Biochemical Markers of Bone Turnover in Saudi Females of Reproductive Age Using Oral Contraceptive Pills

Nadia N. Osman[1,2], Manal M. Alzahrani[1,3*]

[1]1Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia,
[2]Food Irradiation Research Dep. National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt,
[3]Department of Chemistry, Faculty of Science, Albaha University, Baljurashi, Saudi Arabia.

ABSTRACT

Oral contraceptives (OCs) are mostly used by women for a long period in their life during early reproductive years. According to the close relationship between estrogen and bone metabolism, the question of the impact of OCs on bones needs to be addressed. The purpose of this study was to evaluate the changes of bone turnover markers (BTMs) among healthy premenopausal Saudi women using OCs. Seventy-five females (24-40 years) were categorized as OCs users (N=40) for at least one year, and (N=35) non-contraceptive users (NOC), i.e. controls. All Subjects completed a questionnaire on lifestyle characteristics at the Maternity and Children's Hospital in Almasadeya in Jeddah City, which included demographics, age, height, weight, BMI, smoking history, caffeine use, milk consumption, vitamins D intake, supplement use, sun exposure, and exercise. Women with previous fractures, thyroid disease, hypertension and family history of osteoporosis were excluded. Main outcome measures included three markers of bone formation (osteocalcin, bone alkaline phosphatase (Bone ALP) and N-terminal propeptide of type I procollagen (PINP), and two markers of bone resorption that are Carboxy-terminal crosslinked telopeptide of collagen type I and N-terminal crosslinked telopeptide of type I collagen (NTXI). In addition, concentration of bone minerals (Calcium, Phosphorus and Magnesium), hormones and vitamin D_3 levels were assessed. The mean serum bone minerals, parathyroid hormone (PTH) and vitamin D_3 levels obtained from OCs users were slightly lower than those of the control group. Serum indicators of bone turnover were raised slightly among OCs users compared to the age-mate controls. However, increases in Bone serum ALP and serum CTX observed in OCs users were compared with NOC controls, but the results were still in normal range. It was concluded that BTMs were not significantly influenced by using OCs in premenopausal Saudi women.

Key Words: Bone Turnover Markers, Oral Contraceptive Pills, Bone Formation, Bone Resorption.

eIJPPR 2018; 8(3):1-11

HOW TO CITE THIS ARTICLE: Nadia N. Osman, Manal M. Alzahrani (2018). "Biochemical markers of bone turnover in saudi females of reproductive age using oral contraceptive pills", International Journal of Pharmaceutical and Phytopharmacological Research, 8(3), pp.1-11.

INTRODUCTION

As the concept of family planning and birth control has been able to reach almost every corner of the world, the usages of hormonal contraceptives are becoming more prevalent. Contraceptives are methods which can help individuals to control the timing, birth spacing and number of children they want to have, as necessary [1].

According to a 2015 United Nations' (UN) report, it was found that 36.8% of Saudi women (who are married in unions and of reproductive age) use some forms of contraception, and 6.5% of them use birth control pills and 24.1% of them have unmet family planning needs [2]. Nevertheless, it should be noted that the widely used contraceptive by Saudi women is the oral pills [3].

The oral contraceptives (OCs) are the most in demand method of contraception. The pills can prevent ovulation; thicken cervical mucus, which helps block sperm from reaching the egg; or thin the lining of the uterus [4]. OCs

Corresponding author: Manal M. Alzahrani
Address: Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
E-mail: ✉ manal-z-10 @ hotmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest .
Received: 15 January 2017; **Revised:** 02 May 2018; **Accepted:** 16 May 2018

are usually a combination of an oestrogen and a progestogen (combined oral contraceptives, COCs), although they are sometimes a 'progestogen-only' pill (POPs) which is used.

Despite the fact that, combined oral contraceptives are generally effective in preventing pregnancy, a lot of investigators have proposed that the use of contraceptives is beneficial but also has adverse effects. Through study, it is believed that the usage of contraceptives has been of great importance in analyzing the levels of progesterone and egesterone on several biochemical parameters among people who consume it. It was found a correlation between sooner bone formation shrinkage among low amount estrogen COC formulation users than the controls [5]. Hormonal contraceptives have been related to bone changes in women. While some studies have informed negative relations between COC use and bone turnover or BMD [6, 7]. However, various findings showed no relationship between them [8-12].

In women, the effects of estrogen on the skeleton appear most notably during adolescence and after menopause. During adolescence, there is a surge in estrogen levels associated with adolescent growth and sexual development, and during menopause, estrogen levels rapidly decline, exposing women to a hypoestrogenic state [5]. Thus, the exposure to estrogen through these key periods may dramatically influence a woman's bone health [13]. Moreover, the experimental studies have shown that progesterone has an influence on bone quality. Young women who start using progesterone-based oral contraceptives have a significant decline in bone quality [14].

Biochemical markers of bone turnover have been revealed offering valued data for the monitoring plus the diagnosis of the bone diseases which show quotients of bone resorption, bone formation, and they are thought to provide changes in the number of bone remodelling positions [15]. BTMs have revealed the metabolic consequences of medications on bone turnover [16]. Therefore, they may reflect a more illustrative guide of the skeletal loss of bone that would be determined by evaluating the amounts of alteration in BMD at particular skeletal spots [15, 17]. Several analyses are available that measure bone turnover markers (BTMs). Markers that are specific to bone formation include bone-specific alkaline phosphatase (BALP), osteocalcin, and N-terminal propeptide of type 1 procollagen (P1NP); markers specific to bone resorption include N-telopeptide of type 1 collagen (NTX), C-terminal telopeptide of type 1 collagen (CTX), and pyridinoline cross-links [18]. This study was designed to evaluate changes in the markers of bone turnover in the Saudi premenopausal women using OCs.

