Effect of Hormonal Contraceptives on Bone Profile

Raad Y. Al-Hamdani* **MBChB ; M.Sc**

Wahda B.Al-Youzbaki** **MBChB ; M.Sc; Ph.D**

Janan Gazzi*** **BSc. M.Sc**

Atyaf A.Al-Jabbar**** **BSc.**

Abstract:

Objective: To evaluate the effect of hormonal contraceptives (combined oral contraceptive pills (COCPs) and depot medroxy progesterone acetate injection (DMPA) on bone profile (serum calcium, phosphorous and alkaline phosphatase) in relation to age, and duration of usage.

Design: Case control study.

Setting: Family Planning Clinic in Al-Batool Teaching hospital, during the period from February 2006 to June 2006 .

Subjects & Method: Ninety four (94) women using hormonal contraceptives (64 women of them were on COCPs and 30 women on DMPA injection) with mean ± SD age (31.01± 5.686) years for a period range from 0.25-10 years , and another 94 women who were not using any hormonal contraceptives (non user) as control group, with mean ± SD age (31.79±5.68) years, were all investigated by measuring serum calcium, phosphorous and alkaline phosphatase (and albumin to calculate corrected calcium) by spectrophotometric method using commercial Kits. While corrected Calcium were calculated by the following equation:

Corrected Calcium = measured Ca + 0.02 (40_ albumin).

Results: There was a non significant difference in the biochemical parameters of bone profile (serum calcium, phosphorous and alkaline phosphatase) of the hormonal contraceptives (COCPs and DMPA) users group and the non users (control) group, also a non significant difference in the biochemical parameters of bone profile of the COCPs and DMPA users except that serum alkaline phosphatase of DMPA users was significantly higher than that of the COCPs users. Beside that it was found that there was no relationship between the measured biochemical parameters of bone profile of the of the hormonal contraceptives (COCPs and DMPA) users and the age nor with duration of use.

Conclusion: The use of hormonal contraceptives (COCPs or DMPA) up to ten years did not appear to affect serum levels of the minerals related to bone.

Keywords: Hormonal Contraceptive, serum calcium, phosphorous and alkaline hosphatase.

Introduction:

Oral contraceptives are the most common form of birth control among women between age 18-39[1]. In Mosul / Iraq, the most common contraceptives method is the oral pills, 42.6% (including combined oral contraceptives pills (COCPs) in 32.3% and progesterone only pills in 10.3%) in women age range 15-45 years.[2]

The COCPs are a safe and acceptable form of contraception in pre menopausal women and may be effective in maintaining bone mass prior to menopause. Studies of the bone sparing properties of the COCPs are difficult to interrupt because of confounding variables such as age, smoking, duration of use, exercise, menstrual function and endocrine diseases [3].

The decrease in both estrogen and progesterone content in COCPs over the past decades, especially in the recent formulation of COCPs, has led to reduction in both side effects and cardiovascular complications by potentially lower serum estrogen concentration[4]. The shift toward lower dose COCPs preparations, could therefore potentially have greater effect on bone mineral density (BMD) due to a decreased concentration of circulating sex steroids, as estrogen and testosterone can profoundly affect bone metabolism [5].

Injectable contraceptives are safe, highly effective convenient and reversible birth control method. Depot medroxy progesterone acetate (DMPA) is one of them[6]. It was approved by United State Food and Drug Administration (FDA) for use in October 1992. Recent World Health Organization (WHO) data suggest that it is safe contraceptive method and there is no increase of breast or ovarian cancer in DMPA users and a fivefold decrease in endometrial cancer[7,8,9], however, the effect of long term use of DMPA on BMD remain controversial [10].

The DMPA works by inhibiting the pituitary gonadotrophin and thus ovulation is suppressed. With long term use, most women become amenorrheic and ovarian estradiol production is reduced. It is postulated that this low estradiol state may predispose these women to osteoporosis[10].

Life expectancy in most populations of the world is rising, with a consequent increase in the proportion and number of elderly people. Population projections indicate that there will be 1200 million women aged fifty years or older in the year 2030, compared with 467 million in 1990[11], so diseases associated with ageing will become increasingly important in public health. One condition that affects women in particular is osteoporosis. Factors that modify the strength of the skeleton need to be identified, and much attention has been given to hormonal contraceptives, with their diverse effects on bone mass [12].

