Research Article

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Association of oxidative stress marker ischemia modified albumin and polycystic ovary syndrome in adolescent and young girls Adölesan ve genç kızlarda oksidatif stres belirteci iskemi modifiye albümin ve polikistik over sendromu arasındaki ilişki

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Abstract

Objective: The pathophysiologic features of polycystic ovary syndrome (PCOS) seem to be a combination of genetic predisposition and environmental factors. However, data regarding the exact effect of oxidative stress on PCOS is conflicting. This cross sectional and case-control study was designed to compare the serum ischemia

modified albumin (IMA) levels in adolescent and young girls with and without PCOS.

Methods: A total of 41 non-obese adolescents and young girls (15–21 years) diagnosed as PCOS and 41 age and body mass index (BMI) matched controls were enrolled to study. The main features of PCOS and markers of chronic inflammation were determined together with serum IMA levels at the time of study enrollment.

Results: The C-reactive protein and neutrophil-to-lymphocyte ratio were within the normal ranges and also there were no significant difference between the two groups (p > 0.05). Serum levels of IMA were significantly increased in adolescents with PCOS respect to healthy controls (0.44 ± 0.12 versus 0.35 ± 0.10 absorbance units, p < 0.001). And also there was a significant positive correlation between serum IMA and BMI in all groups (r = 0.274, p = 0.013).

Conclusion: Serum IMA levels were higher in PCOS patients than in the healthy controls. This elevation may contribute to the increased cardiovascular diseases risk in PCOS patients.

Keywords: Adolescent medicine; Biomarkers; Cardiovascular diseases; Inflammation; Insulin resistance.

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Öz

Amaç: Polikistik over sendromunun (PKOS) patofizyolojik özellikleri genetik yatkınlık ve çevresel faktörlerin bir kombinasyonu gibi görünmektedir. Bununla birlikte, oksidatif stresin PKOS üzerindeki net etkileri ile ilgili veriler çelişkilidir. Bu kesitsel ve vaka-kontrol çalışma, PKOS olan ve olmayan adölesan ve genç kızlarda serum iskemi modifiye albümin (İMA) seviyelerini karşılaştırmak için tasarlanmıştır.

Metod: Calismava PKOS tanisi alan toplam 41 obez olmayan adölesan ve genc kız (15–21 yas) ile 41 yas ve vücut kitle indeksi (VKİ) bakımından eşleştirilmiş sağlıklı kontrol dâhil edildi. Tüm katılımcılarda, çalışmaya kayıt sırasında, PKOS'un ana özellikleri ve kronik inflamasyon belirteçleri ile birlikte serum İMA seviyeleri belirlendi.

Bulgular: C-reaktif protein ve nötrofil-lenfosit oranı her iki grupta normal sınırlardaydı ve gruplar arasında anlamlı fark yoktu (p>0.05). Sağlıklı kontrollere göre PKOS'lu adölesanlarda serum İMA düzeyleri anlamlı olarak artmıstı $(0.44 \pm 0.12 \text{ ve } 0.35 \pm 0.10 \text{ absorbans birimi},$ p<0.001). Avrıca tüm grupta serum İMA ve VKİ arasında anlamlı pozitif korelasyon vardı (r = 0.274, p = 0.013).

Sonuç: PKOS hastalarında serum İMA düzeyleri sağlıklı kontrollere göre daha yüksektir. Bu artış, PKOS hastalarında artmış kardiyovasküler hastalık riskine katkıda bulunabilir.

Anahtar Kelimeler: Adölesan tıbbı; Biyobelirtecler; Kardiyovasküler hastalıklar; Inflamasyon; Insülin direnci.

Introduction

Polycystic ovary syndrome (PCOS) is a commonly encountered endocrinological disorder affecting at least 5% of women at reproductive age [1]. Despite being partially understood, its pathophysiology seems to be a combination of genetic predisposition and environmental factors. A significant number of women with PCOS have been diagnosed with impaired insulin secretion and insulin resistance (IR) (50-70%) and also increased IR is believed to be one of the leading factors of metabolic disturbances frequently encountered in women with PCOS like hypertension, dyslipidemia, metabolic syndrome, impaired glucose tolerance, and type 2 diabetes mellitus (T2DM) [2]. Additionally and specifically PCOS was previously associated with increased risk of cardiovascular disease (CVD) [3]. Beside those metabolic comorbidities, obesity is frequently (up to 80%) encountered in women with PCOS. The presence of obesity is important as it impacts the expression of metabolic features and their clinical manifestations [4].

