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ORIGINAL ARTICLE

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Fractalkine: an inflammatory chemokine elevated in subjects with polycystic ovary syndrome

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Abstract

Purpose Fractalkine (FKN) is an inflammatory chemokine related to reproductive system and glucose metabolism. There is a link between FKN and steroidogenesis as FKN induces progesterone synthesis. Polycystic ovary syndrome (PCOS) is a common reproductive and metabolic disorder associated with low progesterone production and insulin resistance. We aimed to explore whether women with PCOS have any difference in FKN levels compared to women without PCOS. We also focused on determination of any association between FKN levels and hormonal-metabolic parameters in women with PCOS.

Methods The current research was designed as a case–control study. Eighty subjects with PCOS and 80 age- and body mass index (BMI)-matched subjects with normal menstrual cycle were taken into the study. We measured circulating FKN levels via ELISA methods.

Results Circulating FKN levels were higher in women with PCOS than controls (1.93 ± 0.61 vs. 1.22 ± 0.33 ng/ml, $P < 0.001$). FKN levels showed a positive correlation with body mass index (BMI), insulin resistance, inflammatory marker hs-CRP, total testosterone, and free-androgen index (FAI), whereas it showed a negative correlation with sex hormone-binding protein in women with PCOS. Linear regression analyses revealed that the link of FKN with BMI, insulin resistance, hs-CRP, and FAI was independent. Binary logistic regression analysis showed that the risk of having PCOS was associated with high levels of FKN.

Conclusions Increased FKN levels related to insulin resistance, inflammation and androgens in women with PCOS. FKN may have an inter-related role in different pathophysiologic pathways of PCOS.

Keywords Polycystic ovary syndrome · Fractalkine · Insulin resistance · Inflammation · Body mass index · Hyperandrogenism

Abbreviations

BMI	Body mass index
CI	Confidence interval
CV	Coefficient of variability
DBP	Diastolic blood pressure
DHEA-S	Dehydroepiandrosterone sulfate
FAI	Free-androgen index
FBG	Fasting blood glucose
FG	Ferriman–Gallwey
FKN	Fractalkine
GDM	Gestational diabetes mellitus
GnRH	Gonadotropin-releasing hormone
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment of insulin resistance
hs-CRP	High-sensitivity C-reactive protein
KO	Knockout
LDL-C	Low-density lipoprotein cholesterol
OR	Odds ratio
PCOS	Polycystic ovary syndrome

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ROC	Receiver operator characteristic
SBP	Systolic blood pressure
SHBP	Sex hormone-binding protein
T2DM	Type 2 diabetes mellitus
VIF	Variance inflation factor
WT	Wild type

Introduction

Fractalkine (FKN)/CX3CL1 is a pluripotent inflammatory chemokine, which plays a vital role in different pathophysiological processes such as inflammation, atherosclerosis, glucose metabolism, and reproduction [1–8]. FKN contributes to inflammation through subsiding of chemotaxis and adhesion of leukocytes and natural killer cells. The effect of FKN is activated following attachment to its receptor CX3CR1, a G-protein coupled receptor. FKN and its receptor are mainly expressed in activated endothelial, activated fibroblast, monocytes, T-cells, natural killer cells, adipocytes, and ovaries [1–6]. In addition, accumulating data confirmed the presence of a link between FKN and reproductive system [5, 7]. FKN plays a role in production of ovarian steroidogenesis including progesterone. It was reported that hCG-stimulated progesterone levels were found to be elevated after treatment with FKN in cultured rodent and human ovaries in a dose-dependent manner. FKN expression was also increased following hCG treatment in rodent ovaries [5, 7]. Besides, FKN involves in glucose metabolism through stimulating glucose-dependent insulin secretion, as well as improving glucose hemostasis [8].

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of the reproductive aged women. PCOS is associated with reproductive and metabolic abnormalities, as well as chronic low-grade inflammation. The disorder is characterized by clinical and/or laboratory hyperandrogenism, menstrual dysfunctions, and polycystic ovaries. Albeit the pathophysiology of PCOS has not been clearly identified, it is implicated that hypothalamic-pituitary-ovarian axis abnormality and insulin resistance play a major role in development of the disorder [9–13]. In women with PCOS, increased frequency of hypothalamic gonadotropin-releasing hormone (GnRH) pulses increase secretion of luteinizing hormone frequency by which it induces production of androgens in ovarian theca cells. Progesterone suppresses GnRH pulse acts as a part of feedback mechanism. PCOS subjects have relatively low progesterone levels affecting inappropriate secretion of GnRH [13, 14].

