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Levels of methylated arginines and L-arginine in patients with polycystic ovary syndrome: a promising approach in clinical evaluation

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ABSTRACT

Aim: Polycystic ovary syndrome (PCOS) is a prevalent disease in women. PCOS is related with metabolic syndrome and associated with cardiovascular disorders. Methylated arginine is involved in endothelial dysfunction, inflammation and atherosclerosis pathophysiology. Our aim is to determine the association between PCOS and methylated arginine metabolites in order to investigate the role of vascular factors in the etiopathogenesis of PCOS.

Material and Method: This is a case-control study. The study group is consisted of 45 PCOS cases and 45 controls. The case group is patients who applied to Department of Gynecology and diagnosed with PCOS. Control group is consisted of healthy volunteers who applied to the outpatient clinics for other reasons. The study took place in Department of Gynecology in XX hospital. Data collection was held between 2018 October to 2019 June. Methylated arginine derivatives such as ADMA, SDMA, L-NMMA (L-NG-monomethyl Arginine Acetate) and also arginine and citrulline were determined.

Results: ADMA, SDMA, L-NMMA, arginine, citrulline, Arginine/ADMA, SDMA/ADMA and total methylarginine parameters showed statistically significant differences between groups. Strong positive relation was determined between scoring of Ferriman-Gallway (FGS) and luteinizing hormone (LH), glucose, insulin, ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels.

Conclusions: High levels of methylated arginine/NO pathway metabolites in PCOS patients may be related with cardiovascular outcomes of PCOS.

Keywords: PCOS, ADMA, SDMA, methylated arginine

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogenous disorder characterized by increased androgen levels, menstrual disorders, and cysts in the ovaries and affects 7% of adult women (1). Health complications in PCOS include menstrual dysfunction, infertility, hirsutism, acne, obesity, and metabolic syndrome (2-4). It is one of the most common endocrine disorders in women of reproductive age (5). The effects of lifestyle on clinical features reveals that the etiopathogenesis of the disease is affected not only by genetics but also by environmental factors. Intrauterine exposure to Bisphenol A and phthalates is the most commonly accused environmental factors in the development of the disease (6,7). Despite the accusation of genetic and environmental factors in etiology, the factors causing the disease have not been determined exactly.

Major criteria of PCOS are hyperandrogenism and / or hyperandrogenemia, oligo-ovulation and the exclusion of the other known disorders and they were defined at

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the NIH-National Institute of Child Health and Human Development Conference in 1990. A consensus was reached in Rotterdam in 2003 that at least two of the following three criteria: clinical and/or biochemical hyperandrogenism, oligo/anovulation and polycystic ovaries, excluding other endocrinopathies, would be sufficient for the diagnosis of PCOS (8,9).

Symptoms are in chronic nature in PCOS. It usually starts during adolescence and progresses gradually over time. Some conditions can cause changes in the characteristics of these symptoms. For example, weight gain, anovulation and hirsutism can lead to aggravation in symptoms (10,11). The levels of "follicular-stimulating hormone" (FSH) do not change in the hormonal profile but it is typical for "luteinizing hormone" (LH) and "gonadotropin-releasing hormone" (GnRH) levels to increase (12,13).

Insulin resistance is the most important cause of reproductive disorders and metabolic problems (14). This causes an increased risk of impaired glucose tolerance, type 2 diabetes mellitus and cardiovascular disorders (15,16). It has been shown in studies that women with PCOS have increased cardiovascular mortality, especially in the postmenopausal period (17,18).

As an endogenous inhibitor of nitric oxide synthetase (NOS), asymmetric dimethylarginine (ADMA) is an important amino acid that play role in a wide range of human diseases especially cardiovascular diseases (19). It has been demonstrated by many studies that ADMA and SDMA (Symmetric dimethylarginine) are involved in endothelial dysfunction, inflammation and atherosclerosis pathophysiology (20-22). On the other hand, arginine / homoarginine pathway is also known to play a role in endothelial dysfunction and atherosclerosis (23,24).

In our study, we aimed to determine the relation between PCOS and methylated arginine/NO pathway metabolites in order to investigate the role of vascular factors in the etiopathogenesis of PCOS. These metabolic markers can be used to show the severity of the endothelial dysfunction when there is no apparent clinical outcome. This may help taking preventing measures before the worse clinical outcomes become overt. With the results of this study, we will be able to understand the biochemical pathways in development of PCOS.

MATERIAL AND METHOD

The study was carried out with the permission of Liv Hospital Ankara Ethics Committee (Date: 2018, Decision No: 2018-003-005). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Participants and setting

The study group is consisted of 45 PCOS cases and 45 controls. The study took place in Department of Gynecology in Liv Hospital Ankara hospital. Data collection was held between 2018 October to 2019 June. The case group is patients who applied to Liv Hospital Ankara Hospital, Department of Gynecology and diagnosed with PCOS. Control group is consisted of healthy volunteers who applied to the outpatient clinics for other reasons.

