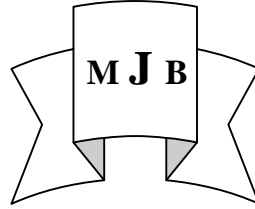# The Effect of Combined Oral Contraceptive Pills and Copper Bearing Intrauterine Contraceptive Devices on The Oxidative Stress, Lipid Profile and Some Trace Elements in Women Sera in Hilla City

Moaed E. Al-Gazally Melal M. Al-Jeborry Ghufran M. Al-Asadi
College of Medicine / University of Babylon, Hilla, Iraq.

M J B

Abstract

The study was conducted on sixty three healthy women used combined oral contraceptive pills (COCPs) and copper bearing intrauterine contraceptive devices (IUDs). Thirty women were used Microgynon [R] (low dose COCPs, each pill containing levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg) and thirty three women used copper T 380A IUDs. Thirty apparently healthy women were taken as control group. Blood samples obtained from women used contraceptives and control group from Babylon maternity and pediatric hospital in Hilla city. The sera obtained from the blood were used to determine the effect of COCPs and IUDs on of malondialdehyde (MDA), total cholesterol, high density lipoprotein (HDL), triglycerides (TGs), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and some trace elements (copper, zinc and iron) concentration.

The results of the present study showed (significant increase in MDA, copper, iron, total cholesterol, TGs, VLDL and LDL concentration), significant decrease in HDL and non significant decrease in zinc concentration in sera of women who used COCPs when compared to both women who used IUDs and the control group. Also this study showed non significant difference in MDA, copper, zinc, total cholesterol, HDL, TGs, VLDL and LDL concentration and significant decrease in iron concentration in sera of women who used IUDs when compared to those of the control group.

تأثير حبوب منع الحمل الفموية المركبة و أجهزة منع الحمل الرحمية الحاملة للنحاس على الاجهاد ألتأكسدي و صورة الدهون وبعض العناصر النزرة في مصول النساء في مدينة الحلة

الخلاصة

أجريت الدراسة على ثلاث وستين امرأة سوية استخدمن حبوب منع الحمل الفموية المركبة و أجهزة منع الحمل الرحمية الحاملة للنحاس. ثلاثون امرأة بعمر 20 -42 سنة استخدمن مايكروجينون (حبوب منع الحمل الفموية المركبة المنخفضة الجرعة, كل قرص يحتوي على ليفوجستريل 15, 0 ملغم وايثينايل استرادايول 03, 0 ملغم) وثلاث وثلاثون امرأة بعمر 21 -44 سنة اســتخدمن أجهزة منع الحمل الرحمية الحاملة للنحاس نوع T380. ثلاثون امرأة سوية بعمر 20 -45 سنة أخذت كمجموعة سيطرة. عينات الدم تم الحصول عليها من النساء المستخدمات لموانع الحمل ومجموعة السيطرة في مستشفى بابل للنسائية والاطفال فـــي مدينة الحلة. المصول تم الحصول عليها من الدم لقياس تأثير حبوب منع الحمل الفموية المركبة و أجهزة منع الحمل الرحميــة الحاملة للنحاس على تركيز مالون ثنائي الالدهايد و الكولسترول الكلي و البروتينات الدهنية عالية الكثافة و الكليسيريدات الثلاثية والبروتينات الدهنية واطئة الكثافة جدا و البروتينات الدهنية واطئة الكثافة وبعض العناصر النزرة (نحاس و خارصين وحديد). أظهرت نتائج هذه الدراسة (زيادة ملحوظة في تركيز مالون ثنائي الالدهايد والنحاس والحديد و الكولسترول الكلي و الكليسيريدات الثلاثية والبروتينات الدهنية واطئة الكثافة جدا و البروتينات الدهنية واطئة الكثافة) و نقصان ملحوظ في البروتينات الدهنية عالية الكثافة و نقصان غير ملحوظ في تركيز الخارصين في مصول النساء المستخدمات لحبوب منع الحمل الفموية المركبة عند مقارنتها

بكل من النساء المستخدمات لأجهزة منع الحمل الرحمية و مجموعة السيطرة. كذلك هذه الدراسة أظهرت اختلاف غير ملحوظ في تركيز مالون ثنائي الالدهايد والنحاس و الخارصين و الكولسترول الكلي و البروتينات الدهنية عالية الكثافة و الكليسيريدات الثلاثية والبروتينات الدهنية واطئة الكثافة جدا و البروتينات الدهنية واطئة الكثافة) و نقصان ملحوظ في تركيزالحديد في مصول النساء المستخدمات لأجهزة منع الحمل الرحمية عند مقارنتها بمجموعة السيطرة.

