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ISSN: 0423-104X

e-ISSN: 2299-8306

The Impact of Dicer, Drosha and Exportin-5 levels in Polycystic Ovary Syndrome (PCOS) Diagnosis and Phenotyping

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DOI: 10.5603/EP.a2021.0096

Article type: Original paper

Submitted: 2021-06-24

Accepted: 2021-09-20

Published online: 2021-11-04

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Running title: Dicer, Drosha, and Exportin-5 levels in PCOS

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is a very common heterogeneous endocrine and gynaecological disease in reproductive women. Early identification and treatment of patients are necessary to prevent future cardiometabolic and reproductive complications. In our study, we aimed to investigate whether Drosha, Exportin-5, and Dicer, which are involved in miRNA formation, are useful markers in the diagnosis of the disease.

Material and methods: Patients who presented to our clinic with complaints such as menstrual irregularity, hirsutism, and acne were diagnosed with polycystic ovary after excluding other possible diagnoses, and if they meet two-thirds of the Rotterdam diagnostic criteria, they were included in the study. Thirty patients with polycystic ovaries and 35 healthy controls were included in this study.

Results: The mean values of Exportin-5, Drosha, and Dicer markers were significantly higher in the PCOS group when compared with the control group. With an Exportin-5 value > 1.70, we found the PCOS with 94% probability, 86.7% sensitivity, and 91.4% specificity. Moreover, if the Drosha value was > 0.166, it was expected that the patient would be diagnosed as PCOS with a probability of 75%, with 66.7% sensitivity and 71.4% specificity. A statistically significant cut-off value could not be obtained for Dicer.

Conclusions: In our study, the levels of all three markers were found to be significantly higher in the PCOS group compared to the control group. It suggests that they can be used in the early diagnosis of PCOS patients without full-blown disease. However, this preliminary study should be supported by larger-scale studies.

Key words: polycystic ovary syndrome (PCOS); miRNA; Dicer; Exportin-5; Drosha

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine and gynaecological syndrome that is found in 1 in 15 women of reproductive age (8.7–17.8%), the aetiology is not fully clarified, and it has a heterogeneous character [1]. It is a systematic disease defined by menstrual cycle disorders (oligo-amenorrhoea), anovulation, hyperandrogenaemia, low fertility success, hirsutism, acne, obesity, severe and painful menstrual cycles, pelvic pain, and multiple follicle cysts in the ovaries in ultrasonography [2].

MicroRNAs (miRNAs) are single-stranded non-coding RNA molecules with 22–24 nucleotides. These bind to the non-coding 3′ region of the target mRNA, inhibiting translation or initiating the destruction of target mRNAs. They perform this process through posttranslational regulation in target genes [3]. Gene expressions have been identified in all animal species and > 1000 miRNAs, whose significance is well understood. Circulating miRNAs can be detected in whole blood, serum, plasma, and follicular fluids of patients with PCOS [3, 4]. Transcription of miRNAs in the nucleus is done with RNA polymerase II. The resulting pre-miRNA is processed in the nucleus with DROSHA enzyme and DGCR8 (DiGeorge Syndrome Critical Region 8) protein complex to form 60–70 nucleotide-long pre-miRNA [5]. This molecule is transported to the cytoplasm by a transport protein called Exportin 5 (XPO5). Here, it is transformed into mature miRNA with the ribonuclease III enzyme called DICER1 [6]. Dicer converts double-stranded pre-miRNA into mature single-strand miRNA. This enzyme plays a key role in miRNA functions [7]. miRNAs are also associated with many biological events such as growth, development, differentiation,

apoptosis, neuronal development, muscle differentiation, and metabolism, and pathological events such as heart diseases, various cancer types, and autoimmune, psychiatric, and neurological diseases [8]. Abnormal expressions have been found in many miRNAs in polycystic ovary syndrome [9].

Studies have shown that miRNAs have a regulatory effect on steroid metabolism. It has been determined that these miRNAs reduce progesterone, testosterone, and oestradiol from human granulosa cells, but only miR-107 increases testosterone. This specific miRNA is also upregulated in granulosa cells of women with PCOS [10]. While miRNAs such as miR-15a and miR-72 were upregulated in some PCOS rat models, miRNAs such as miR-182 and miR-17 were found to be downregulated. Some miRNAs are bunched in the early follicular stage and disappear in the mature oocyte stage [11, 12].