SUBJECTS AND METHODS

Subjects

This study was carried out on a total number of 75 Saudi women between 20 - 40 years of age, eligible for the study inclusion, who volunteered and satisfied the study criteria. A total of 40 OCs users were currently taking pills for at least 12 months and were compared to 35 non - oral contraceptive (NOC) users, and healthy control subjects were not using any contraception at least more than a year. All candidates had normal menstrual cycles. The criteria for inclusion in this study were: no evidence of pregnancy, absence of menstrual abnormalities, not suffering from bone diseases or disorders of bone metabolism, and not taking any drugs that impact on hormone levels. Informed written consent was obtained from all subjects who participated in the present study, and the study was approved by the Ethics Committee of the Ministry of Health of Saudi.

Data collected

Each woman completed an interviewer-administered questionnaire including sociodemo-graphic, lifestyle, and medical history information. Lifestyle characteristics included demographics, smoking history, caffeine use, vitamin supplement use, ultraviolet radiation exposure (sun), sleep habits, and weight changes within the last year. A reproductive history included average menstrual cycle, if one had been pregnant or not, period of no menstrual flow, and if the person had undergone some treatment that caused her periods to be regular and used hormonal (estrogen and progesterone) contraception. Medical history was assessed, including thyroid disease, hypertension, and family history of osteoporosis. Dietary intake of calcium and vitamin D had to be evaluated by filling in a food frequency questionnaire.

Blood Specimens

Morning fasting blood samples were obtained from the volunteers from 8 to 11am, after 8 hours of overnight fast. Collection was done in tubes with a clot activator and a serum gel separator. Samples were allowed to clot for 2 hours at room temperature before centrifugation (by Heraeus Megafuge 16 Thermo Scientific, USA) for 3 minutes at 4000×g. The serum was collected immediately and stored at -24 ºC.

Anthropometric Measurements

The weight of the body and its height were measured where one was expected to be in light clothes and bare-footed. Electronic gadgets to measure weight and height were used. The weight and the height measure were used to estimate the body mass index of an individual. Body mass index (BMI) was calculated as weight (kg) / height $(m)^2$.

Biochemical analysis

Assessment of bone turnover markers (BTMs)

Concentrations of serum minerals (namely Calcium (Ca), Phosphours (P), Magnesium (Mg)) were determined in an automatic device provided for blood chemistry analysis (Cobas Integra with Cobas C 501 autoanalyzer), whose technique is based on atomic absorption spectrometry, by using a chemical method (mineral serum) and available commercial kits (Roche, Mannheim, Germany).

The serum concentrations (Vitamin D3) were measured with an automatic device provided for blood chemistry analysis (Elecsysand Cobas E601 immunoassay analyzers), and were assessed with an electrochemiluminescence immunoassay(ECLIA) by means of the automated Elecsys kits (Roche, Mannheim, Germany).

The serum activity of ALP was determined in an automatic device provided for blood chemistry analysis (Cobas Integra with Cobas C 501 autoanalyzer), whose technique is based on atomic absorption spectrometry, using an enzymatic method (ALP) and available commercial kits (Roche, Mannheim, Germany). In addition, the serum activity of Bone ALP, Osteocalcin, PINP, CTXI and NTXI were assessed by enzyme-linked immunosorbent assay (ELISA) technique by using a commercial available kit provided by Elabscience (Elabscience Biotechnology Co., Ltd, Wuhan, China).

Statistical analysis

The analysis of data was executed by using a Megastat (V ersion 9.4). Results were expressed as mean ± standard deviation SD. Independent-unpaired student (t-test) was applied to evaluate the differences between both oral contraceptives users and control of non-users.

RESULTS

A total of 75 women, 40 (53.3%) oral contraceptive users and 35 (46.6%) who had not used any methods of contraception at least more than a year (control group), completed questionnaires which were recruited from the hospital and used in this study There were no significant differences in the means of age, height, weight, BMI, parity between the subjects using oral contraceptives and control. The mean ± SD of years for participants who were using oral contraceptives was 3.07±2.27 and regarding the type of progesterone, they all currently using types of COCs containing Desogestrel, Gestodene and Drospirenone(27.5%, 50%, 22.5%), respectively. The females who participated in the study included graduated from universities (77.1%, 57.5%), housewives (48.5%, 67.5%) and employed outside the home (51.4%, 32.5%), in the control, and OCs user groups, respectively. Only 10% and 20% had exercised more than a half hour per day in OCs users and control groups, respectively (Table 1).

Most participants had once or twice dietary calcium intake per week (62.85%, 65%) in control and OCs users' groups, respectively. Undeniably, premenopausal women showed high percentages of less than fifteen minutes sun exposure (57.14%, 55%) in control and OCs users groups, respectively. The study found that the prevalence of smoking habit does not differ significantly in the groups.

Table 1. Characteristics, Demographic and habits of OCs users and nonusers

	NOC (n=35)	OCs (n=40)
Characteristics variables	Mean±SD	Mean±SD
Age (Years)	30.34±4.83	31.38±4.00
Height (m)	1.56±0. 507	1.57 ±0.584
Weight (Kg)	65.35±15.18	66.76±12.65
BMI (Kg/m$^{2)}$	26.69±5.81	27.06±4.76
Parity of married	2.98±1.39	2.72±1.49
Years of Using OCs		3.07±2.27
Type of OCs		N (%)
Desogestrel Marvelon		11 (%27.5)
Gestodene (GSD) Gynera		20(%50)
Drospirenone (DRSP)Yasmine		9 (%22.5)
Demographics variables	N (%)	N (%)
Marital status		
Ever married	18(%51.4)	40(%100)
Never married	17(%48.5)	
Education level		
No school		1(%2.5)
Elementary school	2(%5.7)	2(%5)
Middle school	1(%2.9)	3(%7.5)
High school	5(%14.3)	11(%27.5)
University-post graduate	27(%77.1)	23(%57.5)
Occupation		
Yes	18(%51.4)	13(%32.5)
No	17(%48.5)	27(%67.5)
Behavioral variables		
Physical activity (min/day)		
Less than 30 min/day	22(%57.14)	22(%55)
30 min/ day	8(%22.8)	14 (%35)
more than 30 min/ day	7 (%20%)	4 (%10)
Milk and dairy consumption		
Less than once/week	3 (%7.5)	5 (%12.5)
Once or twice /week	22 (%62.85)	26 (%65)
Several times/week	10 (%25)	9 (%22.5)
Sun exposer time		
less15 min	33 (%94.2)	38 (%95)
15min more	2(%5.7)	2(%5%)
Current smokers		
Yes	5(%14.2)	3 (%7.5)
No	30(%85.7)	37(%92.5)