_____

Introduction

Contraception means the ability to control fertility by reliable artificial methods has transformed both social and epidemiological aspects of human reproduction [1]. 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 [2].

Combined oral contraceptive pills (COCPs) are medicines taken by mouth to prevent pregnancy by prevent ovulation by suppressing the release of gonadotropins (follicle-stimulating hormone and greatly luteinizing hormone) [3, 4]. COCPs are highly effective, reversible and popular. They also have noncontraceptive uses, including control of menstrual abnormalities, as these agents decrease blood loss, decrease the incidence of iron-deficiency anemia, decrease dysmenorrhea and decrease the incidence of endometrial and ovarian cancer [5].

COCPs are composed of an estrogens and a progestins. Estrogens involved in combined oral contraceptive pills includes ethinyl estradiol (EE_2) or mestranol. There are nine progestins in combined oral contraceptive pills which are norethindrone, norethindrone acetate, ethynodiol diacetate, levonorgestrel, norgestrel, lynestrenol, desogestrel, norgestimate and gestodene [6, 7].

An intrauterine device (IUDs) is a widely used method of birth control with 99% effectiveness. The IUD once inserted in the uterus is immediately effective as a contraceptive [8]. It is the most widely used reversible form of contraception in the world. Copper bearing IUDs is a small, T-shaped device made of polyethylene. The device has two flexible arms that fold down for insertion and expand to form a T shape when released inside the uterus. The copper bearing IUDs protects against pregnancy by reducing motility and viability of sperm, inhibiting ova development and thereby preventing fertilization [9].

Oxidative stress is defined as an imbalance between prooxidants and antioxidants in the cells which is manifested by elevated levels of free radicals [10]. The free radical mediated peroxidation of membrane lipids, increase membrane fluidity and permeability with loss of its integrity that lead to cell damage [11]. Alterations of these structures are associated with the development of several human pathologies including atherosclerosis, cardiovascular disease, cancer, diabetes complications and arthritis [12]. Malondialdehyde (MDA) is the end product of lipid peroxidation which is utilized as a marker of lipid peroxidation in states of elevated oxidative stress [13].

Trace elements (copper, zinc and iron) are essential nutrients for human beings. They are crucial for the functioning of several enzyme systems and are required in a number of metabolic processes in the body. They are involved in gene expression, RNA and DNA metabolism and cellular immune functions. Thus they are of fundamental importance in living organisms [14].

The major lipids present in the plasma are fatty acids, TGs, cholesterol

and phospholipids, are all transported in plasma as lipoprotein particles [chylomicrons (CM), very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL)] [15].

Materials and Methods
Subjects

This study was conducted over a period of 12 months starting from October 2009 till October 2010. All samples collected from Babylon maternity and pediatric hospital in Hilla city. The practical side of the study was performed at the laboratory of biochemistry department in college of medicine / Babylon University.

The general criteria for all subjects in this study includes all women within reproductive age, fertile, with regular menstrual cycle, not suffering from any disease (e.g. Hypertension, diabetes mellitus, asthma etc.), not lactating and not given any medication (e.g. diuretics, steroid, etc.). Any subjects have not this criteria are excluded from this study.

This study includes ninety three healthy women divided into three groups, the first group includes thirty healthy women aged 20-42 year with mean ± SD 32 ± 6.577 year were used low dose, monophasic, second generation COCPs (Microgynon)[R]. Each pill containing levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg. The women in this group used contraceptive pills regularly for 11-29 month with mean ± SD 18.7 ± 5.423 month, the second group includes thirty three healthy women aged 21-44 year with mean ± SD 31.242 ± 6. 494 year. The women in this group used copper bearing IUDs (Cu T 380A IUDs) for 13-34 month with mean ± SD 23.848 ± 6.764 and the third group includes thirty healthy women aged

20-45 year with mean ± SD 33.033 ± 6.655 year who not used low dose combined oral contraceptive pills or Cu T 380A IUDs (i.e. control group).