As can be understood, various miRNAs have been associated with PCOS today, but the aetiology of PCOS is still largely unknown. However, current evidence suggests that this syndrome is influenced by complex multigenic, strong epigenetic, and environmental factors. Our aim in this study is to examine the relationship between Drosha, XPO5, and Dicer-1, which are involved in miRNA formation, with PCOS.

Material and methods

Patients

This research was carried out in the Department of Endocrinology and Metabolism, Training and Practice Hospital of Cumhuriyet University Faculty of Medicine. Approval was obtained from the Ethics Committee of Cumhuriyet University Faculty of Medicine for the study (Decision no. 2019-02/05). Before the study, informed consent was obtained from all patients.

Thirty PCOS patients and 30 healthy controls were included in the study. The control group participants did not have menstrual cycle irregularities, PCOS morphology in pelvic ultrasound, or hyperandrogenism signs such as acne or hirsutism. In addition, there was no steroid or drug use affecting glucose metabolism and no accompanying chronic diseases.

Patients admitted to the endocrinology outpatient clinic with complaints such as menstrual irregularity (oligo- and/or amenorrhea), hirsutism, or acne were excluded from other possible diagnoses such as congenital adrenal hyperplasia (with a short Synacthen test) and Cushing syndrome (with 1 mg dexamethasone suppression test). Based on the Rotterdam diagnostic criteria, they were included in the study.

The National Institutes of Health (NIH 1990 criteria) defined polycystic ovary syndrome as hyperandrogenic anovulation without polycystic ovaries or LH elevation [13]. The Androgen Excess and PCOS (AE-PCOS) Society defines PCOS as ovarian dysfunction with hirsutism and/or hyperandrogenaemia androgen phasing and oligoanovulation and/or PCO.

The Rotterdam consensus defines PCOS as the presence of two of the following features: 1) oligo- and/or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, and 3) polycystic ovaries. These criteria also recognize that other androgen excess or related disorders should be excluded before assigning the diagnosis of PCOS [14].

According to the 2018 international evidence-based guideline for the assessment and management of PCOS, it can be classified into four main phenotypes [15]:

— phenotype A (classic PCOS):

a) clinical and/or biochemical evidence of hyperandrogenism,
b) evidence of oligo-anovulation,
c) ultrasonographic evidence of a polycystic ovary;

— phenotype B (essential NIH criteria):

a) clinical and/or biochemical evidence of hyperandrogenism,
b) evidence of oligoanovulation;

— phenotype C (ovulatory PCOS)

a) clinical and/or biochemical evidence of hyperandrogenism,
b) ultrasonographic evidence of a polycystic ovary;

— phenotype D (non-hyperandrogenic PCOS):

a) evidence of oligoanovulation,
b) ultrasonographic evidence of a polycystic ovary.

Patients included in the study were grouped according to the above phenotype characteristics. Patients with smoking and/or alcohol use, body mass index > 35, chronic liver or kidney disease, and rheumatological or malignant disease were excluded from the study.

Laboratory measurements

Blood samples obtained after the women had fasted for at least 12 hours between 08:00 and 10:00 a.m 3–7 days into their menstrual cycle. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol (E2), androstenedione, total testosterone, dehydroepiandrosterone sulphate (DHEAS), 17-OH progesterone, thyroid-stimulating hormone (TSH), insulin, and glucose levels were measured in all patients. FSH, LH, E2,

androstenedione, total testosterone, DHEA-S, 17 OH progesterone, thyroid-stimulating hormone (TSH), and insulin levels were measured with standard radioimmunoassay. Glucose levels were measured with glucose oxidase method.

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol (E2), total testosterone, thyroid-stimulating hormone (TSH), dehydroepiandrosterone sulphate (DHEAS), insulin, and 25(OH) vitamin D levels were measured by electrochemiluminescent method (Roche Diagnostics, e801, Germany). Glucose levels were determined by the hexokinase method (Roche Diagnostics, e801, Germany). 17-OH progesterone, 11-deoxycortisol, and 1,4 androstenedione were determined with the liquid chromatography-tandem mass spectrometry method (Thermo Scientific, TSQ Quantum Acess Max).

Blood samples (10 mL) were taken from each patient into sterile gel biochemistry tubes. These blood samples were centrifuged at 4000 rpm for 15 min. The serums obtained were portioned and kept at –40°C until the analysis day in order to determine the Drosha (pg/mL), Dicer (pg/mL), and XPO5 (pg/mL) levels. When the required number of patients for the study was reached, all samples were thawed and then studied in a single session with the ELISA reader (GF-M3000; China).

