Research Article

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Fibroblast growth factor-23 concentrations in polycystic ovary syndrome
Polikistik Over Hastalığında Fibroblast Büyüme Faktörü-23 düzeyleri

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Abstract

Objective: This study was designed to compare the serum concentrations of Fibroblast growth factor 23 (FGF23) among patients with PCOS and healthy subjects and to evaluate the relation between the hormonal and metabolic parameters.

Methods: Forty patients with PCOS were compared with 40 healthy individuals in a case-control study design. The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group 2003 guideline criteria were used in the diagnosis of PCOS. Serum intact FGF23 concentrations were determined by enzyme-linked immunosorbent assay (ELISA).

Results: Mean serum FGF23 concentrations were similar between PCOS group and control group (19.73 ± 16.75 pg/mL and 17.20 ± 9.26 pg/mL, p > 0.05). Waist circumference, hip circumference, total testosterone, Ferriman-Gallwey (FG) score and hsCRP were significantly higher in the PCOS group (p < 0.001). The concentrations of LH, DHEA-S, FSH, insulin, total cholesterol, triglyceride, HOMA-IR were significantly higher in the PCOS group when compared to control group (p < 0.05). FGF23 concentrations did not correlate with BMI, fasting glucose and insulin, HOMA-IR and lipid parameters.

Conclusions: FGF23 concentrations were similar in the PCOS group compared with the non-PCOS control group. The present findings may suggest that FGF23 is not a useful marker of metabolic disturbances including insulin resistance, dyslipidemia, and obesity in PCOS.

Keywords: PCOS; FGF23; Cardiovascular risk; Metabolic parameters; Insulin resistance.

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Significant differences were observed between the groups. FGF23 concentrations were not significantly correlated with BMI, fasting glucose, and insulin, HOMA-IR, and lipid parameters.

Özet

Amaç: PCOS'lu hastalarda FGF23 düzeylerini sağlıklı bireylerdeki ile karşılaştırmak ve FGF23 düzeylerinin hormonal ve metabolik parametreler ile ilişkisini değerlendirilmekti.

Yöntem: Bu vaka-kontrol çalışmasına PCOS'u olan 40 hasta ile sağlıklı 40 kadın alındı. Gruplar demografik, antropometrik ve biyokimyasal veriler ile serum FGF23 düzeylerine göre karşılaştırıldı. Serum FGF23 düzeyleri ELISA yöntemi ile insan FGF23 ELISA kitleri kullanılarak ölçülüldü.

Bulgular: Ortalama serum FGF-23 düzeyleri PCOS grubunda ve kontrol grubunda benzerdi (19.73 ± 16.75 pg/mL ve 17.20 ± 9.26 pg/mL, p > 0.05). PCOS grubunda viçut kitle indeksi (VKİ), bel çevresi, kalça çevresi, Ferriman–Gallwey skoru, total testosteron ve hsCRP düzeyleri kontrol grubuna göre yüksek bulundu (p < 0.001). PCOS grubunda LH, DHEA-S, FSH, insülin, total kolesterol, trigliserid düzeyleri ve HOMA-IR kontrol grubuna göre yüksek bulundu (p < 0.05). FGF23 düzeyleri ile VKİ, açık glukoz düzeyleri, insülin düzeyleri, HOMA-IR ve lipid parametreleri arasında ilişki saptanmadı.
Sonuç: Serum FGF-23 düzeyleri her iki gruba benzerdi. Bu çalışmada elde edilen bulgular, FGF23’ün PCOS’lu hastalarda insulin direnci, dislipidemi ve obezite gibi metabolik parametreler için kullanılabilecek bir serum belirteci olamayacağını düşündürmektedir.

Anahtar Kelimeler: PKOS; FGF23; Kardiyovasküler risk; Metabolik parametreler; Insulin direnci.