Blood Sampling

Venous blood samples were drawn from contraceptive users and control subjects by using disposable syringes (5mL) in the sitting position. Five ml of blood was obtained from each subject by vein puncture and pushed slowly into plain disposable tubes. Blood was allowed to clot at 37˚C for 10-15 minutes, and then centrifuged at 3000 rpm for approximately 10-15 minutes then the sera were obtained and stored at -20˚C until analysis (measure MDA, copper, zinc, iron, total cholesterol, HDL,TG, VLDL and LDL).

Methods

Serum MDA concentration are determined by Carl A. Burtis and Edward R. Ashwood procedure [16]. Serum copper concentration and serum zinc concentration are determined by Via melano (Italy) kit. Serum iron concentration are determined by Human (Germany) kit. Serum total cholesterol, TGs and HDL-cholesterol concentration are determined by Biolabo SA (France) kit. VLDL-cholesterol concentration was calculated by dividing triglycerides value by 2.22 [17]. LDL-cholesterol concentration was calculated by using Friedewald equation [18].

Results

MDA, copper, zinc, iron, total cholesterol, HDL,TG, VLDL and LDL concentration were measured in sera of thirty women used low dose combined oral contraceptive pills, thirty three women used copper bearing intrauterine devices and thirty healthy women as shown in table (1).

Table 1 Serum MDA, Copper, Zinc, Iron, Total Cholesterol, HDL, TG, VLDL and LDL Concentration in Combined Oral Contraceptive Pills Users, Intrauterine Devices Users and Control Group. [P-value of < 0.05 was considered to be statistically significant].

Parameter	Subjects	No.	Mean ± SD	Range
MDA (mmol/L)	COCPs Users	30	4.08 ± 0.72**	3.00-5.54
	IUDs Users	33	2.00 ± 0.41	1.39-2.82
	Control	30	1.88 ± 0.49	1.02-3.08
Copper (µmol/L)	COCPs Users	30	2.47 ± 0.44**	1.45-3.12
	IUDs Users	33	1.61 ± 0.32	1.16-2.53
	Control	30	1.58 ± 0.27	0.97-2.10
Zinc (µmol/L)	COCPs Users	30	8.99 ± 1.45	6.71-13.39
	IUDs Users	33	9.18 ± 1.61	6.41-14.3
	Control	30	9.46 ± 1.17	7.33-11.95
Iron (µmol/L)	COCPs Users	30	17.05 ± 4.66***	8.88-25.57
	IUDs Users	33	9.69 ± 3.97	3.14-19.50
	Control	30	12.78 ± 3.30	7.24-21.76
Total Cholesterol (mmol/L)	COCPs Users	30	4.80 ± 0.53**	3.81 – 5.83
	IUDs Users	33	3.77 ± 0.62	2.28 – 4.90
	Control	30	4.00 ± 0.54	2.66 – 4.91
HDL Cholesterol (mmol/L)	COCPs Users	30	0.98 ± 0.24*****	0.50 – 1.40
	IUDs Users	33	1.13 ± 0.18	0.80 – 1.56
	Control	30	1.17 ± 0.25	0.58 – 1.79
Triglycerides (mmol/L)	COCPs Users	30	1.18 ± 0.39****	0.64 – 2.29
	IUDs Users	33	0.92 ± 0.23	0.56 – 1.61
	Control	30	0.97 ± 0.25	0.49 – 1.71
VLDL- Cholesterol (mmol/L)	COCPs Users	30	0.53 ± 0.17****	0.28 – 1.03
	IUDs Users	33	0.41 ± 0.10	0.25 – 0.72
	Control	30	0.43 ± 0.11	0.22 – 0.77
LDL-Cholesterol (mmol/L)	COCPs Users	30	3.28 ± 0.60**	2.13 – 4.54
	IUDs Users	33	2.22 ± 0.66	0.81 – 3.38
	Control	30	2.39 ± 0.56	1.41 – 3.37

P-value
* COCPs versus Control group (p < 0.001)
** COCPs versus Control group (p < 0.01)

* COCPs versus IUDs group (p < 0.001)
** COCPs versus IUDs group (p < 0.01)
*** COCPs versus IUDs group (p < 0.05)
* IUDs versus Control group (p < 0.01)

The results in table (1) show significant increase in (MDA, copper, iron, total cholesterol, TGs, VLDL and LDL concentration), non significant decrease in zinc concentration and significant decrease in HDL concentration in sera of women who used COCPs when compared with those of the control group.