Statistical analysis

Statistical analysis was performed using SPSS software (Version 23.0, SPSS Inc., Chicago, IL, USA). If continuous variables were normal, they were described as the mean ± standard deviation ($p > 0.05$ in Kolmogorov-Smirnov test or Shapira-Wilk [$n < 30$]), and if the continuous variables were not normal, they were described as the median. Comparisons between groups were applied using Student's T-test for normally distributed data, and the Mann-Whitney U test was used for the data not normally distributed. Correlations were tested by Spearman's correlation test. Values of $p < 0.05$ were considered statistically significant.

Results

There was no statistically significant difference between the age and BMI distributions of the PCOS and control groups included in the study. In the PCOS and control groups the mean age was 24.1 ± 6.4 (17–41) and 26.1 ± 6.2 (18–44), and the mean BMI was 24.9 ± 4.9 (17.0–40.7) and 26.3 ± 5.4 (20.0–40.2), respectively. When the PCOS and control groups were compared, E2 and vitamin D were found to be higher in the control group than in the PCOS group, while

other hormonal parameters were found to be significantly higher in the PCOS group than in the control group (see Tab. 1). The distribution of clinical and laboratory information of PCOS patients and control groups is shown in Table 1.

Among the phenotype groups of PCOS patients, it was found that the most common type was D (53.3%) and the least common type was B. In total, 83.3% of the patients had menstrual irregularity, and 86.7% of them had a positive US result (see Tab. 2). The mean values of XPO5, Drosha, and Dicer markers were found to be statistically significantly higher in the PCOS group compared to the control group (see Tab. 3).

If the XPO5 value of the patient was > 1.709, the patient was determined to have a 94% probability of PCOS, with 86.7% sensitivity and 91.4% specificity. If the Drosha value of the patient was > 0.166, the patient was determined to have a 75% probability of PCOS, with 66.7% sensitivity and 71.4% specificity (see Tab. 4, Fig. 1). A statistically significant cut-off value could not be obtained for Dicer.

When we compared the clinical and laboratory results of those with FGS < 8 to those with FGS ≥ 8, it was found that only insulin values were statistically significantly different. It was determined that the mean insulin value of the group with FGS of ≥ 8 was statistically significantly higher (see Tab. 5). Phenotype results were divided between phenotype D and the other phenotypes due to the insufficient number of subgroups.

When we compared the patients with phenotype D and those with the other phenotypes, it was found that the results of total testosterone and DHEA-SO4 were statistically different between the groups. It was determined that the median total testosterone and DHEA-SO4 values of the patients with phenotype D were statistically significantly lower than the other phenotype groups (see Table 6). Only a negative correlation was found between XPO5 and age. Only a negative correlation was found between Drosha and age. A statistically significant positive correlation was found between Dicer and age and BMI (see Tab. 7).

Table 1. Laboratory and demographic characteristics of patients with polycystic ovary syndrome (PCOS) and healthy controls

	PCOS	**Control**	**p**
Age [year]	24.1 ± 6.4	26.1 ± 6.2	0.227
BMI [kg/m^2]	24.9 ± 4.9	25.2 ± 3.4	0.784
Free T4 [ng/dL]	1.15 ± 0.2	1.09 ± 0.1	0.166

TSH [mIU/L]	1.62 (0.49–10.0)	2.80 (0.5–5.9)	0.218
FSH [mIU/mL]	5.29 ± 1.1	4.41 ± 1.3	**0.041**
LH [mIU/mL]	7.14 ± 2.9	4.79 ± 0.8	**0.005**
Prolactin [ng/mL]	20.3 (9.7–104.1)	15.7 (7.8–26.0)	**0.025**
E2 [pg/mL]	30.8 (5–124)	87.5 (33–146.4)	**0.0001**
Total testosterone [ng/mL]	0.32 (0.07–1.09)	0.20 (0.02–0.45)	**0.007**
Glucose [mg/dL]	81.3 ± 6.3	80.9 ± 7.2	0.851
Insulin [mU/L]	12.4 ± 4.8	7.2 ± 2.9	**0.0001**
DHEA-SO4 [µg/dL]	231.8 (73.9–615.5)	114.0 (63–234)	**0.0001**
17-OH progesterone [ng/mL]	0.87 (0.28–1.8)	0.48 (0.11–1.02)	**0.003**
11-deoxycortisol [ng/dL]	3.15 (0.3–6.0)	0.88 (0.1–2.0)	**0.0001**
25-OH-Vit D [µg/L]	11.3 (5.9–33.1)	19.0 (6.7–32.0)	**0.003**
1,4-D-androstenedion [ng/mL]	1.8 (0.87–4.0)	1.0 (0.12–2.43)	**0.001**

