DLK1 and Nesfatin-1 levels and the relationship with metabolic parameters in polycystic ovary syndrome: Prospective, controlled study

Polikistik over sendromunda DLK1 ve Nesfatin-1 düzeyleri ve metabolik parametrelerle ilişkisi: Prospektif kontrolü çalışma

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Abstract

Objective: Delta-like 1 (DLK1) is known to inhibit adipocyte differentiation and nesfatin-1 is a neuropeptide that plays a role in the regulation of nutrition and metabolism. We aimed to assess both the levels of DLK1 and nesfatin-1 in polycystic ovary syndrome (PCOS) and determine the association of DLK1 and nesfatin-1 with metabolic parameters.

Materials and Methods: Forty-four patients with PCOS and 40 healthy women as the control group were included in this study. Venous blood samples of the participants were collected, and hormonal, metabolic parameters, DLK1 and nesfatin-1 blood levels were determined. Anthropometric parameters were also determined. For a double comparison, the Mann-Whitney U test was used for non-parametric numerical data, and Student’s t-test was used for parametric numerical data. Bivariate correlations were investigated using Spearman’s correlation analysis. The diagnostic performance of the parameters was evaluated using receiver operating characteristic curve analysis.

Results: The findings showed that DLK1 and nesfatin-1 levels were lower among the PCOS group, and the differences in these values were found to be statistically significant. A significant negative correlation was found between DLK1 levels and body mass index (BMI), waist/hip ratio, visceral adiposity index (VAI), fasting serum insulin (FSI), homeostasis model of assessment-insulin resistance (HOMA-IR) and triglyceride levels. A significant negative correlation was found between nesfatin-1 levels and BMI, VAI, FSI, HOMA-IR and triglyceride.

Conclusion: The findings showed that DLK1 and nesfatin-1 levels were lower in PCOS. Based on this study, DLK1 may be culpable for metabolic disorders in PCOS and can be a novel marker for PCOS in the future.

Keywords: Polycystic ovary syndrome, delta-like 1, nesfatin-1, visceral adiposity index, insulin resistance

Öz

Amaç: Delta benzeri 1’in (DLK1) adiposit farklılaşmasını inhibe ettiği bilinmektedir ve Nesfatin-1 beslenme ve metabolizmanın düzenlenmesinde rol oynayan bir nöropeptiddir. Polikistik over sendromunda (PCOS) hem DLK1 hem de Nesfatin-1 düzeylerini değerlendirirken ve DLK1 ve Nesfatin-1’ in metabolik parametrelerle ilişkisini belirlemeye çalıştık.

Gereç ve Yöntemler: Kırk dört PCOS tanısı alan kadın ve kontrol grubu olarak da 40 sağlıklı kadın çalışmaya dahil edildi. Katılımcıların venöz kan örnekleri alınarak hormonal, metabolik parametreler, DLK1 ve Nesfatin-1 kan düzeyleri belirlendi. Antropometrik parametrelerde de belirlendi. İkili karşılaştırma için parametrik olmayan sayısal veriler için Mann-Whitney U testi, parametrik sayisal veriler için Student t-testi kullanılıldı. İki değişkenli korelasyonlar, Spearman’nın korelasyon analizi ile araştırıldı. Parametrelerin tanısal performansı alıcı işletim karakteristiği eğri analizi ile değerlendirildi.

Bulgular: Bulgular, DLK1 ve Nesfatin-1 düzeylerinin PCOS grubunda daha düşük olduğunu ve bu değerlerdeki farklılıkların istatistiksel olarak anlamlı olduğunu gösterdi. DLK1 seviyeleri ile vücut kitle indeksi (VKİ), bel kalça oranı (BKO), visceral adipozite indeksi (VAI), açlık serum insülini (FSI), HOMA-IR ve trigliserit seviyeleri arasında anlamlı negatif korelasyon bulundu.

Keywords: Polycystic ovary syndrome, delta-like 1, nesfatin-1, visceral adiposity index, insulin resistance
Sonuç: Bulgularımız, DLK1 ve Nesfatin-1 düzeylerinin PCOS’dada daha düşük olduğunu gösterdi. Bu çalışmaya dayanarak, DLK1 PCOS’daki metabolik bozukluklarından sorumlu olabilir ve gelecekte PCOS için yeni bir belirti olabilir.