Also this study showed significant increase in (MDA, copper, iron, total cholesterol, TGs, VLDL and LDL concentration), non significant decrease in zinc concentration and significant decrease in HDL concentration in sera of women who used COCPs when compared with women who used IUDs group.

The MDA, copper, concentration were found to be non significantly increase, iron concentration significantly decrease and non significantly decrease in (zinc, total cholesterol, HDL, TGs, VLDL and LDL concentration) in sera of women who used IUDs when compared with those of the control group.

Discussion
Lipid peroxidation (MDA)

One of the major findings of the present study was the significant increase in serum MDA concentration observed in the group of women taking a COCPs. Some study has shown an increased in serum MDA levels due to the use of estrogen [19]. While other study showed a significant elevation in the lipid peroxide product malondialdehyde (MDA) and decreasing the antioxidant defense represented by decreased glutathion levels in sera of women who used progesterone as a contraceptives when compared with those of the control group [20]. The change in the serum copper concentration is certainly the

major event leading to the increased level of malondialdehyde. The results of present study are in agreement with Pincemail J. *et al.* study [21].

Trace elements (copper, zinc and iron)

An increased concentration of serum copper in COCPs users women due to estrogen. Estrogen-containing combined oral contraceptives caused an increase of plasma copper level that run parallel to that of ceruloplasmin [22]. The estrogen component is mainly responsible for the increased level of serum ceruloplasmin while progesterone causes a less drastic rise [23]. Estrogen acts as an inducer for synthesis of ceruloplasmin RNA templates causing subsequent increase in synthesis of the protein. For this reason the increasing in ceruloplasmin level cause an increase in serum copper concentration [24]. The results of present study are in agreement with Daunter and Elstein study [25].

The decrease in serum zinc concentration may be an important risk factor in oxidant release and the development of DNA damage and cancer, zinc is co-factor in proteins involved in antioxidant defense, electron transport, DNA repair and protein expression [26]. The results of present study are in agreement with Donat D. *et al.* study [27].

The present study show increase in serum iron level in combined oral contraceptive pills users and it is agree with Behrman R.*et al.* study [28]. The COCPs can increase the serum iron concentration by reduce the menstrual period to 3-4 day and reduce the amount of blood that loss in each menstrual cycle to 25 mL (35 mL of blood are looses in normal menstrual cycle). COCPs are reducing the endometrial thickness that shorten the

menstrual period. For all these reasons the COCPs can be used in treatment of anemia that results from menorrhagia [29, 30]. Several studies show that the estrogen can increase the hepatic synthesis of transferrin (iron transporter protein) that lead to increase in the serum iron level [31]. The present study also show a decrease in serum iron level in IUDs users and it is agree with Dangour A. *et al.* study [32]. This decrease in serum iron concentration resulted from increasing in menstrual bleeding (heavier and more prolonged). This increase in menstrual bleeding resulted from either IUDs insertion or endometrial infections [33].

Lipid profile (Total cholesterol, HDL, TG, VLDL and LDL)

The effect of oral contraceptives on lipid levels depends on the estrogen dose relative to the progestin dose, in addition to the androgenicity of the progestin. Estrogen tends to have beneficial effects by decreasing LDL-cholesterol concentration and increasing HDL-cholesterol concentration, however, TGs concentration also increase. Progestins seem to have the opposite effect, that is, they cause HDL-cholesterol concentration to decrease and LDL-cholesterol concentration increase [34].

The progestin constituent is demonstrated to provoke serum lipid changes depending on the androgenicity of the progestogen. More androgenic progestogens such as levonorgestrel have larger effects than less androgenic progestogens such as desogestrel and gestodene. Oral contraceptives with 'second generation' progestogens (levonorgestrel, lynestrenol and norethisterone) show the most unfavorable effects. The androgenic and antiestrogenic properties of levonorgestrel result in the most pronounced effects. Formulations with levonorgestrel increase total-cholesterol, LDL-cholesterol, and TGs concentration, whereas HDL-cholesterol concentration are decreased [35, 36].

Preparations with other 'second generation' progestogens (lynestrenol and norethisterone) show similar effects on total-cholesterol, LDL cholesterol and TGs concentration. However, the effects on HDL-cholesterol may be more pronounced compared with formulations with levonorgestrel [36, 37].