Collection of Biological Samples and Biochemical Measurements

Eight milliliters of venous blood samples were collected into tubes (BD Vacutainer, USA) by venipuncture from each participant and analyzed according to stability procedures. After the centrifugation at 3500 x g for 10 min at 40C, sera were collected and stored at −800C until transfer the samples for biochemical measurements. These samples were transferred to Yozgat Bozok University Medical Biochemistry Laboratory under cold chain conditions. Methylated arginine derivatives such as ADMA, SDMA, L-NMMA (L-NG-monomethyl Arginine Acetate) and also arginine and citrulline (Sigma, Karlsruhe, Germany) of serum were determined via high performance liquid chromatography (Shimadzu LC-20AD system (Tokyo, Japan) tandem mass spectrometry [Applied Biosystems MDS SCIEX (Foster City, CA, USA) API 3200]. This electrospray ionization (ESI) technique was operated in positive mode with a high-resolution chromatography column [Phenomenex (Torrance, CA, USA) Luna C18] (25,26). According to this procedure, 100 µL of internal standard [(deuterated7-ADMA (Cambridge Isotopes, Tewksbury, MA, USA)] dissolved in methanol (Merck, Darmstadt, Germany) were added to 200 µL of sample and proteins were separated after centrifugation via 6000 g for 10 min. The clear supernatant was taken and evaporated under a nitrogen gas flow at 600C. Two hundred microliters of fresh butanol (Merck, Darmstadt, Germany) solution including 5% (vv−1) acetyl chloride (Merck, Darmstadt, Germany) were used for derivatization of samples. The mixture was incubated at 600C for 20 min. The mixture was dried under nitrogen flow at 600C. The bulks were dissolved in 100 µL of water (Merck, Darmstadt, Germany)–methanol (Merck, Darmstadt, Germany) (90:10, vv−1) containing 0.1% (vv−1) formic acid (Sigma, Karlsruhe, Germany) and 40 µL were used for injection to chromatographic column. Mobile phase A and B consist of high-performance liquid chromatography grade water containing 0.1% (vv−1) formic acid and methanol containing 0.1% (vv−1), respectively with a total binary flow of 0.8 mL. Chromatographic separation was performed on Phenomenex Luna C18 column (Torrance, CA, USA) (250 x4.6 mm, 5 µm, 100 Å) in 5 min analysis

time. Mass spectrometric parameters were such as: ion source gas 1: 60; ion source gas 2: 60; entrance potential: 7.5; collision cell exit potential: 4; ion spray voltage: 5500 V; declustering potential: 40; collision gas: 5; collision energy: 24; temperature: 5500C. Methylated arginine derivatives' analyses were assessed by an optimization procedure with an infusion of a 50 μM solution of each molecule. According to this method, either intra-day coefficient variation (CV) or inter-day CV values for methylated arginine molecules were both under 20%. The observed bias for all added concentrations was <±17% and recoveries were between 80 and 92% (80% for L-NMMA).

Statistical Analysis

Statistical analyses were performed using the SPSS version 20.0 statistical software package. The conformity of continuous variables to normal distribution was tested with Kolmogorov-Smirnov test. The descriptive statistics of continuous variables were expressed as mean ± standard deviation. The presence of a statistically significant difference between the groups in terms of continuous variables was examined with Student's t test for parametric parameters. The difference between the groups (case-control) for non-normally distributed parameters was tested with the Mann-Whitney U test. The presence of a correlation between the groups was analyzed with Spearman correlation test. $p<0.05$ and $p<0.01$ values were considered significant.

RESULTS

A total of 90 participants (45 controls and 45 PCOS) were included in the study. Clinical parameters of control and PCOS groups are shown in **Table 1**. Age and Body Mass Index (BMI) levels of the both groups were found similar ($p>0.05$). LDL, Total cholesterol, Luteinizing hormone (LH), glucose, insulin levels and Ferriman-Gallway Scores (FGS) were significantly different between groups ($p<0.01$). However, HDL level was determined statistically lower in the PCOS group than control. On the other hand, gravidity and parity of control and PCOS groups were found statistically significantly different (gravidity: 3 and 1; parity: 2 and 1; respectively, $p<0.01$). Triglyceride (TG), Follicle-stimulating hormone (FSH), Estradiol (E2), Waist-hip ratio (WHR) and Total testosterone (T. Testosterone) did not show statistically significant difference between control and PCOS groups ($p>0.05$).