Anahtar Kelimeler: Polikistik over sendromu, delta benzeri 1, Nesfatin-1, viseral adipoz indeksi, insulin direnci

Introduction

Polycystic ovary syndrome (PCOS) is an endocrinopathy prevalent among women of reproductive age, characterized by oligomenorrhea, amenorrhea, hirsutism, and polycystic ovaries. Its prevalence ranges from 6% to 20%, depending on the diagnostic criteria. Environmental and genetic issues are associated with the etiology of PCOS by combining with obesity, ovarian dysfunction, and hormonal factors. Risk of type 2 diabetes mellitus (T2DM), hypertension, dyslipidemia, cerebrovascular disease, central obesity, and cardiovascular morbidity related to metabolic dysfunction increases in PCOS. In addition to these, insulin resistance (IR) is also a remarkable factor for the pathophysiology of PCOS, which exacerbates underlying metabolic abnormalities.

Delta-like 1 (DLK1) is also called preadipocyte factor 1 or fetal antigen protein and is a transmembrane protein very similar to the Notch/Delta/Serrate family. It is expressed by a gene present in the long arm of chromosome 14 (14q32.2). It has been revealed to be associated with the Temple syndrome, clinical findings of which include prenatal and postnatal growth failure, central precocious puberty, truncal obesity, hypotonia, as well as small hands and feet. The primary function of DLK1 is known to inhibit adipocyte differentiation. DLK1 is highly expressed in preadipocytes, whereas it disappears during adipogenesis and is not detected in adipocytes. Thus, it is also considered a marker for preadipocyte. Adipose tissue development is impacted by genetic background, hormonal balance, diet, and physical activity. An increase in the number of fat cells due to the differentiation of preadipocytes into adipocytes leads to obesity. Moreover, in vitro studies have revealed that DLK1 improves insulin synthesis and secretion. Gomes et al. detected PCOS in 20% of women who had precocious puberty with the DLK1 mutation. Upon this finding, they reported that women diagnosed as having both precocious puberty and PCOS should be examined related to DLK1 mutations. However, levels of DLK1 in PCOS have never been probed before, despite there being common clinical and metabolic characteristics that can be detected in PCOS and with the DLK1 mutation.

Nesfatin-1 is a nucleobindin-2 induced neuropeptide that plays a role in the regulation of nutrition and metabolism. It was primarily identified in the central nervous system and then expressed in gastro-endocrine cells, adipocytes, and pancreatic beta cells. Nesfatin-1 has been shown to increase glucose-induced insulin secretion in pancreatic β cells by increasing Ca²⁺ flow through L-type channels in mice. Furthermore, nesfatin-1 also has an anorexigenic effect by lessening the number of meals and extending the interval between meals. Due to these metabolic properties, levels of nesfatin-1 were assessed in PCOS, which frequently co-exists with obesity and IR, and it was determined to be lower in some studies, whereas it was determined to be higher in others. In our study, we aimed to assess both the levels of DLK1, which have never been evaluated related to PCOS previously, as well as the serum levels of nesfatin-1 in PCOS, which has contradictory results in previous studies regarding its levels in PCOS, both of which are closely associated with obesity and IR.

Materials and Methods

This case-control study was performed between January 2020 and August 2020 in the Department of Obstetrics and Gynecology, Faculty of Medicine, at Yozgat Bozok University after obtaining ethical approval from the ethics committee (protocol number: 2017-KAEK-189_2019.09.11_04). The principles of the Declaration of Helsinki (Fortaleza, Brazil, 2013) were adhered to throughout this study and written informed consent was obtained from all participants. A power analysis was conducted using the G*Power version 3.1.7 software based on the findings of comparable studies. An effect size of 0.81 was used with a power set at 0.95 and alpha at 0.05. We specified the explanations of the abbreviations as soon as they were first written, determining that an n=40 sample size was required in each group.

Study Population

Forty-four patients with PCOS aged between 18 and 39 years who were admitted to the gynecology outpatient clinic and met the 2003 Rotterdam criteria were included in this study, and 40 healthy women who did not meet the PCOS diagnostic criteria and had regular menstrual cycles (26-30 days) were included as the control group. The diagnosis of PCOS was reached based on the Rotterdam criteria, those who met two of the three specified criteria were diagnosed as having PCOS: oligo and/or anovulation (>35 days or <8 spontaneous menstruation/year), biochemical and/or clinical (Ferriman-Gallwey score >8) hyperandrogenism, and polycystic ovary on ultrasound (each 12 or more follicles of 2 to 9 mm in diameter and/or ovarian volume >10 mL in each ovary). Morphologic features of the ovaries in all participants were examined through transabdominal and/or transvaginal ultrasonography (GE Voluson E8; GE Healthcare, Chicago, IL, USA).