During the reproductive years, many women use hormonal contraceptives for 20 years or more years. It is therefore, important to reassure that hormones used for contraception do not have a negative impact on the individual development and maintenance of bone mass[13].

The aim of this study was to evaluate the effects of hormonal contraceptives (COCPs and DMPA) usage on bone profile including serum calcium, phosphorous, and alkaline phosphatase in relation to age, and duration of usage.

Subjects & Method:

This is a case control study, was conducted from February to June 2006 in the Family Planning Clinic in Al-Batool Teaching Hospital. One hundred eighty eight women enrolled in this study, 94 of them were apparently healthy women using hormonal contraceptives with mean ± age of (31.01± 5.686) and age range between (20-41) years, of whom 64 women were on COCPs called Microgynon of Schering Company of Pharmaceutical Division- Fedral Republic of Germany. Each tablet contains 0.03 mg (30 µg) of Ethinyl Estradiol (EE) and 0.15 mg (150 µg) of Levonorgestrel (LNG), for a period range from 0.25-10 years. While 30 women of them were on DMPA injection of Pharmacia NV/SA Puurs-Belgium Company, which contain 150 mg of medroxy progesterone acetate for a period range from (0.25-1.5) years. These all were considered the hormonal contraceptives users group. The nonusers group (control) were 94 apparently However when we were comparing these parameters between hormonal contraceptives (COCPs and DMPA) users, there was a ANOVA test and Duncan's test were applied revealed no significant difference have been found in all biochemical parameters of bone profile between COCPs users, DMPA users and the hormonal contraceptives non users apart from alkaline phosphatase (table 3).

statistical significance difference (p=0.017) of the serum alkaline phosphatase only (table 2).healthy women with a mean ± SD age of (31.79±5.68) and age range from (19-43) years. Non of them were using any hormonal contraceptives nor pregnants.

Blood sample were collected by venipuncture without using tourniquet from both users and non users of hormonal contraceptives, allowed to clot at room temperature, then the serum sample obtained after centrifugation. Bone profile measurements were performed on serum samples including:

1-Serum Calcium was measured by spectrophotometric method without depolarization using methyl thymol blue as indicator[14].
2-Serum inorganic phosphorous was measured by spectrophotometric method[15] using a Kit, of Randox (UK).
3-Serum alkaline phosphatase was measured by Kind and King method[16] using a kit supplied by Biomerux (France).
4-Serum albumin was measured by Bromocresyl green (BCG) method[17] using a kit from Randox (UK).
5-Serum calcium was approximately corrected to take account of abnormal albumin using a formula[18]:

Corrected Calcium = measured Ca + 0.02 (40_ albumin)

Standard statistical methods used to determine the mean, standard deviation (SD) and the range (minimum-maximum).Unpaired z-test was used to compare the results of various biochemical parameters among hormonal contraceptives users and non-users. ANOVA Test (Analysis of Variance) and Duncan's test were used to identify the groups responsible for statistical differences through comparison. All values quoted as the mean ± SD and a P-value of < 0.05 was considered to be statistically significant [19].

Results:

The data of biochemical parameters of bone profile (serum corrected calcium (CCa), phosphorus (P) and alkaline phosphatase (AP) of the hormonal contraceptives users group and non-users group, are shown in table (1). In which no statistically significant difference have been found between biochemical parameters of bone profile of the two groups. Table 4 and 5 shows that there were no effects of the age and duration of use on biochemical parameters of bone profile between COCPs users and DMPA users respectively.

Table (1): Comparison of biochemical parameters between hormonal contraceptives users group & non users group

Parameters	Mean ± SD		p-value
	Users (n=94)	Non users(n=94)	
Corrected calcium	8.72 ± 0.71	8.80 ± 0.76	NS
Phosphorus	4.23 ± 0.44	4.18 ± 0.38	NS
Alkaline phosphatase	9.06 ± 1.84	8.83 ± 1.28	NS

Table (2): Comparison of biochemical parameters between COCPs users & DMPA users

Parameters	Mean ± SD		p-value
	COCPs (n=64)	DMPA (n=30)	
Corrected calcium	8.64 ± 0.68	8.87 ± 0.64	NS
Phosphorus	4.23 ± 0.43	4.28 ± 0.38	NS
Alkaline phosphatase	8.57 ± 1.45	9.69 ± 1.86	S(0.017)

