Implications of premature ovarian failure on bone turnover markers and bone mineral density

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Summary

Introduction: Premature ovarian failure (POF) is the cessation of ovarian function before the age of 40. The loss of ovarian function, whether premature or not, has an overwhelming impact on female skeletal health, leading to an increased risk of developing osteoporosis because of the lengthened time of exposure to reduced estrogen. The objective of this study was to compare the implications of premature ovarian failure on bone turnover markers and bone mineral density in patients under the age of 40. *Materials and Methods*: Sixty-one patients with a diagnosis of POF were selected for this prospective study. Patients were divided into two groups according to age, patients < 30 years old (n = 30), and patients ≥ 30 years old (n = 31). *Results:* Between the two age sub-groups (< 30 and ≥ 30 years old), there was a significant difference in menopause rating scale (MRS), lumbar spine t-score, N-telopeptides crosslinks (NTx), and serum bone specific alkaline phosphatase (bALP) between the two age groups (10.93 ± 7.79 vs 17.38 ± 8.62; -1.84 ± 1.47 vs -1.06 ± 0.93; 58.80 ± 21.32 vs 41.1 ± 11.37; 48.99 ± 42.16 vs 23.76 ± 10.08, respectively). *Conclusion:* It is apparent that bone mineral density (BMD) is commonly less in women with POF than normal healthy women. Therefore, measurement of BMD is warranted. At this time, it is not clear how often the tests should be carried out to evaluate BMD. Further prospective studies are required to establish guidelines. However, it seems reasonable to monitor women with POF yearly for the presence of any endocrine dysfunction and to assess BMD at periodic intervals.

Key words: Premature ovarian failure; Bone mineral density; Osteoporosis; Bone turnover markers.

Introduction

Premature ovarian failure (POF) is the cessation of ovarian function before the age of 40. Recently, POF has been under the spotlight, as more young women have elevated levels of follicle-stimulating hormone (FSH) and decreased circulating levels of estrogens when they present with absent or irregular menses or infertility [1]. There are no accurate estimates of the prevalence of POF. An estimate based on a considerable number of studies showed that 0.3% of women of reproductive age has POF [2].

The cause of POF is still unclear. There is not a single cause that is predominant, which can cover a large percentage of the POF cases. As the ability to treat malignancies successfully with chemotherapy and irradiation increases in the field of oncology, the number of young women presenting with transient or permanent POF increases as well. There are also several genetic causes for POF. Karyotypic abnormalities, single gene mutations, and complex multifactorial polygenic inheritance account for some causes.

The loss of ovarian function, whether premature or not, has an overwhelming impact on female skeletal health, leading to an increased risk of developing osteoporosis because of the lengthened time of exposure to reduced estrogen. Currently, it is estimated that one in every two Caucasian women will experience an osteoporotic fracture during her lifetime, which increases the morbidity rate and cause a considerable economic burden in the aging female population [3]. Consequently, most studies have been conducted in aging postmenopausal women, while fewer studies focused specifically on changes in bone mineral density (BMD) and bone turnover markers in young women with POF.

The objective of this study was to compare the implications of premature ovarian failure on bone turnover markers and bone mineral density in patients under the age of 40.

Materials and Methods

Sixty-one patients, who were admitted to the Infertility Clinic of Istanbul University School of Medicine, with a diagnosis of POF were selected for this cross-sectional study. Patients were divided into two groups according to age, patients < 30 years old (n = 30) and patients ≥30 years old (n = 31). Approval of the ethics committee of Istanbul University and informed consent from all participants were obtained prior to the treatment.

Inclusion criteria were as follows: (1) at least four months of amenorrhea; (2) two FSH values of > 40 mIU/ml obtained at least one month apart; (3) Estradiol (E2) value of < 40 pg/ml; (4) age under 40 years old. Exclusion criteria were pre-existing hepatic, renal, metabolic or bone diseases or the use of drugs in the past three months that could influence bone metabolism, such as oral contraceptives and hormone replacement therapy.