The participants did not receive any medications (ovulation induction agents, glucocorticoids, antiandrogens, oral contraceptives, insulin sensitizers, or anti-obesity drugs) for at least the last 6 months that could influence the biochemical profile and metabolic variables. Pregnant women, smokers, women in early menopause, women who were breastfeeding, and those diagnosed as having Cushing syndrome, hypertension,
adrenal hyperplasia, hyperprolactinemia, diabetes mellitus, thyroid dysfunction, and androgen-secreting tumors were excluded from the study.

Height and weight measurements of all women were recorded, and body mass index (BMI) was calculated as weight/height² (kg/m²). Waist circumference (cm) was determined by measuring the circumference of the midpoint of the junction of the 10th rib and spina iliaca anterior superior. Hip circumference (cm) was measured as the circumference of the greater trochanter line. The waist/hip ratio (WHR) was calculated. All measurements were performed by the same person.

**Laboratory Measurements**

Venous blood samples were collected between 8:00 and 9:00 AM after overnight fasting. All analyses were performed on the same day, except nesfatin-1 and DLK1. Blood samples for nesfatin-1 and DLK1 were centrifuged for 10 min at 3,000 rpm, after which the supernatant was quickly removed and kept frozen at -80 °C until the assays were performed by a specialist who was blind to patient status. Nesfatin-1 was measured in blood samples using a Human nesfatin-1 enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, Cat.No E3063Hu, Shanghai, China), with a measurement interval of 0.3-90 ng/mL. DLK1 was measured in blood samples using a Human DLK1 ELISA kit (Bioassay Technology Laboratory, Cat. No E5959Hu, Shanghai, China), with a measurement interval of 20-6000 ng/L. Fasting glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were analyzed on a Roche COBAS 6000 c501 (Roche Diagnostics) autoanalyzer. Low-density lipoprotein cholesterol was calculated using the Friedewald formula when the TG level was less than 400 mg/dL.  

**Hormonal Assay**

Blood samples were collected on the 2nd or 3rd days of the menstrual cycle. Serum insulin, luteinizing hormone (LH), follicle-stimulating hormone, estradiol, dehydroepiandrosterone, thyroid-stimulating hormone, and prolactin levels were measured using an electrochemiluminescence immunoassay on a Roche COBAS 6000 e601 (Roche Diagnostics, Mannheim, Germany) autoanalyzer. The concentrations of serum nesfatin-1 and DLK1 were measured using commercially available ELISA kits (Bioassay technology laboratory, Shanghai, China), according to the manufacturer's instructions. 

IR was determined using the homeostatic model assessment IR index (HOMA-IR): fasting plasma glucose (mg/dL) x fasting serum insulin (FSI) (mU/mL)/405(17). Visceral adiposity index (VAI) is a sensitive marker of visceral obesity, which uses both anthropometric and metabolic parameters. VAI was calculated using the following formula: [Waist circumference/(36.58 + (1.88×BMI)] x (Triglyceride/0.81) x (1.52/HDL-C).  

**Statistical Analysis**

In this study, data were analyzed using the SPSS 20 software (IBM Corp. released 2011, IBM SPSS Statistics for Windows, version 20.0, Armonk, NY: IBM Corp.). Data are presented as mean ± standard deviation. Continuous variables were examined using analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk’s test) to investigate whether they were normally distributed. For a double comparison, the Mann-Whitney U test was used for non-parametric numerical data, and Student’s t-test was used for parametric numerical data. Bivariate correlations were investigated using Spearman’s correlation analysis. The diagnostic performance of the parameters was evaluated using receiver operating characteristic (ROC) curve analysis. The results were considered to be statistically significant at p<0.05.

**Results**

The demographic characteristics and biochemical values of the PCOS and control groups are shown in Table 1. As shown in Table 1, when the PCOS group was compared with the control group, it was determined that DLK1 and nesfatin-1 levels were lower among the PCOS group, and the differences in these values were found to be statistically significant (p=0.001 and p<0.001, respectively). The correlation analysis of the parameters, which were assessed for the patients with PCOS using DLK1 and nesfatin-1, is presented in Table 2. A significant negative correlation was found between DLK1 levels and BMI/WHRI/VAI (r=-0.368, p=0.014; r=-0.409, p=0.006; and r=-0.359, p=0.017; respectively). Also, a significant negative correlation was determined between DLK-1 levels and FSI/HOMA-IR/TG levels (r=-0.302, p=0.047; r=-0.336, p=0.026; and r=-0.332, p=0.028; respectively). A significant negative correlation was also found between nesfatin-1 levels and BMI/VAI values (r=-0.307, p=0.042; and r=-0.339, p=0.024; respectively). Moreover, a significant negative correlation was also determined between nesfatin-1 levels and levels of FSI/HOMA-IR/TG (r=-0.369, p=0.014; r=-0.355, p=0.018; and r=-0.423, p=0.004, respectively). The optimal ROC cut-off value of DLK1 for PCOS was calculated as 2018.8 ng/L with a sensitivity of 70% and a specificity of 68.1% [area under the curve (AUC): 0.801]. The optimal ROC cut-off value of nesfatin-1 for PCOS was calculated as 18.6 ng/mL with a sensitivity of 72.5% and a specificity of 72.7% (AUC: 0.805) (Figure 1, Table 3).