Table (3): Comparison of biochemical parameters among COCPs users, DMPA users and Non users

Parameters	Mean ± SD			p-value
	COCPs users (n=64)	DMPA users (n=30)	Non users (n=94)	
Corrected calcium	8.46 ± 0.68	8.87 ± 0.64	8.80 ± 0.76	NS
Phosphorus	4.23 ± 0.43	4.28 ± 0.38	4.18 ± 0.38	NS
Alkaline phosphatase	8.57 ± 1.45 a	9.69 ± 1.86 b	8.83 ± 1.28 a	<0.05

NS = Not significant, Means with different letters horizontally have significant difference at $p \leq 0.05$

Table (4): The Relationship between Biochemical parameters of bone profile and the age of COCPs users & DMPA users.

Biochemical Parameters	r		y		P value	
	COCPs	DMPA	COCPs	DMPA		
Corrected calcium	- 0.03	- 0.09	-0.0035x +8.7627	-0.0121x +9.2593	NS	NS
Phosphorus	- 0.03	- 0.24	-0.0026x +4.2751	-0.019 x +4.8818	NS	NS
Alkaline phosphatase	0.22	- 0.06	0.0714x +6.5772	- 0.0225x +10.851	NS	NS

Table (5): The Relationship between Biochemical parameters of bone profile and duration of use of COCPs users & DMPA users.

Biochemical Parameters	r		y		P value	
	COCPs	DMPA	COCPs	DMPA		
Corrected calcium	- 0.08	- 0.003	-0.0265x +8.7133	-0.0047x +8.872	**NS**	**NS**
Phosphorus	0.02	0.20	0.0047x +4.185	0.2291 x +4.1449	**NS**	**NS**
Alkaline phosphatase	0.14	0.11	-0.1093x +9.0299	0.6107x +9.2722	**NS**	**NS**

Discussion:

This study found a non significant difference in the biochemical parameters of bone profile (serum calcium, phosphorous and alkaline phosphatase) of the hormonal contraceptives (COCPs and DMPA) users group and the non users (control) group, also a non significant difference in the biochemical parameters of bone profile of the COCPs and DMPA users except that serum alkaline phosphatase of DMPA users was significantly higher than that of the COCPs users.

The result of this study is in agreement with other older studies which found also that the use of COCPs or injectable progestational contraceptives did not appeare to affects the levels of some minerals like calcium, magnesium and phosphorous[20,21]. The study of Volpe *et al*., 1997[22] in Italy concluded that oral contraceptives have no significant effects on bone metabolism in women during their childbearing years.

In a study done in 1995[23] in Italy, measured serum alkaline phosphatase level & urinary excretion of hydroxyl proline (OH Prolone) and bone mass density (BMD) at baseline and every 12 months for 5 years among 76 women using COCPs containing 20 µg ethinyl estradiol and 0.15 mg desogestrel and 71 women using no COCPs (control), found that neither urinary excretion of OH Prolone levels nor alkaline phosphatase levels differed significantly in the two groups during the five years from baseline levels. Also in a recent prospective cohort study[24] in USA to evaluate the effect of COCPs on BMD over 36 months in reproductive age women concluded that COCPs did not appear to impact BMD.

In a study done in 1998[25] in United Kingdom, to identify any adverse effects on bone density in long term users of DMPA for contraception for one year, found that despite amenorrhea and low serum estradiol, the DMPA users had bone density only minimally below the normal population mean and therefore found no clinically adverse effect on bone density. While The study of Ott *et al*., [26] in USA on bone biochemical markers (serum calcium, parathormon (PTH), osteocalcin and BMD suggested that COCPs had no significant effect on these markers but DMPA did have in the form suggested that in women using DMPA bone resorption exceed bone formation.