Introduction

Polycystic ovary syndrome (PCOS), the most commonly encountered endocrine disease of women, is a disorder characterized by ovulatory dysfunctions, elevated androgen concentrations, small cysts in ovaries, infertility, dermatologic manifestations, insulin resistance (IR), hyperlipidemia and weight gain [1–4]. Peripheral IR is found in the majority of patients with PCOS [5]. PCOS patients carry a higher risk of diabetes mellitus (DM) and cardiovascular disorders/mortality [6]. Fibroblast growth factor 23 (FGF23) is known to regulate phosphorus homeostasis, metabolism of vitamin D and bone mineralization. Elevated serum phosphorus concentration causes FGF23 secretion by osteocytes and osteoblasts. FGF23 is a hormone-like FGF like FGF19 and FGF21. FGF23 has endocrine, paracrine and autocrine effects since it less vigorously binds to the extracellular matrix when compared to other FGFs [7, 8]. Higher FGF23 concentration regardless of renal functions corresponds to an increased cardiovascular mortality risk in the community [9]. A recent meta-analysis of prospective cohort studies reported that higher FGF23 concentrations are associated with an elevated risk of all-cause mortality, cardiovascular disease events, death related to cardiovascular diseases and stroke [10]. Obesity is associated with increased concentration of FGF23 [11]. We aimed to evaluate the serum concentrations of FGF23 in PCOS patients without known cardiometabolic disease and the relation between the hormonal and metabolic parameters.

Materials and methods

Study population

A total of 80 women, aged 18–46 years, were enrolled in the study. Forty of them were diagnosed with PCOS, and the remaining 40 served as healthy controls in a case-control study design. Approval of Ethics Committee and informed consent of participants were obtained prior to the study. The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group 2003 guideline was used for diagnosis of PCOS [12].

Subjects with diabetes mellitus, cardiovascular disease, thyroid disorders, hyperprolactinemia, congenital adrenal hyperplasia, androgen-secreting tumors, Cushing’s syndrome, hypertension, hepatic or renal dysfunction, infectious diseases, drug use within last 3 months such as oral contraceptive agents, lipid lowering drugs, anti-hypertensive drugs, and insulin-sensitizing medications were excluded. Individuals with a history of oophorectomy or a different type of ovarian surgery and changes in weight for no fewer than 3 months were also excluded.

Clinical, biochemical and hormonal measurements

Weight, height, circumferences of waist (WC) and hip (HC), and systolic and diastolic blood pressure (BP) were measured. WC was determined by measuring the narrowest point between the costal margin and iliac crest at the end of a normal expiration. The BMI was calculated as weight (kg)/height (m)² [13].

A venous blood sample was collected after a 12-h overnight fast from each individual who is in the early follicular stage. The blood was obtained regardless of menstrual cycle stage in women with amenorrhea. Blood samples were collected into Becton Dickinson (BD) SST II gel tubes (Becton Dickinson, Plymouth, UK). The samples were preserved at −80°C after centrifugation. Homeostasis model assessment (HOMA-IR) was used for calculation of IR [14].

CKD-EPI creatinine equation; “141 * min (Scr/k, 1)α * max (Scr/k, 1)−1.209 * 0.993Age * 1.018 [if female] * 1.159 [if black]” where SCr is serum creatinine (in mg/dL), k is 0.7 for women and 0.9 for males, α is –0.329 for women and –0.411 for males, “min” is the minimum of SCr/k or 1, and “max” is the maximum of SCr/k or 1” was used for measurement of glomerular filtration rate [15].

Plasma glucose was determined with glucose oxidase method (Siemens ADVIA 2400 Chemistry System, Siemens Medical Solutions Diagnostics Tarrytown, NY, USA). The concentration of total cholesterol was determined by the enzymatic method (Siemens, ADVIA 2400 Chemistry System, Siemens Medical Solutions Diagnostics Tarrytown, NY, USA). Serum triglyceride was determined according to trinder method without serum blank system (Siemens ADVIA 2400 Chemistry System, Siemens Medical Solutions Diagnostics Tarrytown, NY, USA).
Low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured by elimination/catalase method (Siemens ADVIA 2400 Chemistry System, Tarrytown, NY, USA). High-sensitivity C-reactive protein (CRP) was determined by latex-enhanced immunoturbidimetric method (Siemens ADVIA 2400 Chemistry System, Tarrytown, NY, USA).

Thyroid stimulating hormone (TSH), insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, total testosterone, and estradiol were measured with chemiluminescence immunoassays (Advia Centaur XP, Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Serum 17-hydroxyprogesterone was determined by radioimmunoassay. Serum concentrations of total 25-(OH) vitamin D were measured by a chemiluminescent immunoassay (CLIA) method using an autoanalyzer (LIAISON DiaSorin, Italy).

Serum dehydroepiandrosterone sulfate (DHEAS) concentrations were measured with the chemiluminescent immunometric assay (Immule 2000 XP Systems, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). The coefficients of variation for biochemical and hormonal parameters are shown in Table 1.