**Discussion**

We found in our study that the serum levels of DLK1, known as an inhibitor of adipocyte differentiation, and nesfatin-1, an anorexigenic neuropeptide, were decreased among women with PCOS. We also determined a significant negative correlation between both nesfatin-1 and DLK1 levels and BMI, VAI, and HOMA-IR in PCOS. It was found in previous studies that, in mice, which DLK1-null(10) and overexpressed DLK1(20,21), IR developed,
accompanied by weight abnormalities. This finding suggests that favorable development of adipose tissue and function is critical for maintaining glucose/insulin homeostasis. Lee et al. (22) administered DLK1 to mice for 4 weeks and revealed that DLK1 manifested its metabolic effects by activating AMPK, increasing fatty acid oxidation in the liver, and inhibiting gluconeogenesis. Ultimately, they determined a decrease in IR and fasting blood glucose in DLK1-administered mice. IR also plays a remarkable role in the pathophysiology of PCOS and IR has been reported in 50-80% of women with PCOS, and a correlation between insulin levels and PCOS severity has been demonstrated (17). Similarly, we determined a significant negative correlation between DLK1 and HOMA-IR among the PCOS group in our study.

| Variable          | DLK1 r-value | p-value | Nesfatin-1 r-value | p-value |
|-------------------|--------------|---------|--------------------|---------|
| BMI               | -0.368       | 0.014   | -0.307             | 0.042   |
| WHR               | -0.409       | 0.006   | -0.229             | 0.136   |
| FBG               | -0.218       | 0.155   | 0.078              | 0.613   |
| FSI               | -0.302       | 0.047   | -0.369             | 0.014   |
| HOMA-IR           | -0.336       | 0.026   | -0.355             | 0.018   |
| VAI               | -0.359       | 0.017   | -0.339             | 0.024   |
| HDL               | 0.168        | 0.275   | 0.122              | 0.432   |
| LDL               | -0.078       | 0.615   | 0.082              | 0.599   |
| Total cholesterol | -0.094       | 0.543   | 0.034              | 0.825   |
| Triglyceride      | -0.332       | 0.028   | -0.423             | 0.004   |
| FSH               | -0.092       | 0.551   | -0.026             | 0.867   |
| LH                | -0.062       | 0.688   | 0.148              | 0.336   |
| Total testosterone| -0.085       | 0.584   | 0.065              | 0.676   |

Values are given as mean ± standard deviation. *Mann-Whitney U test, **Independent simple t-test. BMI: Body mass index, WHR: Waist-to-hip ratio, FBG: Fasting blood glucose, FSI: Fasting serum insulin, HOMA-IR: Homeostasis model of assessment-insulin resistance, VAI: Visceral adiposity index, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, PCOS: Polycystic ovary syndrome.

In one study, the weight of large fat stores (inguinal, retroperitoneal, and gonadal) was significantly higher in DLK1-null mice compared with the control group. Furthermore,
Table 3. ROC analysis of DLK1 and Nesfatin-1 for patients with PCOS

|          | Cut-off level | AUC  | Sensitivity (%) | Specificity (%) | p-value |
|----------|---------------|------|-----------------|-----------------|---------|
| DLK-1 (ng/L) | 2018.8        | 0.801 | 70              | 68.18           | <0.001  |
| Nesfatin-1 (ng/mL) | 18.6          | 0.805 | 72.50           | 72.73           | <0.001  |