Although IR was considered as the main problem leading to metabolic abnormalities, new evidence points the importance of several pro-inflammatory cytokines, reactive oxygen species (ROS) and free fatty acid intermediates in development of CVD. Special circumstances like tissue ischemia can cause over production of ROS which in turn results in generation of highly reactive hydroxyl radicals and oxidative stress (OS). OS can be summarized

as an imbalance between the increased production of ROS and antioxidant capacity. It was also shown that OS resulting from the over production of ROS is responsible from formation of ischemia modified albumin (IMA) [5]. IMA is a metabolic variant of serum albumin in which the N-terminus has been chemically modified during ischemic conditions causing OS due to a decrease in binding capacity of albumin for transition metals, such as cobalt, nickel, and copper [6]. Currently, IMA is regarded as a marker of OS and is also related to ischemia reperfusion in any body organ and also the evaluation of serum levels of IMA was found to be useful in some metabolic diseases [7]. It has also been reported that increased serum IMA concentrations and some types of cancer are associated [8].

Several large-scale prospective studies have demonstrated that C-reactive protein (CRP) is a strong independent predictor of future CVD and/or stroke and also CRP was demonstrated to be an improved method of identifying women at risk for CVD [9]. The increased inflammation in women with PCOS has not been clearly explained and it is still an unknown fact that whether it is caused by intrinsic factors related with PCOS or accompanying obesity [10]. In a meta-analysis the serum level of CRP was found to be increased in women PCOS without confirming the effect of obesity on increased CRP levels [11]. On the other hand, CRP level was demonstrated to be increased in obese and non-obese women with PCOS [12].

One of the recent remarkable observation derived from a simple, readily available and inexpensive test, revealed that a ratio of neutrophil to lymphocyte count (NLR) was in fact a good marker of CVD and it is started to be used as a newly emerging prognostic parameter in CVDs [13]. Its superiority over white blood cell count and neutrophil count was supposed to be derived from showing alterations in two different complementary immune pathways; one of which responsible from non-specific inflammation (neutrophils) and the other is a marker of physiological stress and general health (lymphocytes) [14]. This simple but at the same time important ratio was demonstrated to be significantly higher in non-obese patients with PCOS compared to obese control subjects, in one of the recent articles [15].

In the present study, we hypothesized that evaluating a cohort of women free from additional metabolic disturbances under the light of chronic inflammation markers would provide us not only identification of purified role of OS in pathogenesis of PCOS but also an exclusive understanding of recent metabolic status of patients/subjects depending on status of low-grade chronic inflammation.

Materials and methods

Study design and patient population

The present study was approved by the Ethical Committee of the Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey (Date and decision number: 08.26.2016/01) and conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants and as well as their parents. A total of 41 non-obese (body mass index (BMI) between 18 and 29.9 kg/m²) adolescents and young girls (age between 15 and 21 years) diagnosed as PCOS were prospectively enrolled to this study concomitant with an age and BMI matched control subject free from signs and symptoms of PCOS (n=41) from a cohort of adolescents attended to outpatient adolescence clinic of the current hospital. The diagnosis of PCOS was made by the presence of all three of the criteria declared in the Rotterdam consensus. Detection of either clinical or biochemical hyperandrogenism was a must for allocation to the study group for adolescents with PCOS. Clinical hyperandrogenism was confirmed if Ferriman-Gallwey-Score (FGS) was calculated higher than eight and/or acne/alopecia was present. The biochemical hyperandrogenism was diagnosed if serum concentration of free testosterone (fT) and/ or dehyroepiandrosterone sulfate (DHEA-S) was detected above the threshold level [16]. Oligo-menorrhea was confirmed if the cycle length was longer than 45 days or spontaneous menstrual cycles were less than eight times per year. Amenorrhea was defined as absence of menstruation for more than 3 months. The ovaries were considered polycystic by transabdominal ultrasonography when increased stromal echogenicity was peripherally surrounded by more than 12 follicles with a diameter of 2-9 mm or an ovarian volume of >10 cm³ detected on each one of the ovaries.

The control group was constituted from the adolescents with normal menstrual cycles, displaying no evidence of hyperandrogenism and normal ovaries in appearance on abdominal ultrasonographic evaluation. Exclusion criteria: having an additional diagnosis of a specific endocrinopathy including hyperprolactinemia, Cushing's syndrome or congenital adrenal hyperplasia and pregnancy, having a history of systemic chronic disorder like diabetes, thyroid disease, adrenal, hepatic or cardiovascular disorders, receiving any therapies potentially affecting carbohydrate and lipid metabolism, and oral contraceptives. Current or past smokers, alcohol consumers, and any antioxidant drug users that could affect serum oxidative stress markers were also excluded from the study.

Demographic, physical and clinical characteristics of the patients were defined during the study enrollment. Anthropometric measurements were performed to all participants wearing light clothes without shoes. BMI was calculated by dividing the weight in kilograms to the square of height in meters (kg/m²). Waist circumference (WC) was measured at the mid-point between the last rib and hip while hip circumference (HC) was measured at the level of greater trochanter. Waist to hip ratio (WHR) was calculated by dividing waist circumference to hip circumference.