BMI — body mass index; TSH — thyroid-stimulating hormone; FSH — follicle stimulant hormone; LH — luteinizing hormone; E2 — oestradiol; DHEA-SO4 — dehydroepiandrosterone sulphate

Table 2. Clinical and phenotype characteristics of patients with polycystic ovary syndrome (PCOS)

	N	**%**
Menstruation irregularity		
No	5	16.6
Yes	25	83.3
US (PCO)		
No	4	13.3
Yes	26	86.7
FGS	30	46.2
< 8	11	36.7

8–16	19	63.3
Phenotype	30	46.2
Phenotype A	6	20.0
Phenotype B	3	10.0
Phenotype C	5	16.7
Phenotype D	16	53.3

US — pelvic ultrasonography; FGS — Ferriman-Gallwey Hirsutism Scoring System

Table 3. Exportin-5 (XPO5), Drosha, and Dicer levels and comparison between patients with polycystic ovary syndrome (PCOS) and control groups

	PCOS (n = 30)		**Control (n = 35)**		**p**
	Mean ± SD	**Min–Max**	**Mean ± SD**	**Min–Max**	
XPO5	1.93 ± 0.21	1.69–2.32	1.72 ± 0.18	1.67–2.74	**0.0001**
Drosha	0.18 ± 0.01	0.14–0.21	0.164 ± 0.02	0.14–0.20	**0.0001**
Dicer	0.15 ± 0.03	010–0.18	0.53 ± 0.04	0.42–0.61	**0.0001**

Table 4. Sensitivity and specificity values of exportin-5 (XPO5) and Drosha

	Cut-off value	**Sensitivity (%)**	**Specificity (%)**	**AUC**	**Asymptotic 95% CI**		**p**
					Lower Bound	**Upper Bound**	
XPO5	1.709	86.7	91.4	0.94	0.868	1.000	0.0001
Drosha	0.166	66.7	71.4	0.75	0.624	0.869	0.001

AUC — area under the curve; CI — confidence interval

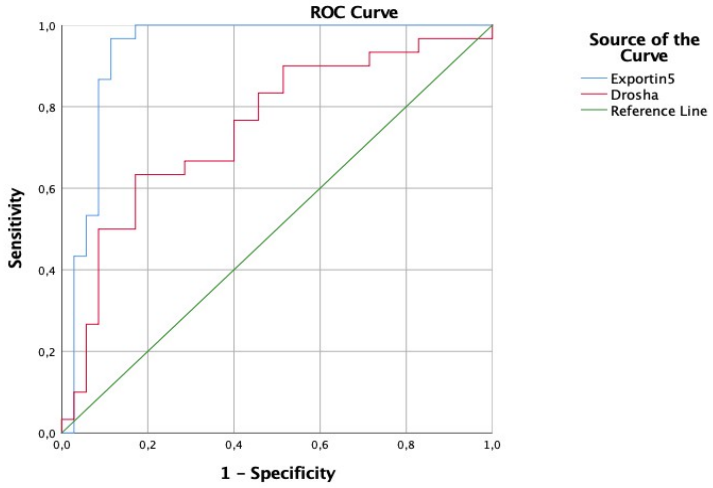

Figure1. ROC analysis of exportin-5 (XPO5) and Drosha in patients with polycystic ovary syndrome (PCOS)