There is no sufficient data regarding to the relationship between FKN and PCOS. FKN secretion in follicular fluid and granulosa cells of women with and without PCOS who were under treatment of GnRH antagonist was investigated. The results showed that FKN secretion was reduced in follicular fluid and granulosa cells of women with PCOS [15]. We aimed to compare circulating FKN levels in women with and without PCOS in the absence of any treatment. We also tried to find any relationship between FKN levels and hormonal-metabolic parameters in women with PCOS.

Materials and methods

Study design for subjects

Two different groups including 80 PCOS subjects and 80 age- and body mass index (BMI)-matched subjects with normal menstrual cycle were recruited into this case–control study. The age range of the involved subjects was between 18–45-years-old. The research lasted for 6 months starting from January 2018 to July 2018 in internal medical and endocrinology departments, Training and Research Hospital in Izmir, Turkey. All subjects' body max index (BMI) was between BMI < 18.5 and BMI ≥ 35 kg/m^2 and none of them were alcohol and tobacco consumers. A single specialist, who was responsible for both physical and detailed history examinations, analyzed the whole subjects. Furthermore, a standard 75 g 2-h oral glucose tolerance test (OGTT) was considered for all subjects.

PCOS group

PCOS subjects were chosen using Rotterdam consensus criteria after excluding other causes of hyperandrogenism and ovulatory dysfunction. Although considering two criteria out of three is sufficient for diagnosis of PCOS disorder, we followed all three following criteria in PCOS diagnosis to reach to a proper homogeneity [16]. The above-mentioned criteria were identifying oligo- and/or anovulation, identification of biochemical and/or clinical signs of hyperandrogenism–use of the Ferriman–Gallwey [FG] for hirsutism determination [17] and occurrence of ≥12 follicles with the size of 2–9 mm in diameter or an ovarian volume of >10 ml (without a cyst or dominant follicle in either ovary) for determination of typical ultra-sonographic symptoms of polycystic ovaries as one ovary is sufficient for diagnosis.

The subjects with FG score ≥8 were shown as hirsute. The allocation of biochemical hyperandrogenism was detected when testosterone (normal range: 0.52–2.42 nmol/l), and/or dehydroepiandrosterone sulfate (DHEA-S) (normal range: 10–248 µg/dl), and/or free androgen index (FAI) ≥5% [18] of serum levels were more than the reference interval limitation.

Control group

The subjects were chosen either from the women who visited endocrinology or gynecology or internal medical clinics for routine checkup or from the employees who were volunteer to join to the study from the hospital. The subjects in control group had normal menstrual cycle. None of the subjects of control group had concomitant health problems, acne, hyperandrogenism, or signs of hirsutism.

Exclusion criteria

The subjects without the proper necessary criteria given below were excluded from this study. Criteria for exclusion were: irregularity in menstrual cycles and/or androgen over-excess (i.e., Hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, or other diseases of the adrenal gland, thyroid disorders, galactorrhea, breastfeeding, and pregnancy), decreased levels of glucose tolerance or having type 1/type 2 diabetes, familial hyperlipidemia, having background of hypertension, suffering from liver/renal disorders or congestive heart failure, having history of coronary artery disease, malignancy or acute infection (within 14 days), gestational diabetes mellitus, presence of any chronic inflammatory or autoimmune disorders and treatment of hormonal contraception and/or anti-androgen (within the preceding 6 months). Moreover, taking medications for dyslipidemia, hypertension, hyperglycemia, insulin resistance or obesity was an excluding criterion for the current study as well.

Anthropometric evaluation

Detailed standards of subjects including anthropometric measurements comprising age, weight (kilogram), and height (centimeter) together with waist circumference were measured while the subjects were barefoot and in casual clothes. The lower rib margin and the iliac crest that was the end of a gentle expiration' midway was measured for waist circumference (cm) estimation. After a 15-minute resting period, the blood pressure of all tested subjects was measured as the subjects were in sitting position. The formula for BMI: weight (kg)/square meter of height (m^2) was used for calculation of BMI value.