BMI body mass index, SD standard deviation, N number, (%) percentage

SD standard deviation, NOC non -oral contraceptives users, OCs oral contraceptives

The data in Table (2) showed no statistically significant differences in the serum levels of calcium, phosphorus, and magnesium between controls and oral contraceptive users respectively. Regarding serum concentrations of vitamin D_3, values were not significant between the two groups (P= 0.170). The Mean ±SD values of vitamin D_3 were (23.53±16.01) and (19.03±11.33) in the healthy control and oral contraceptive user groups, respectively. Despite this, it is noticeable that the arithmetic means for both categories were lower than the minimum of the normal range (30-75) nmol/l.

Table 2. Serum concentrations of calcium, phosphorus, magnesium and Vitamin D3 in OCs users and non-users

Variables	Group	N	Maximum	Minimum	Mean ±SD	t-test	P-value	Sig
Calcium (8.6-10.2) mg/dl	NOC	35	9.9	7	8.973 ±0.530	1.19	0.238	No sig
	OCs	40	9.68	7.58	8.837 ±0.449			
Phosphorus (2.7-4.5) mg/dl	NOC	35	4.4	2.64	3.607 ±0.5014	0.38	0.708	No sig
	OCs	40	4.47	2.58	3.564 ±0.476			
Magnesium (1.6-2.6) mg/dl	NOC	35	2.21	1.39	1.879 ±0.1331	0.08	0.940	No sig
	OCs	40	2.14	1.59	1.880 ±0.1618			
Vitamin D_3(30-75) nmol/l	NOC	40	57.92	5.39	23.53 ±16.01	1.3	0.170	No sig
	OCs	75	54.71	7.25	19.03 ±11.33			

SD standard deviation, NOC non -oral contraceptives users, OCs oral contraceptives, No sig: not significant (p-value ≥ 0.05)
The t-test was used to test the differences in means between three groups; namely the control and oral contraceptives users.

The effect of oral contraceptive on bone formation markers in OCs users and non-users were assessed. As shown in Table 3, no statistically significant differences were observed in the serum levels of ALP and the mean values of Bone ALP which (3140.31±1091.35) and (4001.23±1443.79) in the healthy control and oral contraceptive user group respectively. Bone ALP levels in the oral contraceptives users group were increased significantly as compared to the control group by using the t- test (Table 3). There are no significant changes (P > 0.05) in the serum levels of Osteocalcin and PINP between both groups as shown in Table 3.

Table 3. Blood serum bone formation markers (total ALP, Bone ALP, Osteocalcin and PINP) in OCs users group and non-users group

Variables	Group	N	Maximum	Minimum	Mean±SD	P-value	Sig
ALP (40-150) U/L	NOC	35	85	38	59.74 ±13.27	0.503	No sig
	OCs	40	86	43	61.73 ±12.11		
Bone ALP (78.125-5000) pg/ml	NOC	35	5228.47	966.91	3140.31 ±1091.35	0.004	Sig
	OCs	40	6805.63	482.93	4001.23 ±1443.79		
Osteocalcin (1.25-80) ng/ml	NOC	35	23.6362	0.3668	9.08 ±6.48	0.246	No sig
	OCs	40	49.3427	0.7042	11.57 ±11.58		
PINP (15.63 - 1000) pg/ml	NOC	35	312.00	9.10	121.36 ±83.01	0.207	No sig
	OCs	40	698.29	11.42	155.39 ±143.71		

SD standard deviation, total ALP alkaline phosphatase, Bone ALP bone alkaline phosphatase, (c) The t-test of bone formation marker of PINP N-terminal propeptide of type I procollagen
The t-test was used to test the differences in means between two groups; namely the control and oral contraceptives users.
H Sig: Highly significant (p-value < 0.01), Sig: Significant (p-value < 0.05), No sig: not significant (p-value ≥ 0.05).

As displayed in Table (4), the values of CTXI in normal and OCs users groups ranged from 85.89-552.39 ng/ml, with mean values of 293.94 ng/ml and 143.68 -514.14 ng/ml, and 349.06 ng/ml, respectively. Statistical analysis using t-test showed significant changes in CTXI values between the two groups (P= 0.046).

NTXI mean values were 58.27ng/ml and 68.04 ng/ml in the normal and OCs users group, respectively (Table 4). The t-test analysis exhibited non-significant changes in NTXI values in OCs users group as compared to normal control group (P= 0.070) (Table 4).

Table 4. Blood serum bone resorption markers of CTXI and NTXI in OCs users group and non-users group

Variables	Group	N	Maximum	Minimum	Mean±SD	t-test	P-value	Sig
CTXI (7.81-500) ng/ml	NOC	35	521.93	85.89	293.94 ±119.86	-0.99	0.044	Sig
	OCs	40	501.14	143.68	349.06 ±114.98			
NTXI (3.13-100) ng/ml	NOC	35	106.42	17.63	58.27 ±23.49	-2.07	0.070	No sig
	OCs	40	139.46	31.39	68.04 ±24.24			

SD standard deviation, NOC non -oral contraceptives users, OCs oral contraceptives, CTXI carboxy-terminal cross-linked telopeptide of collagen type I, NTXI amino terminal cross-linked telopeptide of collagen type I.
The t-test was used to test the differences in means between two groups; namely the control and oral contraceptives users
H. Sig: Highly significant (p-value < 0.01), Sig: Significant (p-value < 0.05), No sig: not significant (p-value ≥ 0.05)

The concentrations of serum ALP, Bone ALP, Osteocalcin, PINP, CTXI and NTXI for controls and premenopausal women who were divided into four subgroups according to the duration of OCs use by years, i.e (between 1 and 4 years) are summarized in Table 5.