All methylated arginine parameters such as ADMA, SDMA, arginine, citrulline, homoarginine, L-NMMA, total methylarginine, arginine/ADMA ratio and SDMA/ADMA ratio were found statistically significant different between control and PCOS groups ($p<0.01$). The

Table 1. Clinical parameters of control and PCOS groups

	Group	Mean	Median	STD	Minimum	Maximum	p
Age (year)	0	29.86	29.50	5.56	20.00	39.00	0.094
	1	27.68	26.00	6.17	18.00	39.00	
TG (mg/dL)	0	95.41	89.00	44.27	39.00	297.00	0.686
	1	96.50	82.50	48.38	45.00	298.00	
LDL (mg/dL)	0	80.01	79.00	25.04	34.00	132.00	0.003
	1	63.59	59.50	24.74	27.00	136.00	
HDL (mg/dL)	0	65.75	65.50	9.23	46.00	84.00	0.043
	1	62.00	60.00	7.84	51.00	84.00	
T. Cholesterol (mg/dL)	0	160.27	162.50	33.56	90.00	225.00	0.009
	1	142.57	138.00	28.57	98.00	231.00	
LH (mIU/mL)	0	6.08	4.22	4.24	0.65	17.61	<0.001
	1	9.71	9.13	5.51	2.07	29.35	
FSH (mIU/mL)	0	6.74	6.45	3.08	2.25	21.18	0.238
	1	7.04	6.59	2.14	2.32	14.50	
Estradiol, E2 (mIU/mL)	0	33.55	29.50	21.34	7.00	111.00	0.226
	1	36.62	36.00	18.58	5.00	75.00	
BMI (kg/m2)	0	22.03	21.61	3.13	16.18	29.14	0.157
	1	23.66	22.62	4.59	15.06	35.46	
WHR	0	0.79	0.78	0.07	0.66	0.95	0.887
	1	0.79	0.78	0.07	0.66	0.95	
Glucose (mg/dL)	0	84.00	82.00	7.47	71.00	98.00	<0.001
	1	91.07	91.00	2.54	82.00	99.00	
Insulin (μIU/mL)	0	8.45	6.90	5.86	2.94	38.38	0.003
	1	10.98	9.92	5.68	3.06	33.84	
FGS (%)	0	4.30	5.00	2.22	1.00	10.00	<0.001
	1	11.82	11.50	4.55	1.00	22.00	
T. Testosterone (ng/dl)	0	1.65	1.46	0.79	0.60	3.97	0.263
	1	1.43	1.36	0.57	0.60	3.39	

0: control group (n=45), 1: PCOS group (n=45); PCOS: polycystic ovary syndrome; STD: Standard Deviation; TG: triglyceride; LDL: LDL cholesterol; HDL: HDL cholesterol; T. Cholesterol: Total cholesterol; LH: luteinizing hormone; FSH: follicle-stimulating hormone; E2: Estradiol; BMI: Body Mass Index; WHR: Waist-hip ratio; FGS: Ferriman-Gallway Score; T. Testosterone: Total testosterone

Table 2. The methylated arginine parameters of control and PCOS groups

	Group	Mean	Median	STD	Minimum	Maximum	p
ADMA (µmol/L)	0	0.33	0.37	0.12	0.07	0.50	<0.001
	1	0.52	0.51	0.09	0.41	0.70	
SDMA (µmol/L)	0	0.35	0.38	0.12	0.07	0.52	<0.001
	1	0.48	0.45	0.13	0.31	0.92	
Arginine (µmol/L)	0	171.47	175.00	78.71	55.60	437.00	0.006
	1	205.89	200.50	50.21	91.30	312.00	
Citrulline (µmol/L)	0	23.14	20.75	7.30	11.30	53.80	<0.001
	1	38.86	35.80	14.28	16.50	87.30	
Homoarginine (µmol/L)	0	2.53	2.48	0.81	0.79	4.23	<0.001
	1	4.01	3.75	1.26	1.82	8.78	
L-NMMA (µmol/L)	0	0.04	0.04	0.01	0.01	0.06	<0.001
	1	0.05	0.05	0.01	0.01	0.09	
Total methylarginine (µmol/L)	0	0.73	0.80	0.28	0.14	1.56	<0.001
	1	1.09	1.01	0.24	0.83	1.94	
Arginine/ADMA ratio	0	589.73	519.94	351.10	224.18	2385.09	<0.001
	1	406.39	410.75	112.56	196.77	759.12	
SDMA/ADMA ratio	0	1.09	1.03	0.31	0.83	2.76	<0.001
	1	0.95	0.89	0.29	0.53	2.25	

0: control group (n=45), 1: PCOS group (n=45); PCOS: polycystic ovary syndrome; STD: Standard Deviation.

methylated arginine parameters of control and PCOS groups are shown in **Table 2.**

The correlation between clinical parameters is shown in **Table 3**. Positive correlations were found between LH and FSH (r=0.433; p<0.01), estradiol (r=0.260; p<0.05), ADMA (r=0.250; p<0.05), homoarginine (r=0.238; p<0.05) and total methylarginine (r=0.318; p<0.01). A strong positive relationship was determined between FGS and LH, glucose, insulin, ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels, respectively (r=0.358, r=0.402, r=0.330, r=0.538, r=0.545, r=0.466, r=0.356, r=0.462; p<0.01). **Figure 1** presents relationship between FGS and methylated arginines such as ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels. Strong negative correlations were determined between FGS and Arginine/ADMA, SDMA/ADMA ratios, respectively (r=-0.339, r=-0.355; p<0.01). On the other hand, negative relationship was found with FGS and LDL cholesterol levels (r=-0.258; p<0.05). The relationship between FGS and insulin, glucose, LH, Estradiol (E2), LDL cholesterol levels are shown in **Figure 2**.

