The Predictive Value of Osteocalcin, Granulin, Cathepsin K and Some Other Biomarkers in Women with Premature Ovarian Failure

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Abstract

Background: Premature ovarian failure (POF), often and misleadingly referred to as 'premature menopause', is defined as a loss of ovarian activity before the age of 40 years and is characterized by irregular or absent periods and reduced fertility. Symptoms include those associated with the natural menopause (night sweats and vaginal dryness), and with the long-term adverse effects of estrogen deficiency (osteoporosis and cardiovascular disease): the latter is believed to explain the shorter life expectancy associated with POF.

Aim: To determinethe predictive value of serum osteocalcin(OC), granulin(GRN), cathepsin k (CTK), vitamin D, parathyroid hormones (PTH), calcium and phosphrus as biomarkers for POF.

Method: Sixty (60) women with idiopathic POF with thirty (30) women as control groups were included in the study. Baseline investigation in all subjects included serum Osteocalcin(OC), granulin(GRN), cathepsin k (CTK), vitamin D, parathyroid hormones (PTH), calcium and phosphrus levels were estimated using the appropriate assays for each.

Results: The mean serum levels of Osteocalcin, Ganulin,Parathyroid hormones,vitamin D and phosphorus, were significantly higher in women with POF,when compared to healthy controls (P=<0.05). However, mean serum levels of cathepsin k and calcium in POF group were non significant differences,when compared to healthy controls.

Conclusion: Osteocalcin and granulin serum levels may be used as new biomarker for the diagnosis of premature ovarian failure.

Keywords: *Premature ovarian failure, Vitamin D, parathyroidhormones. Osteocalcin, Granulin, Cathepsin k.*

Introduction

Premature Ovarian Insufficiency (POI) can be spontaneous or iatrogenic and is defined as loss of ovarian function with development of hypergonadotropic hypogonadism in women under the age of 40 years [(1)]. Spontaneous POI affects approximately 1% of women and is associated with genetic defects, autoimmune disorders, environmental factors and infections, but is most commonly idiopathic [(2,3)]. Iatrogenic POI can occur secondary to surgical intervention (E.g. bilateral oophorectomy), chemotherapy and/or radiotherapy [(2,4)]. The effects of oestrogen deficiency include menopausal symptoms such as: vasomotor symptoms, insomnia, mood lability, and vulvo-vaginal atrophy. Longer-term consequences of POI include an increased risk of cardiovascular disease and mortality, accelerated cognitive impairment, infertility and osteoporosis [(2,4–6)].

Osteocalcin (OC), a bone-specific protein synthesized by the osteoblasts in bone, is the major non-collagen protein in the bone matrix. It has a molecular weight of 5,800 Da and contains 49 amino acids, including 3 gamma carboxyl glutamic acid residues that facilitate the binding of OC to hydroxyapatite in bone . The serum OC level, a sensitive marker of bone production, is associated

with a high bone turnover rate and decreased BMD, and correlates well with histomorphometric indices of bone formation[7]. Granulins are a large family of disulfide-rich proteins with diverse biological functions including wound healing, cell growth and proliferation modulation, and angiogenesis[8].Cathepsin K is a papain-like cysteine protease member of the cathepsin family of lysosomal proteases, a family categorized as consisting of cysteine (cathepsins B, C, F, H, K, L, O, S, V, X, and W), aspartate (cathepsins D and E), or serine (cathepsins A and G) proteases depending on the active site amino acid which mediates each member's catalytic activity[9]. An important aspect of cathepsin biology is based on their cellular localization. While cathepsins have greatest activity in acidic environments such as occurs along the endosomal lysosomal continuum, cysteine cathepsins secretion into the extracellular space has also been shown to occur under normal physiologic conditions including skeletal remodeling, wound repair, and prohormone processing[10] .

Vitamin D is synthesized from 7-dehydrocholesterol in the skin via UV-B radiation from the sun. This is an inactive form of vitamin D and requires two enzymatic hydroxylation reactions before activation. These are 25-hydroxylation and 1-a-hydroxylation [11]. While the first reaction takes place mainly in the liver with 25-hydroxylase, the second one occurs primarily in the kidneys with 1-a-hydroxylase (1,25-dihydroxyvitamin D [1,25(OH)2D3]) . This form of vitamin D has a high affinity for binding to vitamin D receptors in target tissue [12]. Renal 1-a-hydroxylase enzyme works under the control of sex hormones and endocrine factors [13]. Vitamin D is a major regulator of calcium phosphorus homeostasis and bone health. Recently, a number of non classical target organs including reproductive ones have been defined for vitamin D. This compilation study explores the potential effects of vitamin D on female reproductive functions. Vitamin D receptors are present in various tissue such as the immune system, endocrine system, and reproductive system [13]. vitamin D receptors are present in both the cytoplasm or nucleus of granulosa cells in ovaries [14]. The presence of vitamin D receptors in female reproductive tissue suggests that vitamin D may have a role in female reproductive functions.