No consistent contraceptive effects on serum ALP, Osteocalcin, PINP and CTXI concentrations were seen in response to the duration of OCs use.

With regards to serum Bone ALP, no significant differences were observed between control subjects and women who had OCs for 1, 2 and 3years (Table 5). However, a significant increase was observed in women used OCs for 4 years as compared with control women p=0.048 (Table 5).

For serum NTXI, the marker of bone resorption, there was no difference between control subjects and women who had OCs for 1 and 4 years (Table 5). While NTXI levels were significantly increased in women used OCs for 2 and 3 years (p=0. 016 and .031, respectively).

Table 5. Biochemical bone marker concentration for Saudi women using oral contraceptives, according to the duration of OCs use by years

	Controls	Years of OCs use			
	(n =35)	1(n =9)	2(n=9)	3(n=9)	4 (n =13)
Serum markers of bone turnover	(mean ±SD)				
ALP (40-150)U/L	52.1 ±14.0	58.3 ±10.1 (0.220) NS	51.1 ±13.7 (0.848) NS	57.4 ±17.0 (0.338) NS	54.6 ± 12.1 (0.562) NS
Bone ALP (78.125-5000) pg/ml	3565.7 ±1325.5	1389.7 ±1400.6 (0.319) NS	4388.2 ±1489.5 (0.114) NS	3583.6 ±1690.0 (0.932) NS	4424.6 ±1242.3 (0.048) a*
Osteocalcin (1.25-80) ng/ml	9.0 ±9.1	8.5 ±6.1 (0.894) NS	12.8 ±13.7 (0.170) NS	13.9 ±14.6 (0.100) NS	10.7 ± 9.9 (0.393) NS
PINP (15.63 -1000) pg/ml	163.1 ±269.6	100.4 ±81.2 (0.498) NS	165.0 ±189.1 (0.983) NS	136.7 ±79.0 (0.775) NS	188.7 ±167.2 (0.828) NS
CTXI (7.81-500) ng/ml	293.4 ±114.4	324.5 ±107.5 (0.466) NS	338.2 ±108.1 (0.297) NS	370.4 ±138.3 (0.092) NS	344.4 ± 114.3 (0.185) NS
NTXI (3.13-100) ng/ml	55.5 ±23.6	59.7 ±15.5 (0.616) NS	79.8 ±33.6 (0. 016) a*	75.4 ±24.9 (0.031) a*	65.9 ± 19.6 (0.135) NS

a Comparison of OCs subjects to controls, values in parentheses are p values, SD standard deviation, (NS) not significant, *the p-value <0.05, t-test is significant.

The bone marker concentrations in the control and OCs groups, who were divided according to their age were shown in Table 6.

It was noticed that in oral contraceptive users between the ages of 24 and 40, the bone formation markers of serum ALP, Bone ALP, Osteocalcin and PINP and bone resorption markers of serum CTXI and NTXI did not adversely change, with the exception of a highly significant increase of CTXI in OCs group with age 30≤34, and significant increase of NTXI in OCs group with age 24≤29

as compared to age-matched controls (P=0.008 and 0.033, respectively).

Table 6. Biochemical bone marker concentration for Saudi women using oral contraceptives, according to their age:

		Age of OCs user		
		24≤29 (n=11)	30≤34 (n=19)	35≤40 (n =10)
Serum markers of bone turnover	groups	(mean ±SD)		
ALP (40-150)U/L	OCs	57.1 ±12.6	54.7 ±14.3	56.8 ±11.2
	NOC	51.3 ±16.3	55.7 ±15.2	61.1 ±12.6
	P-value	(0.342)NS	(0.860)NS	(0.479) NS
Bone ALP (78.125-5000)pg/ml	OCs	3659.2 ±2013.4	3652.2 ±1041.3	56.8 ± 11.2
	NOC	3568.0 ±1492.0	3035.0 ±1248.1	61.1 ± 12.6
	P-value	(0.904)NS	(0.148)NS	(0.479)NS
Osteocalcin (1.25-80)ng/ml	OCs	10.9 ±14.5	15.7 ±12.0	5.2 ±3.2
	NOC	6.9 ±3.8	9.7 ±7.0	10.2 ± 7.0
	P-value	(0.422)NS	(0.087)NS	(0.124)NS
PINP (15.63-1000) pg/ml	OCs	137.2 ±187.4	174. 1±126.2	82.6 ±64.6
	NOC	222.5 ±405.1	124.0 ±96.7	131.0 ±98.2
	P-value	(0.497)NS	(0.213)NS	(0.283)NS
CTXI (7.81-500)ng/ml	OCs	336.8 ±103.2	354.6 ±120.3	372.5 ± 113.9
	NOC	298.9 ±119.2	248.8 ±90.2	392.8 ± 100.2
	P-value	(0.415)NS	(0.008)a**	(0.703)NS
NTXI (3.13-100)ng/ml	OCs	76.4 ±27.13	66.9 ±21.0	73.6 ±27.3
	NOC	53 ±18.8	54.1 ±24.2	67.2 ±32.3
	P-value	(0.033)a*	(0.129)NS	(0.675)NS

a Comparison of OCs subjects to controls, values in parentheses are p values, SD standard deviation, (NS) not significant, *.the p-value <0.05, t-test is significant, **. the p-value <0.01, t-test is highly significant.

DISCUSSION

Osteoporosis is the most important community health problem [19]. Postmenopausal women are the most sufferers of this bone disease and a main contributing factor in bone loss due to insufficiency of estrogen [20]. The major role in bone homeostasis and OCs use which may affect bone strength in females is sex hormones [21]. The effects of oral contraceptives on bone metabolic rate are influenced by covariates such as skeletal maturity, physical activity levels, dose and kind of oral contraceptives, and the period of OCs usage [22]. Therefore, the effects of contraceptives taken orally on bone health persist to be controversial as OCs use has been reported to be connected with lower BMD by [22-24], have positive influence on BMD and no influence on BMD by [22]. Oral contraceptives (OC), because of their capability to moderate concentrations of free testosterone and estrogen, have been supposed to affect bone mass in young adult women. Bone density in young premenopausal women is one of the crucial measurement of osteoporosis risk in aged women. Remarkably, biochemical BTMs reflect alterations in bone metabolism faster than alterations in other medical tests such as BMD, and might possibly be used as indicators in the diagnosis and monitoring of metabolic bone diseases. The metabolic influence of drugs on bone turnover may be reflected by BTMs [16].