Blood lipid profile (total cholesterol, HDL, LDL and TGs) is a useful tool in determining risks for cardiovascular diseases. LDL is bad cholesterol being associated in deposition of cholesterol on the walls of arteries and HDL is good cholesterol being associated in carrying cholesterol out of the blood system and is more compact than LDL [38]. Monophasic-phaic COCPs, have been considered to be an important risk factor for dyslipidemia (increase total cholesterol, decrease HDL, increase LDL and increase TGs levels), contributing to the development of atherogenesis and coronary arterial disease [39].

The results of present study are in agreement with Habibollah M. *et al* . who found a significant increase of total cholesterol, TGs, LDL concentration while HDL concentration significantly decreased in women who used COCPs (ethinyl estradiol and norgesterol) for six months in comparison to the baseline [40].

Conclusions

The study concluded that COCPs (Microgynon) [R] cause undesirable effects on oxidative stress, lipid profile and trace elements, in contrast the copper T 380A IUDs observe no undesirable effects on the same

parameters in sera of women those used contraceptives.

References

1. Ian M. and Malcolm E. (2004). Contraception and sterilization. Essential of Obstetrics and Gynecology; 4th ed. 21: 289.

2. Ash M., Keith E., Ailsa E., *et al.* (2006). Fertility control. Gynecology by Ten Teachers; 18th ed. 8: 59–66.

3. Kaunitz and Andrew M. (2004). Enhancing oral contraceptive success. The Potential of New Formulations. American Journal of Obstetrics and Gynecology; 190 (4): 23–29.

4. Loose, Davis S., Stancel, *et al.* (2006). Estrogens and progestins. Goodman and Gilman's The Pharmacological Basis of Therapeutics; 11th ed. New York: McGraw-Hill. 1541–1571.

5. Nziavake M., Mala S. and Holly L. (2007). Update on hormonal contraception. Cleveland Clinic Journal Of Medicine; 74 (3): 186–198.

6. Hatcher, Robert A., Nelson, *et al.* (2004). Combined hormonal contraceptive methods. Contraceptive Technology; 18th ed. 391–460.

7. Laura B. (2001). Oral contraceptives: An update on health benefits and risks. Journal of American Pharmaceutical Association; 41 (6): 875–886.

8. Al-Ali S., Al-Kandari F. and Al-Hamdan A. (2005). IUCD Threads acting as uterine foreign body following removal of intrauterine contraceptive device. Kuwait Medical Journal; 37 (4): 299–300.

9. Anita L., David G., Raquel A., *et al*. (2006). Intrauterine copper contraceptive: update and opportunities. The Journal of Family Practice; 1–8.

10. Shivananda B., Vinutha R. and Srimathi S. (2004). Serum copper, ceruloplasmin and thiobarbituric acid reactive substance status in patients with ovarian cancer. Indian J physiol pharmacol; 48 (4): 486–488.

11. Abdoljalal M., Gholamreza V. and Mohammad T. (2010). Serum lipid peroxidtion nd leptin level in ml end female type 2 diabetic patients in Gorgan, Iran. Journal of Chinese Clinical Medicine; 5 (1): 26–35.

12. Cutler R. (2005). Oxidative stress profiling: part I. Its potential importance in the optimization of human health. Ann NY Acad Sci.; 1055: 93–135.

13. Abdulkadir Y., Dilcan K., Serap Y., *et. al.* (2007). Increased lipid peroxidation and decreased antioxidant response in serum and cerebrospinal fluid in acute ischemic stroke. Turk J Med Sci.; 37 (2): 75–81.

14. Khanna R., Kumar R., Asthana R., *et al*. (2009). Role of trace elements and antioxidants in free radical mediated injury in neonates. MASAUM Journal of Basic and Applied Sciences; 3 (1): 543–547.

15. Smith A., Beckett G., Walker J., *et al.* (2000). Disorders of plasma lipids and lipoproteins. Lectures Notes on Clinical Biochemistry; 6th ed. Blackwell Science. 101–104.

16. Carl A. and Edward R. (1999). Tietz text book of clinical Biochemistery; 3rd ed. p.1034–1054 and 819–861.

17. Godkar P. (1994). Textbook of Medical Technology, Clinical Biochemistry: Principles and Practice, Bhalani publishing house, Bombay. India. 223–225.

18. Carl A. and Edward R. (2006). Tietz text book of clinical Biochemistery and Molecular Diagnostics; 4th ed. 948.

19. Subakir S., Abdul M., Sabariah S., *et. al.* (2000). Oxidative stress, vitamin E and progestin breakthrough bleeding. Hum Reprod; 3: 18–23.