	FGS < 8 (n = 11)	**FGS > 8 (n = 19)**	**p**
BMI [kg/m^2]	23.6 ± 4.1	25.8 ± 5.2	.248
XPO5 [pg/mL]	1.99 ± 0.2	1.89 ± 0.22	.241
Drosha [pg/mL]	0.18 ± 0.02	0.18 ± 0.02	.925
Dicer [pg/mL]	0.15 ± 0.02	0.15 ± 0.03	.766
Free T4 [ng/dL]	1.15 ± 0.2	1.16 ± 0.2	.897
TSH [mIU/L]	1.6 (0.8–10.0)	1.7 (0.49–4.3)	.350
FSH [mIU/mL]	5.5 ± 0.7	5.2 ± 1.4	.668
LH [mIU/mL]	6.2 (2.8–9.7)	7.6 (1.9–13.1)	.562
Prolactin [ng/mL]	23.2 (15.7–104.1)	19.9 (9.7–56.9)	.255
E2 [pg/mL]	31.9 (15.8–75.7)	28.0 (5–124)	.970
Total testosterone [ng/mL]	0.29 (0.07–0.43)	0.35 (0.14–1.09)	.447
DHEA-SO4 [µg/dL]	295.1 (131.5–615.5)	197.8 (73.9–349.6)	.145
17-OH progesterone [ng/mL]	0.71 (0.3–1.6)	1.03 (0.28–1.8)	.390
11-deoxycortisol [ng/dL]	3.2 (0.3–6.0)	3.1 (2.0–5.5)	.933
25-OH Vit D [µg/L]	22.1 (6.8–33.1)	10.5 (5.9–32.3)	.253
Glucose [mg/dL]	82.7 ± 6.4	80.4 ± 6.4	.434
Insulin [mU/L]	9.6 ± 1.3	14.2–5.3	**.027**

Table 5. Comparison results of patients with polycystic ovary syndrome according to the Ferriman-Gallwey Hirsutism Scoring System (FGS)

FGS —Ferriman-Gallwey Hirsutism Scoring System; BMI — body mass index; XPO5 — exportin-5; TSH — thyroid-stimulating hormone; FSH — follicle-stimulating hormone; LH — luteinizing hormone; E2 — oestradiol; DHEA-SO4 — dehydroepiandrosterone sulphate; E2 — oestradiol; DHEA-SO4 — dehydroepiandrosterone sulphate

Table 6. Clinical and laboratory evaluation of phenotype D and other phenotype groups (A, B, C)

	Phenotype A–B–C (n = 14)	**Phenotype D (n = 16)**	**p**
BMI	24.1 ± 3.5	25.7 ± 5.9	.637
XPO5	1.89 ± 0.2	1.96 ± 0.2	.697
Drosha	0.8 ± 0.02	0.18 ± 0.02	.473
Dicer	0.16 ± 0.03	0.16 ± 0.02	.154
Free T4	1.17 ± 0.2	1.14 ± 0.2	.390
TSH	1.78 (0.8–4.0)	1.33 (0.5–10.0)	.264
FSH	5.4 ± 1.3	5.1 ± 0.9	.442
LH	6.9 (2.2–9.8)	5.9 (1.9–13.1)	.657
Prolactin	20.9 (12.8–104.1)	20.2 (9.7–41.2)	.605
E2	38.8 (5–124)	25.9 (15.8–45.0)	.129
Total testosterone	0.43 (0.26–0.83)	0.25 (0.07–1.09)	**.004**
DHEA-SO4	308.0 (163.7–615.5)	184.7 (73.9–310.2)	**.013**
17-OH progesterone	1.1 (0.3–1.6)	0.6 (0.3–1.8)	.074

11-Deoxycortisol	4.0 (2.3–6.0)	2.9 (0.3–3.6)	.081
1,4-D-androstenedion	2.3 (0.9–3.4)	1.6 (0.9–4.0)	.277
Glucose	81.0 ± 7.1	81.8 ± 5.5	.882
Insulin	10.8 ± 3.6	14.3 ± 5.4	.223
25-OHVit D	13.3 (6.0–30.8)	10.9 (5.9–33.1)	.943

BMI — body mass index; XPO5 — exportin-5; TSH — thyroid-stimulating hormone; FSH — follicle-stimulating hormone; LH — luteinizing hormone; E2 — oestradiol; DHEA-SO4 — dehydroepiandrosterone sulphate; E2 — oestradiol; DHEA-SO4 — dehydroepiandrosterone sulphate

Table 7. Exportin-5 (XPO5), Dicer, and Drosha markers evaluated in patients with polycystic ovary syndrome (PCOS) correlations between other laboratory parameters

		XPO5	**Drosha**	**Dicer**
Age	r	**–.32***	**–.25***	**.31***
	p	**0.01**	**0.043**	**0.012**
BMI	r	–0.19	–0.09	**0.41***
	p	0.322	0.652	**0.026**
Free T4	r	–0.12	0.01	–0.19
	p	0.564	0.94	0.347
TSH	r	–0.03	0.09	–0.22
	p	0.87	0.638	0.278
FSH	r	0.09	0.34	–0.23
	p	0.71	0.156	0.351
LH	r	–0.058	0.0	0.009
	p	0.814	1.00	0.972
Prolactin	r	–0.23	–0.23	–0.12
	p	0.316	0.313	0.606