ROC: Receiver operating characteristic; DLK1: Delta-like 1, PCOS: Polycystic ovary syndrome, AUC: Area under the curve

free fatty acids, cholesterol, and circulating levels of TGs, which are often associated with obesity, also increased in these mice(7). Similarly, Dauber et al.(21) demonstrated an increase in the percentage of body fat, which was shown by electrical impedance analysis that visceral abdominal fat was predominant in some women with a DLK1 deletion. On the other hand, mice overexpressing DLK-1 had resistance to high-fat diet-induced obesity, which was accompanied by a remarkable reduction in adipose tissue mass(22). These studies corroborate the fact that DLK1 deficiency increases adipogenesis and DLK1 is a negative regulator of adipogenesis. In addition to menstrual irregularity and hyperandrogenism in women with PCOS, another common clinical problem is obesity, and the risk of metabolic syndrome is increased fourfold. Fifty percent of women with PCOS are overweight or obese, and central obesity is common among them as well. Fat accumulation in the central abdominal region has been observed even among women with normal weight PCOS(24-26). VAI is recommended for clinical practice because it is useful in the evaluation of IR and cardiometabolic risk(27). In line with this, we assessed the VAI of all women and determined a higher VAI and WHR among the PCOS group in our study. Also, we found a negative correlation between serum levels of DLK1 and VAI and BMI in the PCOS group, in line with the literature.

Although the above-mentioned studies support our results, some studies suggest otherwise. For instance, Jensen et al.(28) revealed that there was a positive correlation between DLK1 levels and both body fat and HOMA-IR. These contradictory findings suggest that the accurate dosage and timing of DLK1 might be crucial for its metabolic impact on adipose tissue. This concept is also endorsed by a study suggesting that the tightly regulated dosage control of DLK1 is significant for its regulatory function in neurogenesis(29). These varying results could be attributed to differences in populations of the studies and/or the use of different DLK1 test kits.

Given the anorexigenic effect of nesfatin-1, as well as its increasing effect on insulin secretion, it is not surprising that we determined lower nesfatin-1 levels among patients with PCOS in our study. In previous studies, it has been shown that nesfatin-1 is lower in women with both T2DM and gestational diabetes mellitus (GDM) than in healthy individuals. T2DM and GDM show similar properties to PCOS due to weight and IR(30,31). It has been shown that intravenous administration of nesfatin-1 to mice increases the effect of insulin and decreases glucose concentrations(32). However, contrary to our study, nesfatin-1 was found to be higher in PCOS in some studies(9,14), and Ademoglu et al.(9) showed that this result could arise from nesfatin-1 resistance, which is characterized by impaired receptor and post-receptor signaling in target tissues. Moreover, a positive correlation has been revealed in some studies, whereas some studies demonstrated a negative correlation between nesfatin-1 levels and BMI and HOMA-IR, consistent with our study(12,14). We consider that these differences stem from the heterogeneity of the study groups. Kim et al.(33) demonstrated that nesfatin-1 was expressed in the hypothalamus and ovaries of mice and argued that nesfatin-1 might have a regulatory role on the hypothalamo-pituitary-ovarian (HPO) axis. In another study, it was shown that nesfatin-1 injections reduced LH and gonadotropin-releasing hormone expression in the hypothalamus(34). Therefore, it can be thought that nesfatin-1 could prevent the development of PCOS by both reducing BMI with its appetite-inhibiting effect and by its regulatory role on the HPO. In the light of this information, it is an expected result that we found that nesfatin-1 was less in women with PCOS in our study.

Study Limitations

Our study had some limitations. The first limitation was that free testosterone and sex hormone-binding globulin values were not examined. The second limitation was that we did not classify the PCOS group according to phenotypes. Thus, we need more data from patients with PCOS, such as AES/Rotterdam criteria and women with different phenotypes to document whether DLK1 and nesfatin-1 are associated with PCOS, not merely fat metabolism. On the other hand, the fact that we evaluated the DLK1 level for the first time in PCOS is the main strength of our study.

Conclusion

We found that both DLK1 and nesfatin-1 levels were lower in PCOS. Moreover, we determined that both proteins were negatively correlated with BMI, VAI, and HOMA-IR in PCOS. Based on this study, it is considered that DLK1 may be culpable for metabolic disorders in PCOS and could be a novel marker for PCOS in the future. However, more detailed in vivo and in vitro studies are needed to clarify the effects of both molecules on weight gain and ovarian function.

Ethics

Ethics Committee Approval: This case-control study was performed between January 2020 and August 2020 in the Department of Obstetrics and Gynecology, Faculty of Medicine, at Yozgat Bozok University after obtaining ethical approval from the ethics committee (protocol number: 2017-KAEK-189_2019.09.11_04).
Informed Consent: Written informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.D.C., A.C., Concept: M.D.C., A.C., Design: M.D.C., A.C., Data Collection or Processing: S.E.Y., Analysis or Interpretation: S.E.Y., Literature Search: D.A.K., E.B., E.S.Y., Writing: M.D.C.

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