20. Faddah L., Al-Rehany M., Abdel-Hamid N., *et. al.* (2005). Oxidative stress, lipid profile and liver functions

in average Egyptian long term Depo Medroxy Progesterone Acetate(DMPA) Users. Molecules; 10: 1145–1152.

21. Pincemail J., Vanbelle S., Gaspard U., *et. al.* (2007). Effect of different contraceptive methods on the oxidative stress status in women aged 40–48 years from the ELAN study in the province of Lie`ge, Belgium. Human Reproduction; 22 (8): 2335–2343.

22. Benes B., Spevackova V., Smid J., *et al.* (2003). Effects of age, BMI, smoking and contraception levels of Cu, Se, Zn in the blood of the population in the Czech Republic. Cent Eur J Public Health; 13: 202–207.

23. Sontakke A. and Umesh M. (2004). Changes in serum ceruloplasmin levels with commonly used methodsof contraception. Indian Journal of Clinical Biochemistry; 19 (1): 102–104.

24. Kluft C., Leuven J., Helmerhorst F., *et al.* (2002). Pro-inflammatory effects of estrogens during use of oral contraceptives and hormone replacement therapy. Vascul Pharmacol; 39: 149–154.

25. Daunter B. and Elstein M. (1973). Serum levels of copper, ceruloplasmin and ceruloplasmin oxidase activity in women using copper containing intrauterine devices and in women taking combined oral contraceptives. J. Obstet. Gynecol. Brit; Commonwealth 80: 644.

26. Emily H. (2004). Zinc deficiency, DNA damage and cancer risk. J of nutri. Biochem; 15 (10): 572–578.

27. Donat D., Sophin P., Axelle P., *et al.* (2009). Effect of oral contraception with ethinylestradiol and drospirenone on oxidative stress in women aged 18-35 years old. An international Journal. Contraception.

28. Behrman R., Kliegman R. and Jenson H. (2000). Nelson text book of pediatrics; 16th ed. W.B.Saunders company. 577–579.

29. Goldman L. and Bennett J. (2000). Cecil text book of medicine; 21 st ed. W. B. Saunders company. 1341–1344.

30. Dayal M. and Barnhart K. (2001). Non-contraceptive benefits and theraputic uses of the oral contraceptive pill. Semin. Reprod. Med.; 19 (4): 295–303.

31. Mayne P. (1994).Clinical Chemistry in diagnosis and treatment; 6th ed. Oxford University press. Inc. New York. 173–381.

32. Dangour A., Hill H. and Ismail S. (2001). Haemoglobin status of adult non-pregnant kazakh woman living in kzyl-orda region,kazakhstan.Eur.J.Clin.Nutr; 55 (12): 1068–1075.

33. Wang I., Russell P. and Fraser I. (1995). Endometrial morphometry in users of intrauterine contraceptive devices and women with ovulatory dysfunctional uterine bleeding a comparison with normal endometrium.Contraception; 51 (4): 243–248.

34. Christine S., Ellen B., Julie F., *et al.* (2003). Prescribing oral contraceptives for women older than 35 years of age. Ann Intern Med.; 138: 54–64.

35. Godsland I., Crook D., Simpson R., *et al.* (1990). The effects of different formulations of oral contraceptive agents on lipid and carbohydrate metabolism. New England Journal of Medicine; 323 (20): 1375–81.

36. Burkman R., Robinson J., Kruszon-Moran D., *et al.* (1988). Lipid and lipoprotein changes associated with oral contraceptive use: a randomized clinical trial. Obstetric Gynecology; 71: 33–38.

37. Burkman R., Zacur H., Kimball A., *et al.* (1989). Oral contraceptives and lipids and lipoproteins: part I – variations in mean levels by oral contraceptive type. Contraception; 40: 553–61.

38. Faiqa F., Muhammad N., Saman B., *et al.* (2006). Dietary effects on hemo-lipid profile; in university students of middle income group.Professional Med J; 13 (4): 621–626.

39. Santos, Rebelo, Zuttin, *et al.* (2008). Influence of oral contraceptive use on lipid levels and cardiorespiratory responses among healthy sedentary women. Rev Bras Fisioter; 12 (3): 188–94.

40. Habibollah M., Khadijeh A., Najaf Z., *et al.* (1999). Acomparative analysis of three methods of contraception: effects on blood glucose and serum lipid profiles. Annals of Saudi Medicine; 19 (1): 8–11.