The present study showed no significant change in calcium serum levels of OCs women when compared with the control group (Table 2). Studies associating OCs use and calcium are inconsistent, while some linked OCs use to reduction in stress fracture risk [25, 26], others detected no association [27]. Subjects treated with the gonadal steroid combination were reported to achieve a positive calcium balance with an infrequent associated reduction in serum calcium levels [27]. [28] observed a statistically significant difference in calcium level between OCs previous users and never-users in premenopausal women. [29] reported that a critical assessment of the participants on contraceptives demonstrated that OCs users showed significantly improved serum calcium levels over the control group. It is possible that the use of oral contraceptives is frequently connected with expanded absorption of calcium.

The present data showed that there were no significant differences in phosphorous and magnesium levels between the OCs users and non-user control (Table 2). The result of this study is in contrast with the results of [29] who informed that there was a significant reduction in the concentration of serum phosphorous in applicants of oral contraceptive pills as a whole compared to the control group. [30] recorded that a

reduction in the serum levels of zinc, selenium, phosphorus, and magnesium has been reported in OCs users. Such declines were related to the interval of contraceptive intake. These falls may suggest a fall in the possibility of having a pregnancy and/or a rise of a severe illness for the unborn. Accordingly, a supplementation with the previous elements could be beneficial in OCs users, namely for decreasing side effects. In this study, it is obvious that the sum of means of vitamin D for both classifications is lower than the minimum of the typical range (30-75) nmol/l (Table 2). It has been assumed that populations living in sunny countries such as Saudi Arabia would be less likely to be vitamin D deficient due to the abundant daylight throughout the year [31]. The Kingdom of Saudi Arabia (KSA) is among the countries with the highest number of sunny days per year. Exposure to sunlight is an essential factor for vitamin D production by the skin [32]. Vitamin D insufficiency has been associated with osteoporosis, fractures, and falls [33, 34]. Environment, tradition, and religion in KSA call for conservative clothing for both men and women. The traditional dress habits evolved around reducing sun exposure in the hot months and protection from cold during nights in the desert and colder months. Thick clothing and head scarves are used by the Saudi population. With reduced outdoor activities, the population is now less exposed to the sun. Previous studies showed low levels of vitamin D in KSA [35, 36]. [37] have shown that Saudi Arabian women exhibited an extraordinary frequency of vitamin D deficiency [25] (OH) D ≤50 nmol/L] that was associated with secondary hyperparathyroidism in about 18.5% and 24.6% of pre- and postmenopausal women, respectively. Moreover, [38] demonstrated that vitamin D inadequacy is experienced by both male and female at a percentage of 40.6% and 62.65%, respectively.

In this study, there were no significant differences between the oral contraceptive users and non-user control in ALP (Table 3). These conclusions are in agreement with numerous studies [39, 40] who found no significant differences in serum alkaline phosphatase between users and nonusers. In contrast to our results, [41] showed that alkaline phosphates- a marker of bone turnover- was significantly reduced in cases taking ethinyl estradiol/norgestrel. Moreover, [42] showed that oral contraceptive users had lower levels of mean serum alkaline phosphatase and higher mean bone mineral density. [43] demonstrated that two weeks of oral contraceptive therapy rapidly reduces markers of bone resorption and formation.

In this study, there were significant increases in Bone ALP levels in OCs users as compared with non-user control (Table 2). These results agreed with the results of [44, 45] who reported an increase in serum alkaline phosphatase levels in women used oral contraceptive. These raised the blood concentration of serum alkaline phosphatase activity that may indicate elevated activity of the osteoblasts [46]. [47] in his research illustrated a significant rise in serum Bone ALP concentration after a period of 3 years of 20 µg EE and 100 µg levonorgestrel or 30 µg EE and 150 µg levonorgestrel in females aged 20–35 years. Main limiting reason for that study was the absence of the control.

The findings of this study of premenopausal females suggest no indication of a significant difference in Osteocalcin between the oral contraceptive users and non-user control groups (Table 3). These outcomes are in dissimilarity with the findings of [28] who established that the bone turnover markers (osteocalcin and CTXI) were significantly declined in premenopausal women who used OCs compared with nonusers while there was no variation in post-menopausal women. Furthermore, [48] demonstrated that serum osteocalcin was significantly reduced in contraceptive users compared to non-users. [49] compared serum levels of PINP and osteocalcin in 534 premenopausal females not using oral contraceptives, with 83 females using several oral contraceptives commonly being the COC. It was observed that the percentage of spreading levels of the bone formation markers were 26 percent lower in OC users than in non-users ($P < 0{\cdot}001$).

In the current study, there were no significant differences in PINP values between the oral contraceptive users and non-user control (Table 3). Our results are in contrast to [50] and [40] who found that the median concentrations of CTXI and PINP were lower in COCs users compared with non-users. In addition, [43] concluded that low-dose OCs therapy rapidly reduced markers of bone resorption and formation (PINP and CTXI).