Patient and Method

Study population and sample collection: The Women included in the present study were gathered from those attending Obstetrics and Gynecology Clinic in Kalar General Hospital, Kalar. The study conducted during the period from June 2019 to January 2020 and total of 60 POF women and 30 apparently healthy matched were included in the study.In this study, the eligibility criteria for POF cases included: (1) under 40 years of age at the first time of diagnosis; (2) amenorrhea for at least 4 months; (3) an increased FSH level >25 IU/L on two occasions >4 weeks apart; (4) patients with known causes of POF (such as karyotypic abnormalities, ovarian surgery, and autoimmune diseases etc.) were excluded. The eligibility criteria for the controls included: (1) healthy women with regular menstrual cycles; (2) without hormonal therapy in the last six months; (3) without endocrine system diseases, such as polycystic ovary syndrome, thyroid, and hyperprolactinemia etc. Peripheral blood of patients was collected at the time of interview with a structured questionnaire. Venous blood of patient women was collected so that the control . The blood was centrifuged immediately at 3000 r/min for 10 min, and the serum was collected in a polypropylene tube. The samples were stored at 80 C for further chemical analysis and hormone measurement.

Biochemical analysis: The serum osteocalcin, granulin, cathepsin k,vitamin D and PTH levels were measured by an automated Roche Modular Analytics E411 immunoassay system (Roche Diagnostics, Mannheim, Germany). Inter and intra-assay coefficient of variations (CVs) for all the tested hormones was less than 10%. Total calcium and phosphorus,were determined by Roche Cobas C111 Automatic Biochem Station (Roche Diagnostics, Germany).

Statistical analysis: The obtained data were analyzed using IBM SPSS statistical package (version 20). Student's t test was applied to calculate significance of differences between patients and controls groups.

Results

Determination the serum osteocalcin, granulin and cathepsin k: Mean serum level of osteocalcininpatient (47.35± 5.35 ng/ml) was significantly elevated (P=0.001)as compared with the concentration in the normal controls (17.21± 3.07 ng/ml). However, the mean serum level of Cathepsin K in patient group (0.27± 0.05 ng/ml) was not significantly (P=0.69) differ from that in control group (0.24± 0.07 ng/ml). While,a significant elevation (P=0.002)in the mean serum level of Granulin in patients (7.87± 0.53ng/ml) than in controls (5.44± 0.56 ng/ml), Table (1).

Table (1): Serum Biochemical hormones in patient cases & control

Parameter	Patient			Control			P
	Mean±Se	Min	Max	Mean±Se	Min	Max	
Osteocalcin ng\ml	47.35± 5.35	0.54	130.98	17.21± 3.07	0.54	60.21	00.001
Cathepsin K ng\ml	0.27± 0.05	0.01	1.61	0.24± 0.07	0.02	2.16	0.691
Granulin ng\ml	7.87± 0.53	2.55	20.00	5.44± 0.56	1.80	16.25	0.002

Determination the serum PTH, vitamin D,calcium and phosphorus: The Mean serum levels of parathyroid hormones and vitamin D were significantly lower (P<0.01) in patient (35.42±1.34,12.43±0.90 ng/ml), when compared with controls (46.53±1.77, 20.88±1.99 ng/ml) respectively. While the mean serum level of calcium showed a nonsignificant (P>0.05) in patient cases (9.63±0.07), when compared with control group (9.85±0.09), while the serum level of phosphorus demonstrated significant elevation (P=0.001),in patient cases (1.47±0.02), when compared with control group (1.34±0.02), Table (2) .

Table (2) : Serum bone hormones and maniralin patient cases & controle

Parameter	Patient			Control			P
	Mean±Se	Max	Min	Mean±Se	Max	Min	
PTH ng/ml	35.42±1.34	56.98	15.16	46.53±1.77	62.43	28.69	0.001
Vitamin D ng/ml	12.43±0.90	33.12	3.08	20.88±1.99	41.21	5.36	0.003
Calcium mg/dl	9.63±0.07	10.98	8.22	9.85±0.09	10.73	8.81	0.06
Phosphurse mmol/l	1.47±0.02	1.74	1.22	1.34±0.02	1.53	1.16	0.001

Discussion

In the present study, the mean serum osteocalcin level showed a statistically significant increase in women with POI when compared with apparently healthy control. This finding agreed the study of Singh, Sudhir; etal[15]. Additionally, the mean serum granulin level in POI cases was significantly increased in women with POF. This result in disagreement with that reported by Kan, Ozgur;etal[16]. While the mean serum value of cathepsin k did not shows a significant difference between patients and controls group.

Womens with POF show significant mean serum level of parathyroid hormones than in controls. In addition, VitD deficiency to be significantly more prevalent among POI patients than in controls. as well as the levels of phosphorus showing significantly highermean serum value in women with POI than in controls group. However, calcium mean serum level was not significantly different in women with POI as compared to controls group. This result in contrast with that reported by Ersoy, Ebru, et al [17]as they found no significant difference between Caucasian women POI (n=48) as compared to Caucasian women as controls group.

The present study shows no significant difference in the mean serum levels of vitD, PTH, phosphorus and calcium in women with POI as compared with controls. However, Kurabayashi, etal[18], reported a significant difference in serum PTH levels and non significant in the levels of serum calcium and phosphorus. Their finding was in agreement with the levels of PTH and conflicting with the levels of calcium and phosphorus when compared to controls group.

Conclusions

Women with POI demonstrated significantly higher serum osteocalcin and granulin level than healthy control, suggesting their use as biomarker for the diagnosis premature ovrian insufficiency .

Conflict of Interest: Nil

Source of Funding: Self, from Kalar Health Authority.

Ethical Clearance: The study design approved by the Ethical Committee of Tikrit University College of Medicine and informed consent was taken from each participant before enrollment in the study

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