Biochemical evaluation

The venous blood samples of the subjects were taken from the antecubital veins during the early follicular phase of menstrual bleeding (3rd to 5th days), either spontaneous or progesterone-induced menses, in the morning (between 08:00 a.m.–09:00 a.m.) after a 10 h fasting term. The blood samples were kept in room temperature for at least 30 min to be clotted form. The clotted samples were centrifuged at 2000 x *g* for 15 min for separation. The separated serum samples were kept in aliquots at −80 °C for analysis of FKN. Some other criteria such as fasting blood glucose (FBG), serum insulin, glycated hemoglobin $A1_C$ ($HbA1_C$), total cholesterol, low- and high-density lipoprotein cholesterol (LDL-C and HDL-C), total testosterone, DHEA-S, luteinizing hormone (LH), triglycerides, sex hormone-binding-globulin (SHBG), estradiol (E_2), follicle-stimulating hormone (FSH), 2-h plasma glucose, high-sensitivity C-reactive protein (hs-CRP), and FKN levels were also measured. Some dedicated kits (Abbott Diagnostics, Wiesbaden, Germany) from an auto-analyzer (Abbott Architect C 16000, IL, USA) were used for level test of several criteria in serum of test subjects such as FBG and 2-h plasma glucose, serum hs-CRP, total cholesterol, triglyceride and total HDL-C. The calculation of LDL-C was done using the Friedewald equation (LDL-C = total cholesterol-(HDL-C + Triglyceride/5)) [19]. Chemiluminescent microparticle immunoassay (CMIA) with its dedicated kits (Abbott Diagnostics, Wiesbaden, Germany), as well as auto-analyzer, Abbott Architect I2000, IL, USA, were used for measurement of serum insulin levels. High-performance liquid chromatography (ADAMS $A1_C$ HA-8160; Arkray Inc., Menarini Diagnostics, Firenze, Italy) was used for measuring the levels of $HbA1_C$. The following standards including LH, FSH, E_2, DHEA-S, and total testosterone levels were also measured via CMIA (UniCel DXI 800, Beckman Coulter Inc., Brea, CA, USA). In addition, SHBG level was measured using chemiluminescence immunoassay technique (Immulite 2000 XPi, Simens Healthcare Diagnostics, Eschborn, Germany). The formula FAI: (total testosterone/SHBG) × 100 was used for FAI measurement. Each sample tested for insulin resistance via homeostasis model assessment of insulin resistance: HOMA-IR = fasting insulin (μU/ml) × fasting glucose (mg/dl)/405 [20].

Measurement of circulating FKN by ELISA

Human ELISA kits (Catalog number DCX310, R&D systems, Minneapolis, MN, USA) were used to measure serum FKN levels (in duplicate) following the instructions of manufactures. The rates between <6% and <8% were considered as the intra-assay coefficient of variability (CV) and the inter-assay CV respectively. A level of circulation FKN range is between 0.2 and 10 ng/ml.

Statistical analysis

We compared the results of both PCOS and control groups using Social Sciences software version 18.0 (SPSS Inc. Chicago, IL, USA). The distribution of variables was

checked using Kolmogorov–Smirnov test in each group and numeric variables showed normal distribution. The results were shown as mean with standard deviation (SD). The variables were compared using a *t*-test. The correlation of FKN with other parameters was analyzed using Pearson's correlation coefficient. To find independent relationship between FKN and other parameters, a multiple regression analysis was used, PCOS status was added as a covariate into the model. Odds ratio (OR) was calculated to explore the existence of a link between FKN levels (tertile) and having PCOS using a multivariate binary logistic regression analysis. The potential confounders including age, BMI, HOMA-IR, hs-CRP, and FAI were added into the model for appropriate adjustment. The utility for circulating FKN levels to predict PCOS was calculated via the receiver operator characteristic (ROC) curve analysis. The level of 95% for confidence interval (CI) was utilized as well. A two sided *P*-value < 0.05 was considered as statistically significant.