Because osteoclasts affect only bone collagen type I CTX, NTX fragments are the most informative markers of bone collagen destruction [51, 52]. Thus, determination of CTXI and NTXI levels in dynamics is used for diagnosing bone diseases and monitoring bone resorption. In this study, there was a statistically significant increase in CTXI levels in OCs users comparing with the control subjects (Table 4). [53] analyzed the effect of different types of OCs (30 µg EE or 15 µg EE) on bone turnover markers including osteocalcin and C-terminal cross-linking telopeptide of type I collagen (CTXI) in teenage girls. They concluded that the values of osteocalcin and CTXI diminished after 1-year use in both the 30-µg EE and 15-µg EE groups, but the differences between groups were not statistically significant when regulating for confounders. A Cross-sectional research of exercising subjects and inactive controls revealed that the bone resorption markers serum CTXI were significantly greater in exercising women not taking COCs than in non-exercising women not taking COCs [54]. [55] demonstrated that women with a higher

BMI showed significantly lower serum osteocalcin and serum CTXI, and a trend towards lower serum PINP and urinary NTXI values among pre-menopausal women. The current data showed no significant differences in serum NTX levels between the OCs users and nonusers control (Table 4). This finding is in an agreement with a finding of [56] who reported that no significant difference was found between users and non-users in osteocalcin, CTX, and NTX levels

When the premenopausal women were divided into four subgroups according to the duration of OCs use by years, a significant increase was observed in serum Bone ALP in women used OCs for 4 years as compared with control women p=0.048 (Table 5). Moreover, the NTXI showed significant difference values (P=0.016 and 0.031) in women used OCs for two and three years, respectively. This analysis suggests that the bone turnover markers were not affected by the prolonged use of OCs, except significant change was observed in the NTXI level only. In this regards, [57] would suggest that the level of estrogen needed to aid in the stability of cell apoptosis of osteoblasts is still enough despite the decreased circulating amount regulated by OCs use. By contrast, a cross-sectional study of some Nigerian subjects using levonorgestrel implants up to 4 years indicated the concentrations of serum Bone ALP and serum NTXI to be 40–60% lower than in nonuser controls. The reduction in serum NTXI concentrations was significantly associated with the usage period, with the lowest serum NTXI levels seen in ladies via the implants for 4 years [58]. In [59], comparison was made using 20 controls and DMPA was examined in accordance to its usage. Levels of serum Osteocalcin were higher in users than the non-users, the deviation was due to DMPA usage. Thus, subjects who had been on DMPA for more than 5 years had Osteocalcin concentrations double as high as those using DMPA for less than a year.

In the present study, no significant correlation was observed with age and bone ALP. The levels of CTXI reached the maximum in the age range of 30-34, while the level of NTXI reached the maximum in the age range of 24-29. These results are in congruence with [60]. However, the present results are in contrast to [61], who reported an increase in bone turnover (Bone ALP and CTXI) with the increase in the age showing the minimal level in the 30-39, and maximum in 40-59 group. In a case-control study including 100 DMPA users, [62] detected a significantly greater level of serum PINP and urinary NTXI. Remarkably, the variances between users and non-users were more obvious in younger females (18– 25 years) than in older women (35–45 years).

CONCLUTION

In conclusion, OCs use does not impact BTMs significantly in premenopausal Saudi women. It is recommended that further research should be carried out using a larger sample who are divided according to the different types of contraceptive pills and other factors such as exercise and local nutrition.

ACKNOWLEDGEMENTS

We gratefully acknowledge the staff at the Maternity and Children's Hospital in Almasadeya in Jeddah City and the King Abdulaziz City for Science and Technology for their financial support.

REFERENCES

[1] Shaw, D. (2010). The ABCs of family planning. The Globe and Mail. Medical Journal, 5(1), 29-36.
[2] Trends Contraceptive Use 2015 Report (Accessed February 16, 2018, at http://www.un.org/en/development/desa/population/publications/pdf/family/trendsContraceptiveUse2015Report.pdf.
[3] Bamufleh, R. A., Al-Zahrani, A. E., & Yousuf, S. A. (2017). Systematic Review: Contraceptive Knowledge and Use in Saudi Arabia. Journal of Gynecology and Obstetrics, 5(6), 69.
[4] Food and Drug Administration. (2011). Birth control guide, (Accessed February 10, 2018, at https://www.fda.gov/forconsumers/byaudience/forwomen/freepublications/ucm313215.htm#Hormonal_Methods.
[5] Trémollieres, F. (2013). Impact of oral contraceptive on bone metabolism. Best Practice & Research Clinical Endocrinology & Metabolism, 27(1), 47-53.
[6] Scholes D, Ichikawa L, LaCroix AZ, et al. Oral contraceptive use and bone density in adolescent and young adult women. Contraception. 2010; 81(1):35–40.
[7] Scholes D, Hubbard RA, Ichikawa LE, et al. Oral contraceptive use and bone density change in adolescent and young adult women: a prospective study of age, hormone dose, and discontinuation. J Clin Endocrinol Metab. 2011; 96(9): E1380–7.
[8] Reed SD, Scholes D, LaCroix AZ, et al. Longitudinal changes in bone density in relation to oral contraceptive use. Contraception. 2003; 68(3):177–82.
[9] Massaro M, Di Carlo C, Gargano V, et al. Effects of the contraceptive patch and the vaginal ring on bone metabolism and bone mineral density: a prospective, controlled, randomized study. Contraception. 2010; 81(3):209–14.
[10] Gai L, Jia Y, Zhang M, et al. Effect of two kinds of different combined oral contraceptives use on bone mineral density in adolescent women. Contraception. 2012; 86(4):332–6.