E2	r	0.09	–0.33	0.22
	p	0.721	0.161	0.371
Total testosterone	r	–0.28	–0.01	0.11
	p	0.213	0.946	0.635
Glucose	r	0.01	0.11	0.27
	p	0.954	0.634	0.258
Insulin	r	0.04	0.12	0.05
	p	0.987	0.61	0.982
DHEA-SO4	r	–0.12	0.03	–0.05
	p	0.602	0.889	0.984
17-OH progesterone	r	–0.04	0.06	0.07
	p	0.852	0.808	0.779
11-deoxycortisol	r	–0.03	–0.27	–0.50
	p	0.905	0.345	0.067
25-OHVit D	r	0,06	0,24	0,24
	p	0.845	0.426	0.426
	r	–0.14	–0.22	0.023
1,4-D-androstenedion	p	0.596	0.397	0.931

r — correlation coefficient; BMI — body mass index; XPO5 — exportin-5; TSH — thyroid-stimulating hormone; FSH — follicle-stimulating hormone; LH — luteinizing hormone; E2 — oestradiol; DHEA-SO4 — dehydroepiandrosterone sulphate; E2 — oestradiol; DHEA-SO4 — dehydroepiandrosterone sulphate

Discussion

In polycystic ovary syndrome, abnormal expressions have been found in many miRNAs. miR15a and miR-182 are essential factors in maintaining the physiological functions of granulosa cells, such as proliferation, apoptosis, and steroidogenesis. However, in the PCOS rat model treated with dihydrotestosterone, miR-182 was found to be significantly reduced in ovarian tissues [11, 12]. Hossain et al. (2013) found that in this rat model, 72 miRNAs were

upregulated and 17 were downregulated. These expressed miRNAs are densely present in granulosa and theca cells and in the early follicular stages of oocytes. miR-222 is expressed in the granulosa cells and follicular membranes of the early follicles and disappears in the mature stage [12].

The expression of miRNAs variously increases or decreases in plasma or tissue. Our study investigated Dicer, Drosha, and XPO5, which play a role in miRNA formation and regulation in PCOS patients. Dicer, Drosha, and XPO5 levels were found to be significantly higher in polycystic ovarian patients than in controls.

Exportin-5 regulates Dicer expression required for premiRNA maturation. Exportin-5 is directly associated with Dicer mRNA. With XPO5 inactivation, nuclear Dicer mRNA accumulates. Dicer protein expression is reduced. As a result, XPO5 is involved in miRNA biogenesis, pre-miRNA transport, and regulation of Dicer expression [16]. Based on our studies in PCOS patients it was observed that the amount of export-5 protein increased significantly compared to the control group.

The relationship of Drosha, XPO5, and Dicer-1 with various diseases has been investigated. The absence of Dicer-1 in mice disrupted miRNA maturation, resulting in early foetal loss [17]. Expression of Drosha and Dicer mRNA in ovarian cancer cells was found to be 50% lower compared to normal ovarian cells [18]. In human ovarian cancer, in vivo and in vitro oestrogen receptor and Dicer-1 expression were found to be associated [19]. In a study performed in human fallopian tubes, it was shown that Dicer-1 expression, specific to cycle phases and cells, correlates with steroid hormone receptor genes and protein expressions [20]. In our study, the amount of Dicer-1 increased significantly in PCOS patients compared to the control group, supporting the literature.

In another study, it was observed that Dicer protein decreased significantly in the adipose tissue of patients with PCOS. Therefore, the miRNA 223 responsible for adipose tissue differentiation was found to be significantly reduced, and thus Dicer was thought to play a prominent role in obesity in PCOS patients. Dicer expression has been shown to decrease significantly in the presence of high glucose and insulin [21, 22]. In our study, insulin levels were found to be higher in PCOS patients with FGS > 8 than in those with FGS < 8, and higher in the PCOS group than in controls.

Target gene analysis showed that miRNA-9, -18b, -32, -34c, and -135a, which are effective in the carbohydrate metabolism and beta cell function of PCOS patients, are upregulated, while miR-132 and miR-320 are downregulated [23, 24]. The importance of adipose tissue in PCOS

aetiology is known. Dicer and miRNAs are responsible for many physiological events in adipose tissue, and many of them are abnormally expressed in patients with PCOS [25,26]. Conversion from preadipocytes to adipocytes is impaired in Dicer-deficient mice, and mature adipose tissue is markedly reduced in these mice [27]. In a study that analysed subcutaneous omental tissue samples via Western blot, it was found that Dicer and Ago proteins decreased significantly in PCOS patients compared to controls. As a result, while serum levels of miRNA 15b regulated by Dicer decreased, no change was observed in the serum levels of follicular fluid or granulosa cells. In our correlation analysis, no relationship was found between Drosha, XPO5, and Dicer and glucose and insulin levels.