### Measurement of FGF23

The concentration of FGF23 was measured using enzyme-linked immunosorbent assay (ELISA) method. Commercially available human FGF23 ELISA kit (Merck Millipore, MO, USA) was used. The procedure for the ELISA method was carried out based on the manufacturing company obtained instructions. The concentrations of FGF23 are presented as pg/mL. The intra-assay and interassay coefficient of variation were <11% and <11%, respectively.

### Table 1: The clinical, biochemical and hormonal results in women with polycystic ovary syndrome (PCOS) patients and healthy controls.

| Parameters                      | PCOS group | Control group | p-Value |
|---------------------------------|------------|---------------|---------|
|                                 | Mean       | SD            | Mean    | SD    |
| BMI (kg/m²)                     | 29.85      | 7.01          | 23.12   | 3.74  | <0.001 |
| Waist circumference (cm)        | 94.3       | 15.77         | 77.67   | 8.63  | <0.001 |
| Hip circumference (cm)          | 112.77     | 22.9          | 89.39   | 9.69  | <0.001 |
| Age (years)                     | 28.78      | 5.02          | 29.25   | 8.27  | 0.757  |
| Total testosterone (ng/dL)      | 66.01      | 25.16         | 41.28   | 15.79 | <0.001 |
| Estradiol (pg/mL)               | 75.28      | 48.33         | 128.11  | 75.66 | <0.001 |
| LH (IU/L)                       | 8.95       | 6.21          | 5.46    | 3.62  | 0.02   |
| DHEA-S (mg/dL)                  | 250.82     | 160.76        | 183.43  | 76.73 | 0.014  |
| 17-OH progesterone (nmol/L)     | 1.3        | 0.8           | 1.96    | 0.89  | <0.001 |
| FSH (IU/L)                      | 6.18       | 1.49          | 4.87    | 2.66  | 0.003  |
| FPG (mg/dL)                     | 85.79      | 9.34          | 82.59   | 8.96  | 0.126  |
| Insulin (mU/L)                  | 17.74      | 12.24         | 13.83   | 11.41 | 0.013  |
| Prolactin (ng/mL)               | 12.53      | 6.72          | 13.8    | 9.77  | 0.952  |
| TSH (mU/L)                      | 2.17       | 1.22          | 1.9     | 0.82  | 0.251  |
| FG score                        | 15.34      | 6.81          | 0.83    | 0.81  | <0.001 |
| Total cholesterol (mg/dL)       | 183.29     | 31.61         | 164.9   | 28.49 | 0.015  |
| Triglyceride (mg/dL)            | 116.34     | 59.94         | 83.42   | 35.93 | 0.011  |
| LDL-C (mg/dL)                   | 103.34     | 26.11         | 93.35   | 29.75 | 0.135  |
| HDL-C (mg/dL)                   | 55.68      | 24.56         | 55.82   | 13.61 | 0.172  |
| FGF-23 (pg/mL)                  | 19.73      | 16.75         | 17.2    | 9.26  | 0.406  |
| 25-OH vitamin D (ng/mL)         | 7.78       | 4.31          | 22.96   | 8.06  | <0.001 |
| Creatinine (mg/dL)              | 0.78       | 0.07          | 0.76    | 0.08  | 0.588  |
| GFR (mL/min/1.73 m²)            | 104.32     | 10.52         | 105.27  | 12.90 | 0.720  |
| HsCRP (mg/L)                    | 5.93       | 5.07          | 2.29    | 1.91  | <0.001 |
| SBP (mmHg)                      | 112        | 12.03         | 113.93  | 10.84 | 0.454  |
| DBP (mmHg)                      | 73.63      | 7.59          | 74.88   | 5.37  | 0.398  |
| HOMA-IR                         | 3.92       | 3.23          | 2.86    | 2.38  | 0.025  |

Bold values indicate clinically significant p-values. BMI, Body mass index; LH, luteinizing hormone; DHEA-S, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; FPG, fasting plasma glucose; TSH, thyroid stimulating hormone; HDL-C, high-density lipoprotein cholesterol; FG score, Ferriman–Gallwey score; LDL-C, low-density lipoprotein cholesterol; FGF-23, fibroblast growth factor 23; GFR, glomerular filtration rate; hs-CRP, high-sensitive C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment insulin resistance index.
Statistical analysis

All statistical analyses were performed using SPSS Version 15.0 (SPSS, Inc, Chicago, IL, USA). Data were expressed as the mean and standard deviation. Normality assumption was checked by Shapiro-Wilk test. Student’s t-test and Mann-Whitney U-test were used to compare differences between two independent groups with normal and non-normal distributions, respectively. Bivariate correlation coefficients (r) were calculated using the Pearson product moment formula or Spearman’s rank test, depending on normality of distribution.