[11] Nappi C, Bifulco G, Tommaselli GA, Gargano V, Di Carlo C. Hormonal contraception and bone metabolism: a systematic review. Contraception. 2012; 86(6):606–21.
[12] Farr, J. N., Khosla, S., Achenbach, S. J., Atkinson, E. J., Kirmani, S., McCready, L. K., ... & Amin, S. (2014). Diminished bone strength is observed in adult women and men who sustained a mild trauma distal forearm fracture during childhood. Journal of Bone and Mineral Research, 29(10), 2193-2202.
[13] Jackowski, S. A., Baxter-Jones, A. D., McLardy, A. J., Pierson, R. A., & Rodgers, C. D. (2016). The associations of exposure to combined hormonal contraceptive use on bone mineral content and areal bone mineral density accrual from adolescence to young adulthood: a longitudinal study. Bone reports, 5, e333-e341.
[14] West, C. (2009) Oral Contraceptives: The Risks, Benefits, and Dilemmas a Senior Thesis submitted in partial fulfillment of the requirements for graduation in the Honors Program Liberty University Fall 2009.
[15] Reid, D. (2011). Handbook of osteoporosis. Springer Science & Business Media.
[16] Khan, A., Hanif, M., Ahmed, A., Habib, S., & Kamal, S. (2016). Correlation between Bone Turnover Markers and Bisphosphonates Treatment in Postmenopausal Osteoporosis. International Journal of Pathology, 14(3), 3.
[17] Agrawal, B. K., Jain, V., & Varshney, A. (2017). Bone turnover markers in women can predict low bone mineral density. International Journal of Research in Medical Sciences, 3(4), 876-879.
[18] Hlaing, T. T., & Compston, J. E. (2014). Biochemical markers of bone turnover–uses and limitations. Annals of clinical biochemistry, 51(2), 189-202.
[19] Caliri, A., De Filippis, L., Bagnato, G. L., & Bagnato, G. F. (2007). Osteoporotic fractures: mortality and quality of life. Panminerva medica, 49(1), 21.
[20] Riggs, B. L., Khosla, S., & Melton III, L. J. (2002). Sex steroids and the construction and conservation of the adult skeleton. Endocrine reviews, 23(3), 279-302.
[21] Wei, S., Winzenberg, T., Laslett, L. L., Venn, A., & Jones, G. (2011). Oral contraceptive use and bone. Current osteoporosis reports, 9(1), 6-11.
[22] Liu, S. L., & Lebrun, C. M. (2006). Effect of oral contraceptives and hormone replacement therapy on bone mineral density in premenopausal and perimenopausal women: a systematic review. British journal of sports medicine, 40(1), 11-24.
[23] Prior, J. C., Kirkland, S. A., Joseph, L., Kreiger, N., Murray, T. M., Hanley, D. A., Adachi, J.D., Vigna, Y.M., Berger, C., Blondeau, L., Jackson, S. A., & Tenenhouse, A. (2001). Oral contraceptive use and bone mineral density in premenopausal women: cross-sectional, population-based data from the Canadian Multicentre Osteoporosis Study. Canadian Medical Association Journal, 165(8), 1023-1029.
[24] Weaver, C. M., Teegarden, D., Lyle, R. M., Mccabe, G. P., Mccabe, L. D., Proulx, W., Kern, M., Sedlock, D., Anderson, D.D., Hillberry, B.M., Peacock, M., & Johnston CC (2001). Impact of exercise on bone health and contraindication of oral contraceptive use in young women. Medicine and science in sports and exercise, 33(6), 873-880.
[25] Barrow, G. W., & Saha, S. (1988). Menstrual irregularity and stress fractures in collegiate female distance runners. The American journal of sports medicine, 16(3), 209-216.
[26] Emokpae, M. A., & Uadia, P. O. (2014). Serum Calcium Level is Associated with Lipids in Young Nigerian Women Using Low Dose Oral Contraceptive Pills. Journal of Basic and Clinical Reproductive Sciences, 3(2), 105-110.
[27] Birge, S. J., & Avioli, L. V. (1969). The effect of oral contraceptives on calcium absorption. In Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (pp. 485-492). Springer, Boston, MA.
[28] Allali, F., El Mansouri, L., zohra Abourazzak, F., Ichchou, L., Khazzani, H., Bennani, L., & Hajjaj-Hassouni, N. (2009). The effect of past use of oral contraceptive on bone mineral density, bone biochemical markers and muscle strength in healthy pre and post-menopausal women. BMC women's health, 9(1), 31.
[29] Akinloye, O., Adebayo, T. O., Oguntibeju, O. O., Oparinde, D. P., & Ogunyemi, E. O. (2011). Effects of contraceptives on serum trace elements, calcium and phosphorus levels. West Indian Medical Journal, 60(3), 308-315.
[30] Dante, G., Vaiarelli, A., & Facchinetti, F. (2016). Vitamin and mineral needs during the oral contraceptive therapy: a systematic review. International Journal of Reproduction, Contraception, Obstetrics and Gynecology, 3(1), 1-10.
[31] Ardawi, M. S., Sibiany, A. M., Bakhsh, T. M., Qari, M. H., & Maimani, A. A. (2012). High prevalence of vitamin D deficiency among healthy Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. Osteoporosis International, 23(2), 675-686.
[32] Holick, M. F. (2004). Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. The American journal of clinical nutrition, 80(6), 1678S-1688S.
[33] Holick, M. F. (2009). Vitamin D status: measurement, interpretation, and clinical application. Annals of epidemiology, 19(2), 73-78.
[34] DeLuca, H. F. (2004). Overview of general physiologic features and functions of vitamin D. The American journal of clinical nutrition, 80(6), 1689S-1696S.
[35] Lips, P. (2004). Which circulating level of 25-hydroxyvitamin D is appropriate?. The Journal of steroid biochemistry and molecular biology, 89, 611-614.
[36] Autier, P., & Gandini, S. (2007). Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Archives of internal medicine, 167(16), 1730-1737.

[37] Ardawi, M. S., Qari, M. H., Rouzi, A. A., Maimani, A. A., & Raddadi, R. M. (2011). Vitamin D status in relation to obesity, bone mineral density, bone turnover markers and vitamin D receptor genotypes in healthy Saudi pre-and postmenopausal women. Osteoporosis International, 22(2), 463-475.

[38] Tuffaha, M., El Bcheraoui, C., Daoud, F., Al Hussaini, H. A., Alamri, F., Al Saeedi, M., & Mokdad, A. H. (2015). Deficiencies under plenty of sun: Vitamin D status among adults in the kingdom of Saudi Arabia, 2013. North American journal of medical sciences, 7(10), 467.

[39] Rodin, A., Chapman, M., & Fogelman, I. (1991). Bone density in users of combined oral contraception. Preliminary reports of a pilot study. British Journal of Family Planning, 16(4), 125-9.