Results

Clinical and laboratory characteristics of the study population

The results of the parameters are given in Table 1. Circulating levels of FKN were elevated in subjects with PCOS when compared with controls (1.93 ± 0.61 vs. 1.22 ± 0.33 ng/ml, $P < 0.001$) (Fig. 1a). We divided PCOS patients into two subgroups as being insulin resistant or not (HOMA-IR > 2.71 and HOMA-IR ≤ 2.71) [21]. Out of 80 subjects, 49 were diagnosed as being insulin resistant. As

Table 1 Comparison of the demographic and laboratory characteristics of the subjects

Variables	PCOS $n = 80$	Controls $n = 80$	P^a
Age, years	30.00 ± 6.92	29.88 ± 6.97	0.911
BMI, kg/m^2	26.28 ± 4.53	26.99 ± 4.73	0.336
Waist circumference, cm	93.11 ± 12.70	92.04 ± 14.95	0.626
SBP, mmHg	108.76 ± 12.66	107.53 ± 11.60	0.523
DBP, mmHg	74.32 ± 6.71	73.24 ± 5.92	0.285
Ferriman–Gallwey score	14.64 ± 2.80	4.28 ± 1.25	<0.001*
FBG, mg/dl	83.77 ± 8.26	81.22 ± 6.01	0.028*
2-h OGTT, mg/dl	123.93 ± 12.82	121.14 ± 11.67	0.152
$A1_C$, %	5.28 ± 0.18	5.26 ± 0.19	0.610
Insulin, μIU/ml	17.37 ± 6.32	10.96 ± 4.50	<0.001*
HOMA-IR	3.62 ± 1.45	2.19 ± 0.89	<0.001*
Total cholesterol, mg/dl	206.13 ± 34.30	201.99 ± 43.51	0.505
LDL-C, mg/dl	136.01 ± 29.10	131.19 ± 27.40	0.282
HDL-C, mg/dl	41.57 ± 9.82	48.85 ± 10.83	<0.001*
Triglycerides, mg/dl	143.34 ± 33.48	109.72 ± 29.73	<0.001*
Hs-CRP, mg/l	1.22 ± 0.57	0.67 ± 0.20	<0.001*
FSH, mIU/ml	6.91 ± 1.84	7.23 ± 1.91	0.272
LH, mIU/ml	13.89 ± 4.15	8.41 ± 2.92	<0.001*
Estradiol, pg/ml	50.71 ± 12.58	49.05 ± 8.11	0.323
Progesterone, ng/ml	1.08 ± 0.22	1.13 ± 0.25	0.188
Total testosterone, nmol/l	2.90 ± 0.43	1.69 ± 0.35	<0.001*
SHBG, nmol/l	37.58 ± 12.10	68.43 ± 14.82	<0.001*
FAI, %	8.21 ± 1.74	2.48 ± 0.11	<0.001*
DHEA-SO_4, μg/dl	184.32 ± 73.34	152.62 ± 39.10	0.001*

Results are given in mean ± SD

A1c glycosylated hemoglobin, *BMI* body mass index, *DHEA-S* dehydroepiandrosterone sulfate, *DBP* diastolic blood pressure, *FAI* free androgen index, *FBG* fasting blood glucose, *FSH* follicle-stimulating hormone, *HDL-C* high-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance, *Hs-CRP* high-sensitivity C-reactive protein, *LDL-C* low-density lipoprotein cholesterol, *LH* luteinizing hormone, *PCOS* polycystic ovary syndrome, *SBP* systolic blood pressure, *SHBG* sex hormone-binding globülin, *2-h OGTT* 2-h oral glucose tolerance test

[a]Independent samples *t*-test was used. A *P*-value of < 0.05 was considered significant (*)