Results

Forty patients with PCOS (mean age; 28.78±5.02 years) and 40 age-matched healthy controls (mean age; 29.25±8.27 years) were enrolled in the study. Clinical and biochemical characteristics of PCOS group and control group are shown in Table 1. Waist circumference, hip circumference, total testosterone, Ferriman-Gallwey (FG) score and hsCRP were significantly higher in the PCOS group (p < 0.001) (Table 1). The concentrations of LH, DHEA-S, FSH, insulin, total cholesterol, triglyceride, HOMA-IR were significantly higher in the PCOS group than the control group (p < 0.05). 25-OH vitamin D, estradiol and 17-OH progesterone concentrations were significantly lower in the PCOS group (p < 0.001). BMI was significantly higher in the PCOS group than the control group (p < 0.001). Mean FGF23 concentrations of the PCOS group were 19.73±16.75 pg/mL, while mean FGF23 concentrations were 17.20±9.26 pg/mL in the control group (p = 0.406). Mean creatinine and glomerular filtration rate (GFR) were similar between groups (p > 0.05). FGF-23 concentration was not correlated with the clinical, biochemical and hormonal parameters (Table 2). The PCOS group was divided into two groups according to the HOMA-IR concentrations. The cut-off point of 2.7 was assumed. Twenty four patients had an HOMA-IR greater than 2.7 (Mean FGF-23 concentration: 21.51±20.30), while 16 patients had an HOMA-IR lower than 2.7 (Mean FGF-23 concentration: 17.29±9.50). FGF23 concentrations were not different in both of the PCOS groups (p = 0.535).

Discussion

Our study shows that FGF23 concentrations were similar in patients with PCOS compared with those in age-matched control subjects. FGF23 concentrations did not correlate with BMI, fasting glucose and insulin, HOMA-IR and lipid parameters. To our knowledge, this study is the first to evaluate the FGF23 concentrations in PCOS patients.

PCOS patients carry a higher risk for DM and cardiovascular disease/mortality. Higher FGF23 concentration regardless of renal functions corresponds to an increased cardiovascular mortality risk in the community. In addition, individuals who have a combination of elevated FGF23 (>60 pg/mL), GFR lower than 60 mL/min and micro-/macro-albuminuria had an almost eight-fold elevated risk for cardiovascular mortality [9]. Mirza MA notified that elevated serum FGF23 concentrations correspond to impaired arterial vasoreactivity and increased the stiffness of arteries in the community [16]. Elevated serum FGF23 corresponds to increased total body atherosclerosis in the community and this association was stronger in subjects who have eGFR lower than 60 mL/min/1.73 m² [17]. Higher serum FGF23 concentrations are related to increased risk for left ventricular mass, hypertrophy, and geometry in elderly individuals and this relation was stronger in individuals who have eGFR lower than 60 mL/min/1.73 m² [18]. It is well-documented that PCOS carries an elevated cardiovascular risk and we aimed to evaluate FGF23 concentrations in PCOS without known

### Table 2: The correlation between FGF-23 concentrations and clinical, biochemical and hormonal parameters in PCOS group.