For each study subject, the authors obtained information on age, height, weight, sexual activity level, obstetric-gynecological history (para/abortus, age at menarche, regularity of menstrual periods, cessation of periods, use of hormone replacement), med-

Revised manuscript accepted for publication January 3, 2013

ical history (previous chemotherapy or radiotherapy, pelvic surgery, existence of systemic diseases as thyroid, hyperprolactinemia, rheumatoid arthritis, hypertension, malignancy), and family history (POF in either mother or sister). Each patient underwent gynecologic examination, Pap smear and vaginal ultrasound. Body mass index (BMI) for each patient was calculated as weight (kg) divided by the square of the height (m^2). Menopause rating scale (MRS II), developed by Potthoff *et al*., was utilized to measure the severity of menopause symptoms [4]. Complete blood count, hormone profile (including luteinizing hormone (LH), FSH, E2, progesterone, prolactin (PRL), thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), dehydroepiandrosterone sulfate (DHEA-SO4), sex hormone binding globulin (SHBG), testosterone, thyroid peroxidase antibody (TPOAb), antithyroglobulin antibody, liver and kidney function, blood glucose test, and blood lipid tests were ordered for each patient. Karyotyping was performed in patients under the age of 30.

BMD Measurements

Both lumbar spine (L2-L4) and the total hip were chosen as measurement sites for BMD. BMD was measured by dual X-ray absorptiometry in the Osteoporosis Screening and Treatment Clinic of Istanbul University School of Medicine.

Blood biochemical analyses

Blood samples were obtained between 8:00-10:00 a.m. after an overnight fasting. Samples were centrifuged at 2,000 rpm for ten minutes at room temperature. The separated sera were stored at −80°C until analyzed.

Complete blood test count was performed on an autoanalyzer. Hormone profile, TPOAb ,and antithyroglobulin antibody were assayed by electrochemiluminescence immunoassay.

Serum bone-specific alkaline phosphatase (bALP) and procollagen type I C-peptide (PICP) were measured as markers of bone formation. BALP was assayed using an enzyme immunoassay kit and PICP was measured with two-step enzyme immunoassay. The interassay coefficients of bALP ranged from 5.0% to 7.6%. The intra-assay coefficients of bALP ranged from 3.9% to 5.8%. The interassay and intra-assay coefficients of PICP were 6.6% and 5.4%, respectively.

Cross-linked N-telopeptides of type I collagen (NTX) was measured as a marker of bone resorption. It was determined by enzyme immunoassay kit. The interassay coefficients of NTX ranged from 3% to 5%. The intra-assay coefficients of NTX ranged from 5% to 8%.

Statistical analysis

All values were expressed as mean ± SD. The corresponding data from BMD and bone turnover markers measurements were compared statistically using the Mann-Whitney U test. To determine the potential association of BMD and bone turnover markers, bivariate correlation analysis was used first to calculate Pearson's correlation coefficients. Multiple linear regression analysis was used to estimate which parameter was the most important index to predict BMD. BMD at the lumbar spine and the total hip were chosen as the dependent variable separately. A SPSS 15.0 software package was used for all statistical procedures; $p < 0.05$ was considered significant.

Results

All participants of the study were Caucasian. The mean age of the patients at the time of the study was 27.9 years (ranging 16 to 38). Out of 61 patients, 54 patients (89%) were diagnosed with POF and seven patients (11%) with hypogonadotropic hypogonadism. In 54 POF patients, seven were diagnosed with Turner syndrome (gonadal dysgenesis), while two patients experienced surgical menopause. 35% of the patients who were younger than 30-years-old were either married or had a partner, while 86% of the patients who were 30 years of age or older were either married or had a partner.