DISCUSSION

All methylated arginine parameters such as ADMA, SDMA, arginine, citrulline, homoarginine, L-NMMA, total methylarginine, arginine/ADMA ratio and SDMA/ADMA ratio were found statistically significant different between control and PCOS groups (**Table 2**). With the results of this study, we will be able to understand the biochemical pathways in development of PCOS. Krishna et al. (27) reported that women with PCOS showed reduced plasma NOx (nitrate plus nitrite), high ADMA synthesis and reduced arginine bioavailability. Similarly, in our study, serum ADMA levels are high in the PCOS group. ADMA, which is the most analyzed member of methylarginines, is frequently analyzed in the detection of metabolic changes and cardiovascular risk resulting from endothelial dysfunction in PCOS. One of the advantages in our study is that all methylarginine in the ADMA pathway were analyzed. The high level of total methylarginine (ADMA+SDMA+L-NMMA) in the PCOS group supports this thesis. Reduced arginine bioavailability reduces nitric oxide formation. In our study, although the serum ADMA, SDMA, arginine, citrulline, homoarginine, L-NMMA and total methylarginine levels were higher in the PCOS group compared to the control group, this difference was statistically significant. One explanation for this could be a compensator increasing of the levels of arginine, the precursor molecule, to eliminate endothelial dysfunction and to increase nitric oxide synthesis again. Elci et al. (28) found that serum ADMA levels were significantly higher in the PCOS group, both obese and non-obese, compared to the control group. In our PCOS group, obesity or non-obesity classification has not been made, however, similar to this study, serum ADMA levels were high. In spite of that BMI levels in our study of the both groups were found similar.

Obesity and weight gain are factors that can speed up the process of developing insulin resistance and the formation of vascular damage. In our study, serum glucose and insulin levels were found to be high in the PCOS group with high ADMA levels. In a study conducted by Burchall et al. (29), in support of our data, it was found that in patients with insulin resistance and high serum glucose values in the PCOS group, serum ADMA value

Table 3. The correlation between all clinical parameters

	TG	LDL	HDL	T. Cholesterol	LH	FSH	Estradiol, E2	BMI	WHR	Glucose	Insulin	FGS	T.Testosteron	ADMA	SDMA	Arginine	Citrulline	Homoarginine	L-NMMA	Total methylarginine	Arginine/ ADMA ratio	SDMA/ADMA ratio
Age	.069	.012	.001	-.008	.022	.015	-.081	-.191	-.174	-.135	-.106	-.192	-.045	-.103	-.059	.047	-.128	-.185	-.131	-.098	.048	.083
TG	1	.309**	-.085	.466**	.130	-.125	-.007	.069	.059	.015	-.010	.025	.107	-.037	-.074	.124	.038	-.022	-.016	-.082	.038	-.080
LDL		1	.287**	.786**	-.164	-.047	-.101	.067	.063	-.117	-.122	-.258*	.101	-.315**	-.212*	-.121	-.250*	-.243*	-.249*	-.233*	.139	.127
HDL			1	.428**	-.257*	-.113	-.009	-.138	-.036	-.123	.183	-.097	.152	-.254*	-.298**	-.109	-.137	-.228*	-.227*	-.321**	.116	.069
T. Cholesterol				1	-.172	-.085	-.120	.176	.168	-.130	-.037	-.182	.159	-.315**	-.220*	-.084	-.144	-.214*	-.160	-.315**	.196	.126
LH					1	.433**	.260*	-.001	-.075	.138	.020	.358**	-.181	.250*	.125	.066	.118	.238*	.105	.318**	-.114	-.045
FSH						1	.195	-.053	-.056	-.043	.073	.105	-.094	.081	.127	.128	-.080	.187	.152	.245*	.048	.049
Estradiol, E2							1	.045	-.158	.035	.108	.212*	-.021	.161	.083	-.065	.140	.052	.187	.164	-.166	-.122
BMI								1	.341**	.062	-.042	.088	-.008	.064	.051	-.015	.192	-.055	.206	.027	.026	-.050
WHR									1	.025	-.054	-.011	.017	.073	.203	.143	-.058	.060	.150	.018	.065	.068
Glucose										1	.519**	.402**	-.065	.277**	.156	.004	.236*	.148	.149	.209	-.198	-.146
Insulin											1	.330**	.016	.223*	.083	.088	.194	.068	.137	.165	-.096	-.126
FGS												1	-.117	.538**	.250*	.094	.545**	.466**	.356**	.462**	-.339**	-.355**
T.Testosteron													1	-.148	.029	.112	.015	-.043	.073	-.186	.154	.186
ADMA														1	.606**	.322**	.457**	.586**	.505**	.869**	-.599**	-.592**
SDMA															1	.520**	.328**	.419**	.521**	.759**	-.197	.156
Arginine																1	.184	.238*	.362**	.440**	.413**	.041
Citrulline																	1	.346**	.306**	.437**	-.210*	-.247*
Homoarginine																		1	.444**	.558**	-.369**	-.298**
L-NMMA																			1 0	.528**	-.224*	-.171
Total methylarginine																				1	-.412**	-.320**
Arginine/ ADMA ratio																					1	.577**