Conclusion

In a recent study the levels of Dicer, Drosha, and XPO5 were found to be significantly higher in PCOS patients compared to the control group. If polycystic ovarian patients do not meet all diagnostic criteria, diagnosis remains difficult. There is value in finding new markers that can be used to diagnose this complex disease that carries with it many cardiometabolic and reproductive complications. Dicer, Drosha, and XPO5 proteins, which were significantly more prevalent in our study's PCOS patients, are promising diagnostic markers. This small-scale study of these markers needs to be further supported by larger prospective studies.

Conflict of interest

The authors declare that they have no potential conflicts of interest with the contents of this article.

Financial support

The authors received no financial support for the research, authorship, and/or publication of this article.

Authors' contribution

GD and BS conceived and designed the experiments. GD wrote the first draft of the manuscript. BS contributed to the writing of the manuscript and agreed with manuscript

results and conclusions. GD made critical revisions and approved the final version. All authors reviewed and approved the final manuscript.

Ethical approval

The study protocol was approved by the Human Research Ethics Committee of Sivas Cumhuriyet University, Sivas, Turkey (Registry No. 2019-02/06).

References

1. Andrade VH, Mata AM, Borges RS, et al. Current aspects of polycystic ovary syndrome: A literature review. Rev Assoc Med Bras (1992). 2016; 62(9): 867–871, doi: 10.1590/1806-9282.62.09.867, indexed in Pubmed: 28001262.

2. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012; 33(6): 981–1030, doi: 10.1210/er.2011-1034, indexed in Pubmed: 23065822.

3. Hammond SM. An overview of microRNAs. Adv Drug Deliv Rev. 2015; 87: 3–14, doi: 10.1016/j.addr.2015.05.001, indexed in Pubmed: 25979468.

4. Li SC, Chan WC, Hu LY, et al. Identification of homologous microRNAs in 56 animal genomes. Genomics. 2010; 96(1): 1–9, doi: 10.1016/j.ygeno.2010.03.009, indexed in Pubmed: 20347954.

5. Lee EJ, Baek M, Gusev Y, et al. Systematic evaluation of microRNA processing patterns in tissues, cell lines, and tumors. RNA. 2008; 14(1): 35–42, doi: 10.1261/rna.804508, indexed in Pubmed: 18025253.

6. Anfossi S, Fu X, Nagvekar R, et al. MicroRNAs, Regulatory Messengers Inside and Outside Cancer Cells. Adv Exp Med Biol. 2018; 1056: 87–108, doi: 10.1007/978-3-319-74470-4_6, indexed in Pubmed: 29754176.

7. Luense LJ, Carletti MZ, Christenson LK. Role of Dicer in female fertility. Trends Endocrinol Metab. 2009; 20(6): 265–272, doi: 10.1016/j.tem.2009.05.001, indexed in Pubmed: 19646895.

8. Ha TY. MicroRNAs in Human Diseases: From Cancer to Cardiovascular Disease. Immune Netw. 2011; 11(3): 135–154, doi: 10.4110/in.2011.11.3.135, indexed in Pubmed: 21860607.

9. Sørensen AE, Wissing ML, Salö S, et al. MicroRNAs Related to Polycystic Ovary Syndrome (PCOS). Genes (Basel). 2014; 5(3): 684–708, doi: 10.3390/genes5030684, indexed in Pubmed: 25158044.

10. Sirotkin AV, Ovcharenko D, Grossmann R, et al. Identification of microRNAs controlling human ovarian cell steroidogenesis via a genome-scale screen. J Cell Physiol. 2009; 219(2): 415–420, doi: 10.1002/jcp.21689, indexed in Pubmed: 19194990.

11. Sirotkin AV, Laukova M, Ovcharenko D, et al. Identification of microRNAs controlling human ovarian cell proliferation and apoptosis. J Cell Physiol. 2010; 223(1): 49–56, doi: 10.1002/jcp.21999, indexed in Pubmed: 20039279.

12. Hossain MdM, Cao M, Wang Qi, et al. Altered expression of miRNAs in a dihydrotestosterone-induced rat PCOS model. J Ovarian Res. 2013; 6(1): 36, doi: 10.1186/1757-2215-6-36, indexed in Pubmed: 23675970.

13. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR. ed. Polycystic ovary syndrome. Blackwell Scientific Publications, Boston 1992: 377–384.

14. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004; 19(1): 41–47, doi: 10.1093/humrep/deh098, indexed in Pubmed: 14688154.

15. Kiconco S, Teede HJ, Azziz R, et al. International PCOS Network, International PCOS Network, International PCOS Network, International PCOS Network, International PCOS Network, International PCOS Network, International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Clin Endocrinol (Oxf). 2018; 89(3): 251–268, doi: 10.1111/cen.13795, indexed in Pubmed: 30024653.

16. Bennasser Y, Chable-Bessia C, Triboulet R, et al. Competition for XPO5 binding between Dicer mRNA, pre-miRNA and viral RNA regulates human Dicer levels. Nat Struct Mol Biol. 2011; 18(3): 323–327, doi: 10.1038/nsmb.1987, indexed in Pubmed: 21297638.

17. Bernstein E, Kim SY, Carmell MA, et al. Dicer is essential for mouse development. Nat Genet. 2003; 35(3): 215–217, doi: 10.1038/ng1253, indexed in Pubmed: 14528307.

18. Merritt WM, Lin YG, Han LY, et al. Dicer, Drosha, and Outcomes in Patients with Ovarian Cancer. New Engl J Med. 2010; 363(19): 1877–1877, doi: 10.1056/nejmx100079, indexed in Pubmed: 19092150 .

19. Faggad A, Budczies J, Tchernitsa O, et al. Prognostic significance of Dicer expression in ovarian cancer-link to global microRNA changes and oestrogen receptor expression. J Pathol. 2010; 220(3): 382–391, doi: 10.1002/path.2658, indexed in Pubmed: 19960504.

20. Shao R, Norström A, Weijdegård B, et al. Distinct expression pattern of Dicer1 correlates with ovarian-derived steroid hormone receptor expression in human Fallopian tubes during ovulation and the midsecretory phase. J Clin Endocrinol Metab. 2011; 96(5): E869–E877, doi: 10.1210/jc.2010-2353, indexed in Pubmed: 21346072.

21. Lee EJ, Baek M, Gusev Y, et al. Systematic evaluation of microRNA processing patterns in tissues, cell lines, and tumors. RNA. 2007; 14(1): 35–42, doi: 10.1261/rna.804508, indexed in Pubmed: 18025253.

22. Qin L, Chen J, Tang Li, et al. Significant Role of Dicer and miR-223 in Adipose Tissue of Polycystic Ovary Syndrome Patients. Biomed Res Int. 2019; 2019: 9193236, doi: 10.1155/2019/9193236, indexed in Pubmed: 31828146.

23. Roth LW, McCallie B, Alvero R, et al. Altered microRNA and gene expression in the follicular fluid of women with polycystic ovary syndrome. J Assist Reprod Genet. 2014; 31(3): 355–362, doi: 10.1007/s10815-013-0161-4, indexed in Pubmed: 24390626.

24. Sang Q, Yao Z, Wang H, et al. Identification of microRNAs in human follicular fluid: characterization of microRNAs that govern steroidogenesis in vitro and are associated with

polycystic ovary syndrome in vivo. J Clin Endocrinol Metab. 2013; 98(7): 3068–3079, doi: 10.1210/jc.2013-1715, indexed in Pubmed: 23666971.

25. Murri M, Insenser M, Fernández-Durán E, et al. Effects of polycystic ovary syndrome (PCOS), sex hormones, and obesity on circulating miRNA-21, miRNA-27b, miRNA-103, and miRNA-155 expression. J Clin Endocrinol Metab. 2013; 98(11): E1835–E1844, doi: 10.1210/jc.2013-2218, indexed in Pubmed: 24037889.

26. Fujimoto Y, Nakagawa Y, Shingyouchi A, et al. Dicer has a crucial role in the early stage of adipocyte differentiation, but not in lipid synthesis, in 3T3-L1 cells. Biochem Biophys Res Commun. 2012; 420(4): 931–936, doi: 10.1016/j.bbrc.2012.03.110, indexed in Pubmed: 22475483.

27. Mudhasani R, Puri V, Hoover K, et al. Dicer is required for the formation of white but not brown adipose tissue. J Cell Physiol. 2011; 226(5): 1399–1406, doi: 10.1002/jcp.22475, indexed in Pubmed: 20945399.