( )	أحيانا ( )

18. هل تعاني من ألم في المعدة	نعم ( )	لا ( )	أحيانا ( )
19. هل تعاني من ضيق في التنفس	نعم ( )	لا ( )	أحيانا ( )
20. هل تعاني من ألم في اليدين و القدمين	نعم ( )	لا ( )	أحيانا ( )
21. هل تعاني من انتفاخ في الأعين	نعم ( )	لا ( )	أحيانا ( )
22. هل تعاني من حكة في الصدر	نعم ( )	لا ( )	أحيانا ( )
23. هل تشعرين بالحمى منذ تناول حبوب منع الحمل	نعم ( )	لا ( )	أحيانا ( )
24. هل تشعرين بفتح الشهية	نعم ( )	لا ( )	أحيانا ( )
25. هل تعاني من أورام في الأرجل	نعم ( )	لا ( )	أحيانا ( )
26. هل تعاني من طفح جلدي	نعم ( )	لا ( )	أحيانا ( )
27. هل تعاني من التقيؤ	نعم ( )	لا ( )	أحيانا ( )
28. هل تعاني من الصداع	نعم ( )	لا ( )	أحيانا ( )

نتائـــج تحليـل عينة الدم

cholesterol	
HDL-c	
LDL-c	
TAG	
Leptin	
CBC	
CRP	

و إذا كنا قد جئنا إلى نهاية هذا البحث فإننا نرجو من الله سبحانه و تعالى أن يكون قد هدانا إلى ما يثبت الإيمان في القلوب و يسخر هذا العلم في خدمة الدين و الوطن و نرجو من الحق جل جلاله أن يتقبل منا، **إنه هو السميع العليم .**

و آخر دعوانا أن الحمد لله رب العالمين.

الباحثة

إصلاح محمد دياب أبو هاني