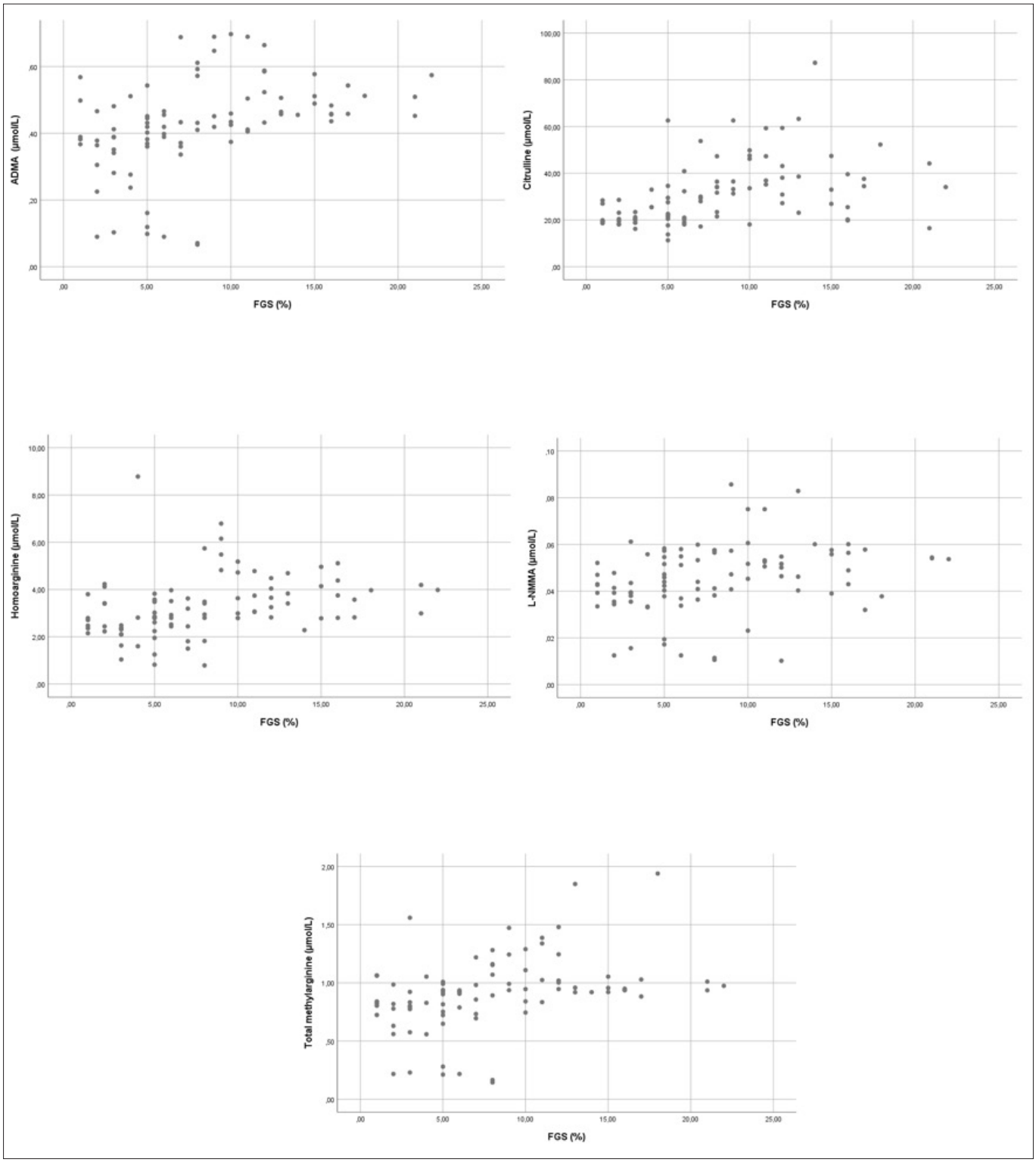

Figure 1. The relation between FGS with ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels

was higher than the control group, regardless of age and BMI. None the less age and BMI levels in our study of the both groups were obtained similar.