Table 1. — *Characteristics of patients.*

	Age		Levene's test		t-test
	< 30 n = 30	≥ 30 n = 31	F	*p*	*p*
Weight (kg) [a]	59.03 ± 12.14	65.33 ± 13.13	0.051	0.822	0.059
BMI (kg/m^2) [a]	23.13 ± 4.46	24.6 ± 5.21	0.206	0.652	0.221
MRS (0-44) [a]	10.93 ± 7.79	17.38 ± 8.62	0.357	0.552	0.004
BMD lumbar spine (t-score) [a]	-1.84 ± 1.47	-1.06 ± 0.93	5.465	0.023	0.021
BMD total hip (t-score) [a]	-0.83 ± 1.30	-1.07 ± 1.15	0.225	0.637	0.489
NTX (nmol) [a]	58.80 ± 21.32	41.1 ± 11.37	5.403	0.024	0.000
bALP (U/l) [a]	48.99 ± 42.16	23.76 ± 10.08	10.990	0.002	0.004
PICP (ng/ml) [a]	221.19 ± 136.93	171.65 ± 95.68	1.250	0.269	0.147
FSH (mIU/ml) [a]	58.79 ± 42.34	66.44 ± 34.99	2.024	0.160	0.450
LH (mIU/ml) [a]	27.56 ± 25.87	35.58 ± 20.75	0.625	0.433	0.209
E2 (pg/ml) [a]	22.80 ± 25.76	20.23 ± 11.86	1.721	0.195	0.631
Prolactin (ng/ml) [a]	13.52 ± 6.66	13.46 ± 8.89	1.265	0.266	0.978
TSH (mIU/l) [a]	2.57 ± 3.04	1.83 ± 0.991	0.536	0.468	0.242
DHEA-SO4 (µg/dl) [a]	170.39 ± 90.10	180.06 ± 72.89	2.918	0.098	0.736

[a] Values are expressed as mean ± SD.

Table 2. — *Correlation of BMD, BMI, and bone turnover markers.* [a]

	BMD (Total hip)	BMI	NTX	BALP	PICP
BMD (lumbar spine)	0.511	0.313	-0.665	-0.595	-0.340
BMD (total hip)		0.333	-0.098	-0.327	-0.009
BMI			-0.373	-0.304	-0.197
NTX				0.553	0.404
bALP					0.359

[a] Spearman correlation was used.

Between the two age sub-groups (< 30 and ≥ 30 years), there was not a significant difference in weight, BMI, period of menopause, total hip t-score, PICP, FSH, LH, E2, prolactin, TSH, and DHEA-SO4 (Table 1). There was a significant difference in MRS, lumbar spine t-score, NTX, and bALP between the two age groups (10.93 ± 7.79 vs 17.38 ± 8.62; -1.84 ± 1.47 vs -1.06 ± 0.93; 58.80 ± 21.32 vs 41.1 ± 11.37; 48.99 ± 42.16 vs 23.76 ± 10.08, respectively) (Table 1).

Table 2 presents correlations between BMI, BMD (lumbar spine and total hip t-scores), and bone turnover markers (NTX, BALP, and PICP). There was a significant negative correlation between lumbar spine t-score and NTX (p = -0.665), between lumbar spine t-score BALP (p = -0.595) and between lumbar spine t-score and PICP (p = -0.340). A significant negative correlation was also observed