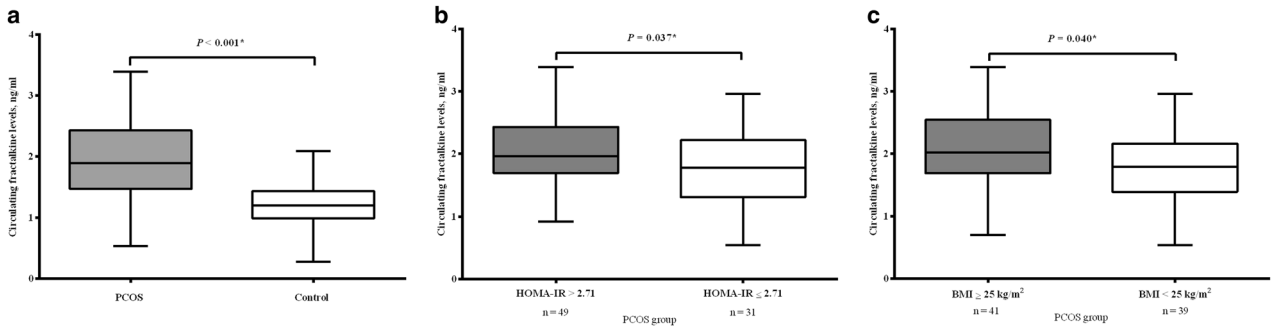

Fig. 1 **a** Circulating fractalkine levels in PCOS and control groups. **b** Circulating frackalkine levels in PCOS women with and without insulin resistance. **c** Circulating fractalkine levels in PCOS women according to BMI (normal-weight: BMI < 25 kg/m^2; overweight/obese: BMI ≥ 25 kg/m^2). A *P*-value of <0.05 was considered significant (*)

Table 2 Correlation coefficient between fractalkine levels and clinical parameters

	Fractalkine			
	PCOS		Control	
	r	*P*	*r*	*P*
Age	0.091	0.134	0.058	0.201
BMI	0.269	0.021*	0.253	0.034*
Waist circumference	0.278	0.018*	0.244	0.037*
Insulin	0.314	0.007*	0.115	0.035*
FBG	0.209	0.013*	0.122	0.044*
2-h OGTT	0.102	0.215	0.092	0.108
HOMA-IR	0.265	0.011*	0.113	0.041*
A1c	0.103	0.089	0.076	0.212
FSH	0.115	0.108	0.128	0.164
LH	0.087	0.125	0.110	0.234
Estradiol	0.175	0.102	0.093	0.255
Progesterone	0.141	0.098	0.107	0.114
SHBP	−0.138	0.003*	−0.067	0.066
Total testosterone	0.317	0.016*	0.101	0.054
FAI	0.218	0.009*	0.098	0.063
DHEA-S	0.105	0.118	0.045	0.366
Hs-CRP	0.274	0.007*	0.132	0.031*
Total cholesterol	0.148	0.113	0.107	0.122
LDL-C	0.045	0.412	0.087	0.346
HDL-C	−0.112	0.144	0.042	0.158
Triglycerides	0.189	0.091	0.102	0.118

Pearson's correlation analysis was used. *r*: Pearson's correlation coefficient. A *P*-value of <0.05 was considered significant (*)

A1c glycosylated hemoglobin, *BMI* body mass index, *DHEA-S* dehydroepiandrosterone sulfate, *DBP* diastolic blood pressure, *FAI* free androgen index, *FBG* fasting blood glucose, *FSH* follicle-stimulating hormone, *HDL-C* high-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance, *Hs-CRP* high-sensitivity C-reactive protein, *LDL-C* low-density lipoprotein cholesterol, *LH* luteinizing hormone, *PCOS* polycystic ovary syndrome, *SBP* systolic blood pressure, *SHBG* sex hormone-binding globülin, *2-h OGTT* 2-h oral glucose tolerance test

shown in Fig. 1b, PCOS patients with insulin resistance had significantly elevated circulating FKN levels compared to those PCOS patients without insulin resistance (2.03 ± 0.57 vs. 1.74 ± 0.63 ng/ml, $P = 0.037$). We next stratified PCOS subjects into two subgroups according to their BMI levels (<25 and ≥25 kg/m^2). There were 39 subjects with BMI < 25 kg/m^2 and 41 subjects with BMI ≥ 25 kg/m^2. We compared FKN levels in PCOS subjects according to their BMI. As shown in Fig. 1c, overweight/obese women had significantly higher circulating FKN levels than lean subjects in PCOS groups (2.07 ± 0.62 vs. 1.79 ± 0.57 ng/ml, $P = 0.040$). Moreover, we discovered the elevated levels of serum insulin, FBG, HOMA-IR, and hs-CRP in subjects with PCOS. Circulating FSH, estradiol and progesterone levels did not show significant difference between groups whereas LH levels were significantly higher in PCOS group with respect to controls. SHBG levels were significantly lower in PCOS group than controls.