Taslipinar et al. (30) reported high serum ADMA levels as an endothelial dysfunction indicator in the PCOS patient group. When all parameters are compared in terms of distinguishing PCOS disease from the control group, the use of classical laboratory parameters and the clinical scoring of Ferriman-Gallway (FGS) are less predictive to detect disease compared to serum methylarginine levels which is a new biomarker. The relation between FGS with ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels are shown in **Figure 1**. The measurements of ADMA, SDMA, arginine, citrulline, homoarginine, L-NMMA parameters were found to be more effective in predicting the disease. The reason for this may be that derivatives of methylarginines other

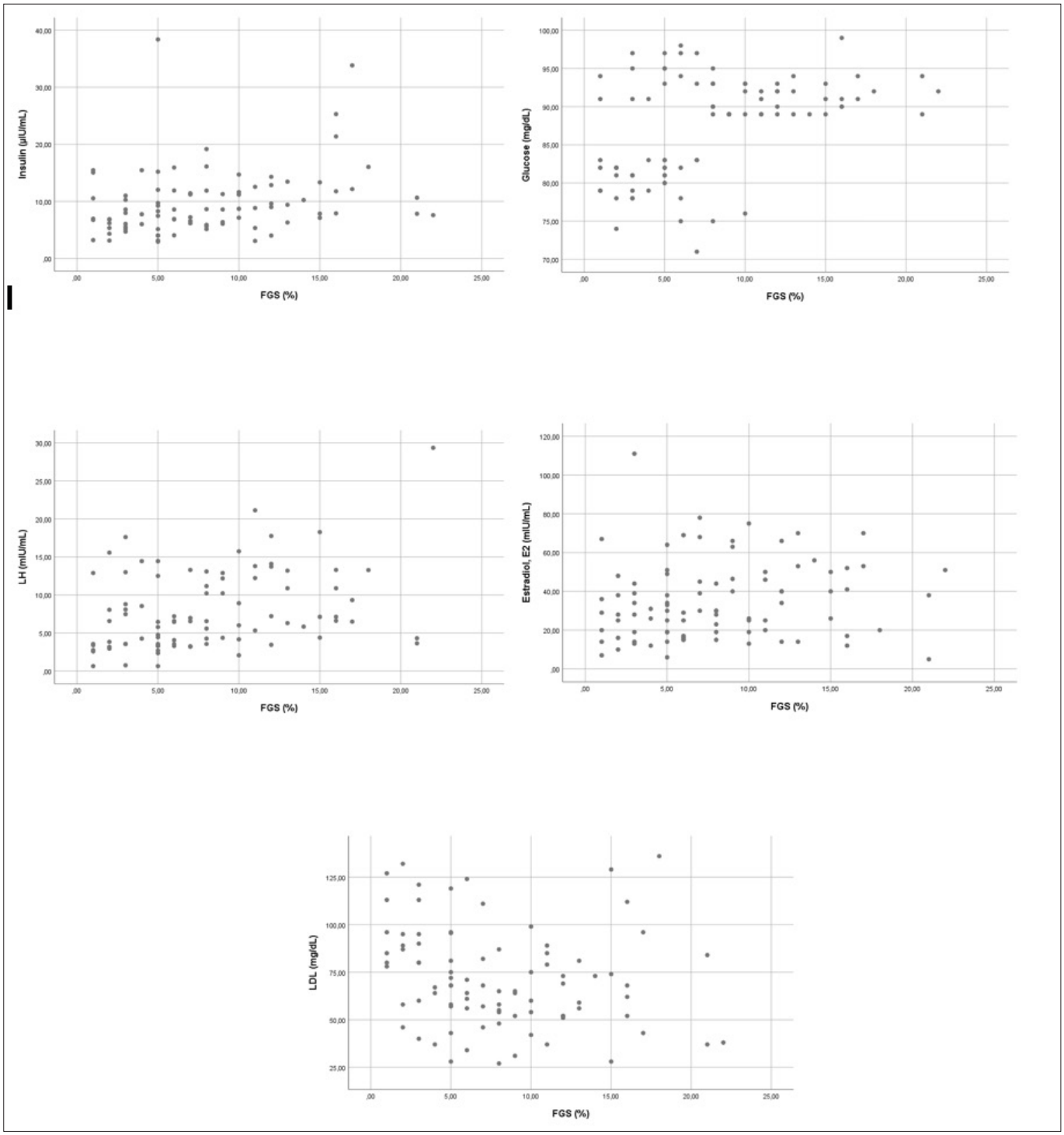

Figure 2. The relation between FGS and insulin, glucose, LH, Estradiol (E2), LDL cholesterol levels

than ADMA are known to be effective in other metabolic variations. Arginine levels even increases with high protein content diet. Also, plasma SDMA levels can increase due to impaired kidney function.