In contrast to the result of this study, a study done by Hameed *et al*.,(2001)[27] in Frontier, aimed to evaluate serum Ca, Mg, Phosphorus levels in women taking oral contraceptive and DMPA injection and found a significant decrease in serum level of calcium, magnesium and phosphorus in women taking oral contraceptive but there was significant increase in these minerals in women taking DMPA. Also the study done on 185 Nigerian women, found a significant reduction in serum Ca level but a significant increase in the serum inorganic phosphorus in women taking oral contraceptives in comparison with the control[28]. While the study of Almstedt Shoepe and Snow[29] in USA found that oral contraceptive use in young women is associated with lower BMD than that of controls.

The different results in different studies may be due to different number of patients investigated, the different COCPs formulations, and to the multifactorial nature of bone loss phenomenon. Furthermore, the use of different techniques and different sites of measuring the bone density may limit the interpretation and reproducibility of the reported findings[22].

The only significant difference in this study is found between the alkaline phosphatase level of COCPs users and DMPA users. This goes with the results of the study of Ohno *et al*.,[30], who found that the use oral contraceptives (containing ethinyl estradiol) in rats induced increased in serum alkaline phosphatase, which is due to increased in the liver type isozyme induced in the liver and to the increased bone-type isozyme, and among gradients of this oral contraceptive, ethinyl estradiol was mainly involved in the increased serum alkaline phosphatase induced by this drug.

Our study found no effects of the age and duration of use of the hormonal contraceptives on the biochemical parameters of bone profile between COCPs users and DMPA users. This is in contrast

to a large case control study[31] from Sweden reported lower risk of fracture in postmenopausal women who had used oral contraceptives in past and concluded the protective effect to be confined to women who had taken COCPs for five years or more after age of forty and to women with body mass index (BMI) < 25Kg / m^2, while a study done by Banks *et al*., [32] about the relationship between use of progesterone only contraception and BMD concluded that using DMPA have average lower BMD than non users in uncertain magnitude but appear to be greater with longer duration of use. Also in a cross-sectional study from USA noted that the reduction of bone density in DMPA users was more pronounced in women aged 18-21 than in older women [33].

Conclusion:

This study concluded that the use of hormonal contraceptives (COCPs or DMPA) up to ten years did not appear to affect serum levels of the minerals related to bone, in relation to the age nor to the duration of use.

References:

1-Piccinino LJ, Mosher WD. Trends in contraceptive use in United States: 1982-1995. Fam Plann Perspec. 1998; 30: 4-10.

2-Al-Youzbaki WB. Contraceptives practice in Mosul. Iraqi J Comm Med. 2004; 17(2):85-90.

3-Corson SL. Oral contraceptives for the prevention of osteoporosis. J Rep Med. 1993; 38(12 supp 1): 1015-1020.

4-Mishell DR. Oral contraceptives and cardiovascular events: summary and application of data. Int J Fertil. 2000; 45(suppl 2): 121-133.

5-Compston JE. Sex steroids and bone. Physiol Rev. 2001; 81:419-447. Abstract.

6-Kaunitz AM, Rosenfield. Injectable contraception with depot medroxy progesterone acetate. Drug. 1993; 45: 854-865.

7-World Health Organization Collaborative Study of Neoplasia and Steroid Contraceptives, Breast cancer. Lancet. 1991; 388: 833-838.

8- World Health Organization Collaborative Study of Neoplasia and Steroid Contraceptives, Endometrial cancer. Int J Cancer. 1991; 49: 186-190.

9-World Health Organization Collaborative Study of Neoplasia and Steroid Contraceptives, Epithelial ovarian cancer. Int J Cancer. 1991; 49: 191-195.

10-Cundy T, Evans M, Roberts H, *et al*., Bone density in women receiving depot medroxyprogesterone acetate for contraception. Br Med J 1991; 303: 13-16. Abstract.

11-World Bank. World development report 1993: investing in health. New York: Oxford University Press, 1993.

12-Meirik O. Hormonal contraceptives and bone mass. IPPF Medical Bulletin. 2000; 34(5): 1-4.

13-Merki-Feld G S, Neff M, Keller PJ. A 2- year prospective study on the effects of depot-medroxyprogesterone acetate on bone mass-response to estrogen and calcium therapy in individual users. Contraception. 2003; 67(2): 79-86.

14-Enders DV, Rude RK. Mineral and bone metabolism. In Tietz Textbook of Clinical Chemistry. 3rd ed., Buritis CA and Ashwood ER editors. W.B. Saunders Company, USA. PP 1398.