Correlation of fractalkine with other parameters

The results of the link between FKN and other parameters gained from Pearson's correlation coefficient are given in Table 2. The results showed that FKN levels were positively correlated with BMI, HOMA-IR, and hs-CRP in both PCOS and control groups. FKN levels were not correlated with FSH/LH and progesterone/estrogen levels. Moreover, FKN showed a positive correlation with total testosterone and FAI, whereas it displayed a negative correlation with SHBG in only PCOS group.

Multivariate regression analysis

The results regarding to independent link between FKN and other parameters obtained from a multiple linear regression are given in Table 3. The results revealed that FKN levels were independently associated with BMI, HOMA-IR, hs-CRP, and FAI.

Table 3 Multiple linear regression analysis of correlated variables with fractalkine levels in all study population ($R^2 = 0.511$)

	β	95% CI		P
		Lower	Upper	
Variables				
Age	0.103	−1.356	1.562	0.216
BMI	1.689	0.442	2.936	0.031*
HOMA-IR	2.467	1.033	3.899	0.019*
FAI	2.243	0.967	3.519	0.011*
Hs-CRP	1.975	0.631	3.319	0.024*

Multiple linear regression analysis was used. β: unstandardized regression coefficient; A P-value of <0.05 was considered significant (*).

BMI body mass index, *CI* confidence interval, *HOMA-IR* homeostasis model assessment of insulin resistance, *Hs-CRP* high-sensitivity C-reactive protein, *FAI* free-androgen index

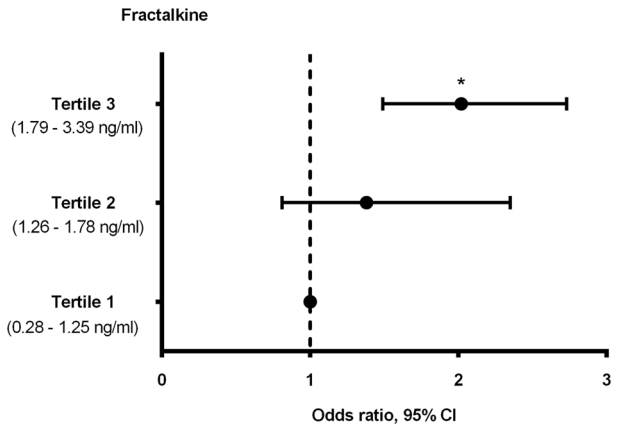

Fig. 2 Association of fractalkine with PCOS in adjusted models. Multivariate adjusted OR for having PCOS according to the tertiles of fractalkine (reference, the lowest tertile). Model adjusted for age, BMI, HOMA-IR, FAI, and hs-CRP. OR: odds ratio; CI: confidence interval. A P-value of <0.05 was considered significant (*)

Multivariate binary logistic regression analysis

The results of finding the link between FKN levels and PCOS risk obtained from multivariate logistic regression analysis are shown in Fig. 2. The results displayed that the highest tertile of FKN levels in subjects tended to high possibility of PCOS risk with respect to the lowest tertile of FKN levels (OR = 2.02, 95% CI = 0.1.49–2.73, $P = 0.006$*).

The receiver operator characteristic (ROC) curve analysis

The ROC curve for circulating FKN levels to predict PCOS is shown in Fig. 3. Based on ROC analysis, the best cutoff value of circulating levels of FKN was calculated to be 1.69 ng/ml with a sensitivity of 82% and a specificity of