It is known that estrogens increase the expression of endothelial-dependent and inducible nitric oxide synthase enzymes in vascular cells. Karakurt et al. (31) reported that high serum ADMA levels decreased with estrogen therapy in the PCOS group. These hormonal changes in PCOS can lead to increased vascular damage and permeability, especially as a result of disruption of nitric oxide synthesis and increased levels of methylarginine. In our study, serum methylarginine levels were found to be higher in the PCOS group compared to the control group. This may be important to prevent occurrence of vascular damage. Moran LJ et al. (32) reported that weight loss did not alter serum ADMA levels statistically when compared in PCOS and healthy groups.

In this study, serum homoarginine parameter, which has not previously been studied in the PCOS group, was analyzed.

Previous literature shows that low circulating homoarginine as well as high levels of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) have been associated with impaired cardiovascular (CV) outcome and mortality in patients at risk and in the general population (33). Another study shows that high L-homoarginine (hArg) levels are directly associated with several risk factors for cardiometabolic diseases (34). ADMA, SDMA, and homoarginine are non-proteinogenic amino acids structurally related to L-arginine. hArg has been shown to serve as an alternative substrate for NOS and to inhibit arginase. Thus, it is considered to increase NO formation. In addition, low circulating concentrations of homoarginine have been proposed as a cardiovascular risk factor (35). In our study, serum homoarginine levels were found to be high in the PCOS group. This may mean suppression of nitric oxide synthesis. To our knowledge, no other study investigated serum homoarginine levels in the PCOS group.

CONCLUSIONS

Radiological imaging and physical examination are important in diagnosis of PCOS; however, these metabolic markers are found to be significantly higher in the study population, therefore, may have shed a light understanding the pathogenesis of PCOS. These markers can be used to show the severity of the endothelial dysfunction when there is no apparent clinical outcome. This may help taking preventing measures before the worse clinical outcomes become overt. Our study is unique as there are no studies investigating all ADMA parameters in patients with PCOS.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Liv Hospital Ankara Ethics Committee (Date: 2018, Decision No: 2018-003-005).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet 2007; 370: 685-97.
2. Archer JS, Chang RJ. Hirsutism and acne in polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2004; 18: 737-54.
3. Dokras A, Bochner M, Hollinrake E, Markham S, Vanvoorhis B, Jagasia DH. Screening women with polycystic ovary syndrome for metabolic syndrome. Obstet Gynecol 2005; 106: 131-7.
4. van der Spuy ZM, Dyer SJ. The pathogenesis of infertility and early pregnancy loss in polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2004; 18: 755-71.
5. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod 2016; 31: 2841-55.
6. Schonfelder G, Friedrich K, Paul M, Chahoud I. Developmental effects of prenatal exposure to bisphenol a on the uterus of rat offspring. Neoplasia 2004; 6: 584-94.
7. Vagi SJ, Azziz-Baumgartner E, Sjodin A, et al. Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol A in polycystic ovary syndrome: a case-control study. BMC Endocr Disord 2014; 14: 86.
8. Cheung AP, Cog F. Polycystic ovary syndrome: a contemporary view. J Obstet Gynaecol Can 2010; 32: 423-5.
9. Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004; 81: 19-25.
10. Ribeiro VB, Kogure GS, Lopes IP, et al. Effects of continuous and intermittent aerobic physical training on hormonal and metabolic profile, and body composition in women with polycystic ovary syndrome: A randomized controlled trial. Clin Endocrinol (Oxf) 2020; 93: 173-86.
11. Balen AH, Morley LC, Misso M, et al. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum Reprod Update 2016; 22: 687-708.
12. Blank SK, McCartney CR, Marshall JC. The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. Hum Reprod Update 2006; 12: 351-61.
13. Willis DS, Watson H, Mason HD, Galea R, Brincat M, Franks S. Premature response to luteinizing hormone of granulosa cells from anovulatory women with polycystic ovary syndrome: relevance to mechanism of anovulation. J Clin Endocrinol Metab 1998; 83: 3984-91.
14. Hansen SL, Svendsen PF, Jeppesen JF, et al. Molecular mechanisms in skeletal muscle underlying insulin resistance in women who are lean with polycystic ovary syndrome. J Clin Endocrinol Metab 2019; 104: 1841-54.
15. Ganie MA, Dhingra A, Nisar S, et al. Oral glucose tolerance test significantly impacts the prevalence of abnormal glucose tolerance among Indian women with polycystic ovary syndrome: lessons from a large database of two tertiary care centers on the Indian subcontinent. Fertil Steril 2016; 105: 194-201.
16. Forslund M, Landin-Wilhelmsen K, Trimpou P, Schmidt J, Brannstrom M, Dahlgren E. Type 2 diabetes mellitus in women with polycystic ovary syndrome during a 24-year period: importance of obesity and abdominal fat distribution. Hum Reprod Open 2020; 2020: hoz042.
17. Merz CN, Shaw LJ, Azziz R, et al. Cardiovascular Disease and 10-Year Mortality in Postmenopausal Women with Clinical Features of Polycystic Ovary Syndrome. J Womens Health (Larchmt) 2016; 25: 875-81.
18. Zhao L, Zhu Z, Lou H, et al. Polycystic ovary syndrome (PCOS) and the risk of coronary heart disease (CHD): a meta-analysis. Oncotarget 2016; 7: 33715-21.