[40] Garnero, P., Sornay-Rendu, E., & Delmas, P. D. (1995). Decreased bone turnover in oral contraceptive users. Bone, 16(5), 499-503.

[41] Milner, M., Harrison, R. F., Gilligan, E., & Kelly, A. (2000). Bone density changes during two years treatment with tibolone or conjugated estrogens and norgestrel, compared with untreated controls in postmenopausal women. Menopause (New York, NY), 7(5), 327-333.

[42] Sultana, S., Choudhury, S., & Choudhury, S. A. (2002). Serum alkaline phosphatase and bone mineral density: to assess bone loss in oral contraceptive pill user. Mymensingh medical journal: MMJ, 11(2), 107-109.

[43] Vescovi, J. D., VanHeest, J. L., & De Souza, M. J. (2008). Short-term response of bone turnover to low-dose oral contraceptives in exercising women with hypothalamic amenorrhea. Contraception, 77(2), 97-104.

[44] Endrikat, J., Mih, E., Düsterberg, B., Land, K., Gerlinger, C., Schmidt, W., & Felsenberg, D. (2004). A 3-year double-blind, randomized, controlled study on the influence of two oral contraceptives containing either 20 µg or 30 µg ethinylestradiol in combination with levonorgestrel on bone mineral density. Contraception, 69(3), 179-187.

[45] Awad, M. H., Mansour, S. M., & Abbas, R. M. (2014). Do combined oral contraceptive pills affect serum alkaline phosphatase level and bone mineral density?. Journal of Evidence-Based Women's Health Journal Society, 4(3), 164-167.

[46] Kaveh, K., Ibrahim, R., Emadi, M., Bakara. (2010).Osteoprosis and bone health. Journal of Animal and Veterinary Advances, 9(6) 1048-1054.

[47] Seibel, M. J. (2005). Biochemical markers of bone turnover part I: biochemistry and variability. The Clinical biochemist. Reviews/Australian Association of Clinical Biochemists., 26(4), 97.

[48] Ott, S. M., Scholes, D., LaCroix, A. Z., Ichikawa, L. E., Yoshida, C. K., & Barlow, W. E. (2001). Effects of contraceptive use on bone biochemical markers in young women. The Journal of Clinical Endocrinology & Metabolism, 86(1), 179-185.

[49] Gargano, V., Massaro, M., Morra, I., Formisano, C., Di Carlo, C., & Nappi, C. (2008). Effects of two low-dose combined oral contraceptives containing drospirenone on bone turnover and bone mineral density in young fertile women: a prospective controlled randomized study. Contraception, 78(1), 10-15.

[50] De Papp, A. E., Bone, H. G., Caulfield, M. P., Kagan, R., Buinewicz, A., Chen, E., Rosenberg, E., & Reitz, R. E. (2007). A cross-sectional study of bone turnover markers in healthy premenopausal women. Bone, 40(5), 1222-1230.

[51] Baim, S., & Miller, P. D. (2009). Perspective: assessing the clinical utility of serum CTX in postmenopausal osteoporosis and its use in predicting risk of osteonecrosis of the jaw. Journal of bone and mineral research, 24(4), 561-574.

[52] Tanzy, M., & Camacho, P. (2011). Effect of vitamin D therapy on bone turnover markers in postmenopausal women with osteoporosis and osteopenia. Endocrine Practice, 17(6), 873-879.

[53] Lattakova, M., Borovsky, M., Payer, J., & Killinger, Z. (2009). Oral contraception usage in relation to bone mineral density and bone turnover in adolescent girls. The European Journal of Contraception & Reproductive Health Care, 14(3), 207-214.

[54] Herrmann, M., & Herrmann, W. (2004). The assessment of bone metabolism in female elite endurance athletes by biochemical bone markers. Clinical Chemistry and Laboratory Medicine (CCLM), 42(12), 1384-1389.

[55] Glover, S. J., Garnero, P., Naylor, K., Rogers, A., & Eastell, R. (2008). Establishing a reference range for bone turnover markers in young, healthy women. Bone, 42(4), 623-630.

[56] Di Giovanni, G., Roy, B. D., Gammage, K. L., Mack, D., & Klentrou, P. (2008). Associations of oral contraceptive use and dietary restraint with bone speed of sound and bone turnover in university-aged women. Applied Physiology, Nutrition, and Metabolism, 33(4), 696-705.

[57] Bradford, P. G., Gerace, K. V., Roland, R. L., and Chrzan, B. G. (2010). Estrogen regulation of apoptosis in osteoblasts. Physiology and behavior, 99(2), 181-185.

[58] Naessen, T., Olsson, S. E., & Gudmundson, J. (1995). Differential effects on bone density of progestogen-only methods for contraception in premenopausal women. Contraception, 52(1), 35-39.

[59] Shaarawy, M., El-Mallah, S. Y., Seoudi, S., Hassan, M., & Mohsen, I. A. (2006). Effects of the long-term use of depot medroxyprogesterone acetate as hormonal contraceptive on bone mineral density and biochemical markers of bone remodeling. Contraception, 74(4), 297-302.

[60] Kumar, A., Devi, S. G., Mittal, S., Shukla, D. K., and Sharma, S. (2013). A hospital based study of biochemical markers of bone turnovers and bone mineral density in north Indian women. The Indian Journal of Medical Research, 137(1), 48–56.

[61] Shan, P. F., Wu, X. P., Zhang, H., Luo, X. H., Cao, X. Z., Xie, H., Liu, S.P., Pi, Y.Z., Fang, T.Y., Liu, H., Chen, Z.H., Zhong, N., & Liao, E. (2006). Age-related changes of serum bone alkaline phosphatase and cross-linked C-telopeptides of type I collagen and the relationship with bone mineral density in Chinese women. Clinica chimica acta, 366(1-2), 233-238.

[62] Walsh, J. S., Eastell, R., & Peel, N. F. (2008). Effects of depot medroxyprogesterone acetate on bone density and bone metabolism before and after peak bone mass: a case-control study. The Journal of Clinical Endocrinology & Metabolism, 93(4), 1317-1323.