15-Correlisson G. Broadly pertinent chronobiology methods quantify phosphate dynamics (chronome) in blood and urine. Clin Chem. 1992; 38: 329-333.

16-Kind PRN, King EJ. Estimation of plasma phosphates by determination of hydrolyzed meth ammonium antipyrine. J Clin Path. 1954; 7: 3222-326.

17-Johnson AM, Rohlfs EM, Silverman LM (1999). Proteins. In Tietz Textbook of Clinical Chemistry. 3rd ed., Buritis CA and Ashwood ER editors. W.B. Saunders Company, USA. PP 477-540.

18-Smith AF, Beckett GJ, Walker SW, Rae PWH. Lecture Notes on Clinical Biochemistry, 6th ed., England Black well Science. 1998. PP 83.

19-Kirkwood BR (1988). Essential of Medical statistics 1st ed. Blackwell Scientific publications, Oxford. PP 43-56.

20-Thin CG. The effect of an oral contraceptive agent on the concentrations of calcium and magnesium in plasma, erythrocytes and platelets in women. Ann Clin Res. 1971; 3(2): 103-106.

21-Simpson GR, Dale E. Serum levels of phosphorus, magnesium, calcium in women utilizing combination oral or long- acting injectable progestational contraceptives. Fertil Steril. 1972; 23(5): 326-330.

22-Volpe A, Amram A, Cagnacci A, Battaglia C. Biochemical aspects of hormonal contraception: effects on bone metabolism. Eur J Contracept Reprod Health Care. 1997; 2(2): 123-126.

23-Polatti F, Perotti F, Filippa N, Gallina D, Nappi RE. Bone mass and long-term monophasic Oral contraceptive treatment in young women. Contraception. 1995; 51(4): 221-224.

24-Reed SD, Scholes D, Andrea Z, *et al*., Longitudinal changes in bone density in relation to oral contraceptive use. Contraception. 2003; 68 (3): 177-182.

25-Gbolade B, Ellis S, Murby B, Randall S, Kirkman R. Bone density in long term users of depot-medroxyprogesterone acetate. Br J Obstet Gynaecol. 1998; 105(7): 790-794.

26-Ott SM, Scholes D, LaCaroix AZ, Ichikawa LE, Yoshida CK, Barlow WE. Effects of contraceptive use on bone biochemical markers

in young women. J Clin Endocrin Metabol. 2001; 86: 179-185.

27-Hameed A, Majeed T, Rauf S, *et al*., Effect of oral and injectable contraceptive on serum calcium, magnesium and phosphorus in women. J Ayub Med Coll Abbottabad. 2001; 13(3): 24-25.

28-Olatunbosun D, Adeniyi FA, Adadevoh BK. Total serum calcium and phosphorus levels in women taking oral contraceptives. Niger Med J. 1976; 6(2): 144-146.

29-Almstedt Shoepe H, Snow CM. Oral contraceptive use in young women is associated with lower bone mineral density than that of controls. Osteoporos Int. 2005; 19: 25-27.

30-Ohno H, Tojo H, Kajimura T, Nomura M. Increased serum alkaline phosphatase induced by DT-5061, an oral contraceptive, in rats. J Toxicol Sci. 1994; 19: 507-518.

31-Michaelsson K, Baron LA, Faramand YF, Persson I, Ljunghall S. Oral contraceptive use and risk of hip fracture: a case-control study. Lancet. 1999; 353: 1481-1484.

32-Banks E, Berrington A, Casabonne D. Overview of the relationship between use of progesterone-only contraceptives and bone mineral density. BJOG. 2001; 108(12): 1214-1221.

33-Schles D, Lacroix AZ, Ott SM, Ichikawa LE, Barlow. Bone mineral density in women using depot-medroxyprogesterone acetate for contraception. Obstet Gynecol. 1999; 93: 233-238.

***Prof. Depart. Of Biochemistry , College of Medicine - University of Mosul – Iraq**

**** Lecturer , Depart. Of Pharmacology , College of Medicine - University of Mosul – Iraq**

***** Lecturer, Depart. Of Biochemistry , College of Medicine - University of Mosul – Iraq**

******Depart. Of Biochemistry , College of Medicine - University of Mosul – Iraq**