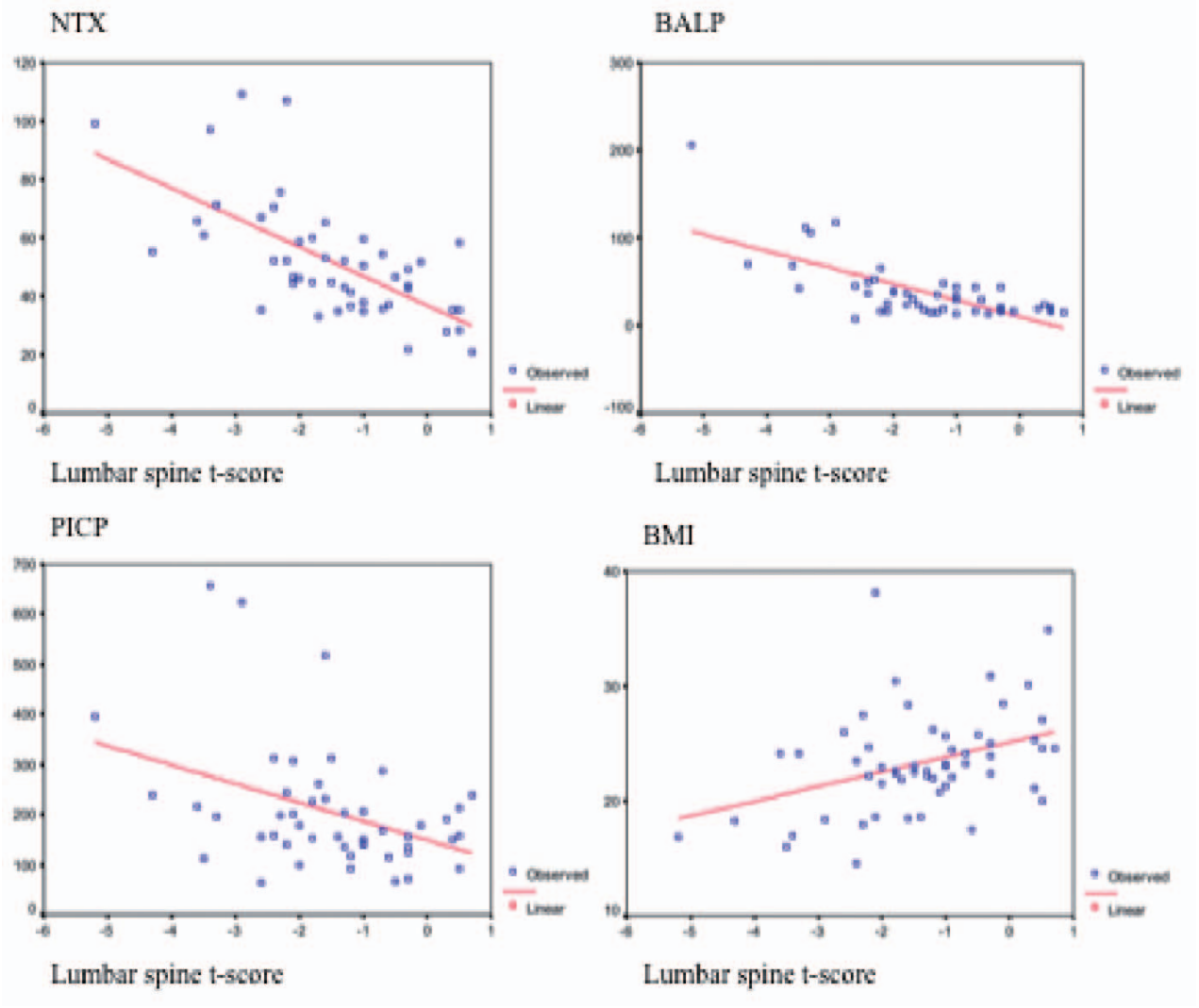

Figure 1. Regression analyses between BMD lumbar spine and NTX, bALP, PICP, and BMI.

between total hip score and bALP (p = -0.327). BMI had a significant negative correlation to NTX and bALP (p = -0.373; p = -0.304, respectively).

When lumbar spine t-score as the BMD measurement was further analyzed, there was a negative correlation between lumbar spine t-score and NTX, bALP, and PICP, while there was a positive correlation between lumbar spine t-score and BMI (Figure 1).

Discussion

Although there is a large body of literature examining BMD among postmenopausal women and a moderate body of literature examining BMD among premenopausal women, fewer studies have monitored BMD in women with POF. According to the present authors' limited knowledge, this study is the first to compare bone turnover markers and BMD in women with POF in two age groups. Thirty years of age was chosen as a benchmark, because it is a well-established fact that peak bone mass is reached around this age. Therefore, the authors wanted to classify and evaluate women with POF that are under 30 and above 30 years separately and compare the outcomes. This study revealed that women under the age of 30 had higher levels of bALP and NTX. On the other hand, MRS was higher in women who were 30 years of age or older.