| Parameters        | Correlation coefficient | p-Value |
|-------------------|-------------------------|---------|
| BMI               | 0.269                   | 0.102   |
| Waist circumference (cm) | 0.211                 | 0.262   |
| Hip circumference (cm) | 0.250                  | 0.182   |
| Age (years)       | 0.192                   | 0.235   |
| Total testosterone (ng/dL) | −0.185                | 0.281   |
| Estradiol (pg/mL) | −0.020                  | 0.902   |
| LH (IU/L)         | −0.206                  | 0.201   |
| DHEA-S (µg/dL)    | −0.191                  | 0.257   |
| 17-OH progesterone (nmol/L) | −0.219                | 0.206   |
| FSH (IU/L)        | −0.030                  | 0.852   |
| FPG (mg/dL)       | 0.129                   | 0.433   |
| Insulin (mU/L)    | 0.143                   | 0.385   |
| Prolactin (ng/mL) | −0.084                  | 0.617   |
| TSH (mIU/L)       | 0.067                   | 0.682   |
| FG score          | 0.098                   | 0.558   |
| Total cholesterol (mg/dL) | 0.205                | 0.238   |
| Triglyceride (mg/dL) | 0.191                  | 0.272   |
| LDL-C (mg/dL)     | 0.066                   | 0.706   |
| HDL-C (mg/dL)     | 0.005                   | 0.979   |
| 25-OH vitamin D (ng/mL) | −0.054                | 0.750   |
| HsCRP (mg/L)      | −0.023                  | 0.892   |
| SBP (mmHg)        | 0.060                   | 0.714   |
| DBP (mmHg)        | 0.143                   | 0.378   |
| HOMA-IR           | 0.175                   | 0.287   |
cardiovascular disease. In this study, we found FGF23 concentrations were similar between groups.

The possible role of FGF23 in the pathophysiology of cardiovascular disorders might partially be explained by its participation in the complex mechanisms of vascular calcification [19]. 1,25(OH)\textsubscript{2}D\textsubscript{3} is the main regulator of FGF23 production via osteoblasts in bone and increased FGF23 concentration cause reduction in 1,25(OH)\textsubscript{2}D\textsubscript{3} concentration [20]. Decrease in 1,25(OH)\textsubscript{2}D\textsubscript{3} can cause elevated angiotensin II production via an increase in the renin expression resulting in hypertension and cardiac hypertrophy [21–23]. FGF23 requires a cofactor known as \(\alpha\)-klotho for activation of FGF signaling [24]. Soluble Klotho protects the heart via inhibition of transient receptor potential cation channel, subfamily C, member six (TRPC6) whose overexpression leads to cardiac hypertrophy and remodeling [25]. Isakova et al. propounded that elevated concentration of FGF23 leads to klotho deficiency [26]. Andrukhova et al. reported that FGF23 increase renal sodium reabsorption thus causing hypertension and cardiac hypertrophy [27]. It has been reported that concentration of FGF23 correlates with different inflammatory markers [28, 29]. Decrease in the concentration of 1,25(OH)\textsubscript{2}D\textsubscript{3}, reduction in expression of soluble klotho, activation of the renin-angiotensin system, an increase in sodium retention in kidney, increase in inflammatory markers could be the indirect effect of FGF23 on the cardiovascular system. Although FGF23 concentrations were higher in PCOS patients, no statistically significant difference was found between groups. This result might be explained by the non-homogenous distribution of the parameters. Increased FGF23 concentration in patients with PCOS may partly explain elevated cardiovascular risk in those patients. A study with a higher number of patients may be able to show a significant difference.

There are conflicting results about the association between FGF23 and IR. A recent study demonstrated a positive correlation between FGF23 and interleukin 6, interleukin 10, hsCRP and insulin resistance in subjects without chronic kidney disease [30]. In one study, it was notified that FGF23 concentrations are directly correlated with IR [31]. Besides, another study showed that FGF23 concentrations were significantly lower in obese patients with IR [32]. Holeciki et al. reported that elevated FGF23 concentrations were not related to IR or obesity [33]. In light of this information, we divided our PCOS patients into two groups according to the HOMA-IR concentrations. FGF23 concentrations were not different in both of the groups. FGF23 concentrations were not correlated with insulin resistance in our study.

FGF23 concentrations were similar between groups and did not correlate with other clinical, biochemical and hormonal parameters. It can be assumed that the possible role of increased FGF23 concentration in cardiovascular disease might be dependent on different mechanisms such as vascular calcification rather than association with clinical, biochemical and hormonal parameters.

FPG concentrations were similar between PCOS and control groups. It is known that, fasting plasma glucose is not a sensitive method of screening for T2DM in patients with PCOS [34] and this is suggested by our results.

A relatively small sample size and being a single center study are limitations of this study.

Conclusions

FGF23 concentrations were similar in the PCOS group compared with the non-PCOS control group. The present findings may suggest that FGF23 is not a useful marker of metabolic disturbances, including insulin resistance, obesity, and dyslipidemia in patients with PCOS however, extensive studies covering larger populations are needed to enlighten the relationship between FGF23 and PCOS.

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