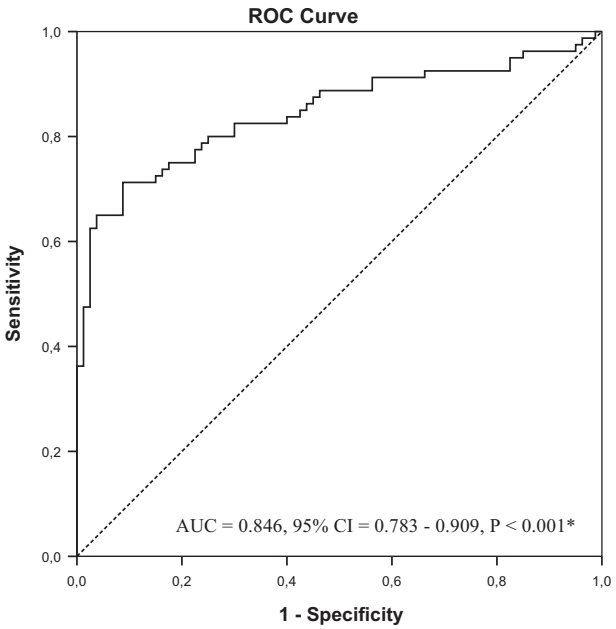

Fig. 3 Receiver operating characteristic (ROC) curve analysis of fractalkine for the prediction of PCOS. The best cutoff value of circulating fractalkine levels was 1.69 ng/ml with a sensitivity of 82% and a specificity of 70% for prediction of PCOS. AUC: area under curve; CI: confidence interval. A P-value of < 0.05 was considered significant (*)

70% and the under curve area of 0.846 (95% CI = 0.783–0.909, $P < 0.001$*) for PCOS prediction.

Discussion

In the current study, for the first time, we evaluated circulating levels of FKN related to glucose metabolism, inflammation and ovarian steroidogenesis in women with PCOS known as metabolic and reproductive disorder of reproductive aged women. We found that circulating FKN levels were higher in women with PCOS than controls. FKN levels also displayed an elevation in PCOS subjects with insulin resistance compared to PCOS subjects without insulin resistance. FKN showed an independent association with insulin resistance, hs-CRP, androgens and BMI whereas FKN levels did not display any relation with FSH/LH and progesterone/estrogen. Moreover, Binary logistic regression analysis revealed that the risk of having PCOS was associated with high levels of FKN. ROC curve analysis showed that the best cutoff value for FKN levels to predict PCOS was 1.69 ng/ml with a sensitivity of 82% and a specificity of 70%.

Proper ovarian follicular development is a complicated process resulted by the multiple interactions between systemic and local influences of some factors such as hormones, adipokines and cytokines [13, 22]. Although the

pathophysiology of PCOS is not completely known yet, hypothalamic-pituitary-ovarian axis dysfunction is related to development of the disorder. GnRH secretion is strongly regulated via feedback mechanisms by gonadal steroids especially progesterone. PCOS subjects have elevated levels of LH and decreased levels of progesterone. Impaired regulation of GnRH is related to defect of progesterone feedback mechanism in PCOS [13]. It is reported that FKN is involved in increasing progesterone synthesis [5, 7]. In a study, FKN levels were found to be decreased in follicular fluid and granulosa cells in women with PCOS when compared with controls as both groups were under treatment of GnRH antagonist for infertility. In the same study, decreased levels of FKN were correlated with low levels of progesterone production and reduced expression of steroidogenic acute regulatory protein (StAR), playing a role in steroid hormone production, in the granulosa cells of patients with PCOS. Following, it was also added that FKN administration increased progesterone and StAR expression in granulose cells [15]. In the present study, we investigated the relation of circulating levels of FKN with LH/FSH, progesterone/estrogen and FAI in women with PCOS. Inconsistently, we found that circulating levels of FKN were elevated in women with PCOS. FKN levels showed a positive correlation with FAI, total testosterone whereas it displayed negative correlation with SHBG. In addition, FKN levels did not show any correlation with FSH/LH and progesterone/estrogen levels in women with PCOS. The discrepancy of the results of our study and above-mentioned study could be due to the difference between local and systemic measurements of FKN as the systemic interactions of FKN with other parameters such as inflammation and metabolic pathways may affect FKN secretion. Additionally, from obtained results of the current study, we could suggest that increased FKN levels in women with PCOS may be the result of impaired CX3CR1 signaling pathway. Apart from this, the different roles of local and systemic FKN in PCOS could be due to the effects of some other involved parameters altered in women with PCOS.