19. Walch K, Kolbus A, Hefler-Frischmuth K. Polymorphisms of the endothelial nitric oxide synthase gene in premenopausal women with polycystic ovary syndrome. Maturitas 2008; 61: 256-9.
20. Sibal L, Agarwal SC, Home PD, Boger RH. The role of asymmetric dimethylarginine (ADMA) in endothelial dysfunction and cardiovascular disease. Curr Cardiol Rev 2010; 6: 82-90.
21. Mookerjee RP, Dalton RN, Davies NA, et al. Inflammation is an important determinant of levels of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) in acute liver failure. Liver Transpl 2007; 13: 400-5.
22. Notsu Y, Yano S, Shibata H, Nagai A, Nabika T. Plasma arginine/ADMA ratio as a sensitive risk marker for atherosclerosis: Shimane CoHRE study. Atherosclerosis 2015; 239: 61-6.
23. Haghikia A, Yanchev GR, Kayacelebi AA, et al. The role of L-arginine/L-homoarginine/nitric oxide pathway for aortic distensibility and intima-media thickness in stroke patients. Amino Acids 2017; 49: 1111-21.
24. Surdacki A, Martens-Lobenhoffer J, Wloch A, et al. Elevated plasma asymmetric dimethyl-L-arginine levels are linked to endothelial progenitor cell depletion and carotid atherosclerosis in rheumatoid arthritis. Arthritis Rheum 2007; 56: 809-19.
25. Di Gangi IM, Pirillo P, Carraro S, et al. Online trapping and enrichment ultra performance liquid chromatography-tandem mass spectrometry method for sensitive measurement of "arginine-asymmetric dimethylarginine cycle" biomarkers in human exhaled breath condensate. Anal Chim Acta 2012; 754: 67-74.
26. Perticone F, Sciacqua A, Maio R, et al. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. J Am Coll Cardiol 2005; 46: 518-23.
27. Krishna MB, Joseph A, Thomas PL, Dsilva B, Pillai SM, Laloraya M. Impaired arginine metabolism coupled to a defective redox conduit contributes to low plasma nitric oxide in polycystic ovary syndrome. Cell Physiol Biochem 2017; 43: 1880-92.
28. Elci E, Kaya C, Cim N, Yildizhan R, Elci GG. Evaluation of cardiac risk marker levels in obese and non-obese patients with polycystic ovaries. Gynecol Endocrinol 2017; 33: 43-7.
29. Burchall GF, Piva TJ, Linden MD, Gibson-Helm ME, Ranasinha S, Teede HJ. Comprehensive assessment of the hemostatic system in polycystic ovarian syndrome. Semin Thromb Hemost 2016; 42: 55-62.
30. Yavuz Taslipinar M, Kilic N, Bayraktar N, et al. Endothelial dysfunction and insulin resistance in young women with polycystic ovarian syndrome. Turk J Med Sci 2014; 44: 787-91.
31. Karakurt F, Carlioglu A, Kaygusuz I, Gumus, II, Uz B, Akdeniz D. Effect of ethinyl estradiol-cyproterone acetate treatment on asymmetric dimethyl-arginine levels in women with polycystic ovary syndrome. Arch Gynecol Obstet 2014; 289: 135-40.
32. Moran LJ, Noakes M, Wittert GA, Clifton PM, Norman RJ. Weight loss and vascular inflammatory markers in overweight women with and without polycystic ovary syndrome. Reprod Biomed Online 2012; 25: 500-3.
33. Atzler D, Schwedhelm E, Nauck M, Ittermann T, Boger RH, Friedrich N. Serum reference intervals of homoarginine, ADMA, and SDMA in the study of health in Pomerania. Clin Chem Lab Med 2014; 52: 1835-42.
34. Seppala I, Oksala N, Jula A, et al. The biomarker and causal roles of homoarginine in the development of cardiometabolic diseases: an observational and Mendelian randomization analysis. Sci Rep 2017; 7: 1130.
35. Loso J, Lund N, Avanesov M, et al. Serum biomarkers of endothelial dysfunction in fabry associated cardiomyopathy. Front Cardiovasc Med 2018; 5: 108.