BMD assessment measured by DXA is currently the "gold standard" for the diagnosis of osteoporosis. According to the World Health Organization (WHO) criteria, osteoporosis is defined by a t-score of -2.5 or below [5]. In the present study, all participants were osteoporotic since their t-score was well below -2.5. Lately, bone turnover markers, which reflect either bone formation or bone resorption cell activities, also play a significant role in assessing

BMD. For postmenopausal women, negative correlations are frequently reported between bone turnover markers and lumbar spine t-score, especially for bALP [6-10] and NTX [6-9, 11-15]. In premenopausal women, NTX is usually the only marker, which is significantly correlated with BMD [6, 7, 11, 13, 16]. The present study revealed a correlation between BMD and bone turnover markers. BALP, NTX, and PICP significantly correlated with lumbar spine t-score, while bALP significantly correlated with total hip t-score. In addition, the present study showed a significant negative correlation between BMI and NTX and between BMI and bALP, which is a further affirmation of the well-known correlation between BMI and BMD [17].

In cross-sectional analyses, bone resorption markers are consistently higher in postmenopausal, while bone formation markers are more variable [18-21]. Higher levels of bone turnover markers have been shown in many, yet not in all studies [19, 22-24]. Only a few number of studies have measured bone turnover markers in perimenopausal women or women with POF. In a cross-sectional analysis, NTX was measured in 2,375 participants who were either premenopausal or early perimenopausal [25]. NTX was slightly higher in the perimenopausal. However, the differences were not significant. In a very recent study, no individual bone turnover marker's increase was predictive for perimenopausal BMD loss [26]. In the present study, bALP and NTX were higher in women with POF who were younger than 30 years of age compared with women with POF who were 30 years of age or older. Reduced estrogen might have a more significant impact on BMD in younger women. On the other hand, it is a well-established fact that bone turnover markers increase significantly during puberty [27]. Since women who are younger than 30 years are still in late adolescent stage, their bone turnover marker levels might still be fluctuating.

When evaluating bone turnover markers, there is also the issue of reference intervals. Presently, studies on reference intervals are very limited and there are no established criteria. Blumsohn *et al.* compared bone turnover markers of healthy premenopausal women living in five different European countries and found no significant difference [28]. However, studies on healthy premenopausal women conducted in UK [29], USA [30], France [31], and Italy [32] reported different reference intervals. These inter-country variations may be related in part to factors such as BMI, alcohol consumption, or smoking as suggested by Glover *et al.* [33]. These authors conducted a study, including 637 healthy young premenopausal women from UK, France, Belgium, and the USA and established reference intervals for some of the bone turnover markers. The reference interval was 5.15–8.68 ng/ml for bALP and 9.22–24.8 nmol BCE/mmol for NTX. Unfortunately, there are no reference intervals regarding PICP as of now. In the present study, both bALP and NTX levels of all participants were well over the suggested reference interval levels.

In the present study, MRS score was significantly higher in women who were 30 years of age or older. The rate of marriage or being in a relationship was also higher in this sub-group. In general, perimenopausal and postmenopausal women present significantly higher rates of menopausal symptoms when compared to premenopausal women and subsequently, MRS scores usually increase in relation to age and the menopausal stage [34]. Moreover, being diagnosed with POF can be an upsetting diagnosis and women often express depression, anxiety, loss, and sadness. In addition, women with POF who are married or in a relationship may be facing the traumatic impact of potential infertility in their relationships or marriages. As quality and expectancy of life increase, even women in natural menopause stages care more about potential health issues they might face during this period. Facing menopause in early ages is devastating for young women with POF. Therefore, consulting patients is extremely important.

Lack of an age-matched, healthy control group was the main limitation of this study. In addition, some of the patients in the study had attempted medical therapy previously. The present authors believe that choosing a group of patients with initial diagnosis of POF may be a better approach for further studies.

Conclusion

It is apparent that BMD is commonly less in women with POF than normal healthy women. Therefore, measurement of BMD is warranted. At this time, it is not clear how often the tests should be carried out to evaluate BMD. Further prospective studies are required to establish guidelines. However, it seems reasonable to monitor women with POF yearly for the presence of any endocrine dysfunction and to assess BMD at periodic intervals.

Acknowledgement

This research received funding from the Scientific Research Projects Coordination Unit of Istanbul University (grant 611/17032005).

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