PCOS subjects have increased levels of a variety of inflammatory markers. Moreover, chronic low-grade inflammation contributes to pathogenesis of PCOS inducing insulin resistance and ovarian dysfunction. Therefore, many researchers focused on inflammatory markers to shed a light to discover the pathogenesis of the disorder [23, 24]. FKN is an inflammatory chemokine, also induced by pro-inflammatory cytokines, affects over adhesion and chemotaxis of leukocytes contributing to inflammation [2, 6]. In a study, FKN showed a positive correlation with various inflammatory markers in subjects with T2DM [25]. In the present study, we found that hs-CRP were highly elevated in subjects with PCOS. Constantly, we found an independent association between hs-CRP and FKN levels. Elevation of FKN levels in women with PCOS may result to occurrence of inflammation.

The link between FKN and glucose metabolism has been reported [8]. In a study CX3CR1 knockout (KO) mice showed defect of insulin secretion in response to glucose and glucagon-like peptide-1. FKN administration increased insulin secretion and improved glucose tolerance in wild-type (WT) mice, whereas CX3CR1 KO mice did not show any remarkable difference [8]. FKN levels were also investigated in metabolic disorders of human. Circulating levels of FKN were found to be elevated in subjects with type 2 diabetes (T2DM) and obesity [26]. In a clinical study, positive association of FKN with insulin levels were also reported [27]. On the other hand, Ebert et al. reported that circulating FKN levels did not show any significant difference between gestational diabetes mellitus (GDM) and controls but FKN levels were negatively associated with insulin resistance in women with GDM [28]. In another study, FKN levels did not show any significant difference among healthy weight, overweight and obese Mexican American children [29]. Alteration of FKN levels was not found in relation with body composition [27]. The relation of CX3CR1 gene polymorphism with T2DM and obesity was also investigated. It was reported that nucleotide gene polymorphism placed over CX3CR1 was associated with low possibility of FKN binding and increased prevalence of T2DM [26]. Gene polymorphism of CX3CR1 was also associated with obesity [30]. PCOS is a metabolic disorder associated with higher levels of insulin as a consequence of insulin resistance. It is inferred that insulin acts together with LH for stimulating androgen production in theca cells in PCOS [13]. Consistently, in the present study, we found that circulating insulin levels and insulin resistance were elevated in subjects with PCOS. FKN levels were elevated in PCOS subjects with insulin resistance compared to PCOS subjects without insulin resistance. Moreover, we discovered that overweight/obese PCOS subjects had significantly elevated levels of FKN with respect to lean PCOS subjects. We also found that elevated FKN levels in women with PCOS were independently associated with insulin resistance and BMI.

We had some limitations in our study. We used HOMA-IR formulation for evaluation of insulin resistance instead of insulin clamp technique, a gold standard method but invasive. We did not evaluate CX3CR1 gene polymorphisms in our study population. Albeit we are aware of this fact that cross-sectional designed study does not give causality, it supplies the discovery of the link between molecules and disorders.

In conclusion, elevated FKN levels in women with PCOS were related to insulin resistance, hs-CRP, BMI, and androgens in women with PCOS. FKN may be considered as an inter-relater in different pathophysiologic pathways of PCOS. Moreover, interaction between FKN and its receptor

CX3CR1 may have the potential role in inflammatory-based metabolic disorders such as PCOS, obesity, and T2DM but this issue needs further investigations.

Author contributions M.C., I.D., A.G., P.A., A.M.I., and O.U. participated in study design and B.A. and M.C. performed ELISA. M.C., I.D., A.G., P.A., A.M.I., and B.A. participated in study design, analyzed the data, wrote, reviewed, and edited the manuscript. M.C., I.D., A.G., O.U., and P.A. provided serum samples and contributed to discussions of data interpretation. All authors reviewed and edited the manuscript. M.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Our study was approved by ethics committee of Bozyaka Training and Research Hospital. The written informed approval was received from each recruited subject. All the subjects were joined to the research adjusting with the principles of Declaration of Helsinki (revised in 2008).

Informed consent The subjects gave their oral and written informed consent before their inclusion in the study. The study adhered strictly to the principles of the Declaration of Helsinki as revised in 2008.

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