Measurement of hormone levels and biochemical parameters

All laboratory studies were performed at the early follicular phase of a spontaneous or progesterone-induced menstrual cycle for each subject after having an overnight fasting period of at least 8 h in the early morning (8-10 am). Serum levels of hormones including; follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), thyroid stimulating hormone (TSH), DHEA-S and insulin were measured using the UniCel D \times I 800 Immunoassay System (Beckman Coulter, Fullerton, CA, USA). Serum 17-hydroxyprogesterone (170H-P) and fT levels were measured by radioimmunoassay (Siemens, Erlangen, Germany). Serum biochemistry including; total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and fasting serum glucose (FSG) were determined with the use of an AU680 Chemistry System (Beckman Coulter, Fullerton, CA, USA) by appropriate reagents. CRP was measured by a nephelometric method with the use of the BN-II-System (Siemens, Erlangen, Germany). For NLR ratio measurements, complete blood counts of the each patient and control subjects were analyzed by using an automated Coulter LH-780 blood analyzer (Beckman Coulter, Brea, CA, USA). IR was calculated using the homeostasis insulin resistance model (HOMA-IR) and this model was computed by the following formula: HOMA- $IR = [Fasting insulin (mIU/mL) \times Glucose (mg/dL)]/405$. IR was considered when the HOMA-IR value was above the cut off value determined for adolescents [17].

Blood samples were placed into plain tubes containing separation gels and allowed to clot for 30 min. The tubes were centrifuged at 3000 rpm (1018 g) for 10 min and stored at -80°C until the analysis. The reduced cobalt-toalbumin binding capacity was analyzed using the rapid and colorimetric method of Bar-Or et al. [18]. The serum IMA measurement was performed by rapid and colorimetric method via using SP-8001UV-VIS Spectrophotometer (Metertech Inc., Taipei, Taiwan) and the results were expressed as absorbance units (ABSU). The intra- and inter assay coefficients of variation were less than 3.1%.

Statistical analysis

Data analysis was performed by using Statistical Package for the Social Sciences (SPSS) for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). The normality of continuous variables and homogeneity of variances was tested by Kolmogorov Smirnov test and by the Levene test, respectively. Continuous variables were shown as mean±standard deviation or median (interquartile range (IQR) 25th–75th percentile), where applicable. The mean differences and median values between the study and control groups were compared by the Student's t test and by Mann-Whitney U-test, respectively. Receiver operating characteristic analysis of the area under the curve was used to determine the discriminative value of IMA. Sensitivity and specificity were calculated according to the highest Youden index

representing best cut off value. Spearman's correlation test was used for the correlation analysis. A p-value less than 0.05 was considered statistically significant.

Results

Table 1 compares the demographic, physical and clinical characteristics with laboratory parameters of the two groups. All subjects enrolled in any one of the two groups were non-obese adolescents as a good determinant of non-obese patient cohort. On the other hand, patients with PCOS had significantly higher fT and FGS compared to control subjects $(2.7\pm0.7 \text{ versus } 1.5\pm0.6 \text{ pg/mL}$ and $12.2\pm5.33 \text{ versus } 7.1\pm3.9$, respectively; both p<0.001). The serum levels of DHEA-S was statistically increased in patients with PCOS respect to the control subjects $(350.4\pm152.2 \text{ versus } 267.2\pm108.9 \text{ µg/dL}, \text{ p}=0.029)$ as a weak determinant of hyperandrogenism in PCOS patients.

All parameters of glucose and lipid metabolism were within normal ranges and there were no statistically significant difference between the two groups. The patients with PCOS were not in an IR state in terms of levels of

Table 1: Clinical and laboratory findings of the subjects.

	PCOS (n=41)	Control (n=41)	p-Value
A. Demographic, physical and clinical	characteristics		
Age (years) ^b	19.4 ± 2.6	19.3 ± 2.7	0.922
BMI (kg/m²)b	23.1 ± 3.9	22.4 ± 3.6	0.431
WHR (×100) ^b	79±8	77 ± 6	0.498
FGS ^a	12 (8–16)	6 (5-9)	< 0.001
B. Parameters of glucose metabolism			
Fasting glucose (mg/dL) ^b	88.8 ± 3.4	86.8 ± 6.0	0.417
Fasting insulin (mIU/mL) ^a	7.9 (6.0-11.4)	7.1 (5.5–9.4)	0.181
HOMA-IR ^a	1.8 (1.4-2.2)	1.6 (1.1–2.0)	0.109
C. Parameters of lipid metabolism			
TG (mg/dL) ^a	83.0 (62.8-112.3)	81.5(66.3-99.8)	0.946
LDL-C (mg/dL) ^a	74.0 (57.5–95.5)	65.9 (44-81)	0.197
HDL-C (mg/dL) ^b	65.0 ± 8.2	66.9±9.3	0.456
T-C (mg/dL) ^b	160.5 ± 31.6	149.6±35.4	0.215
D. Early follicular phase hormone leve	els		
LH (U/L) ^a	8.9 (6.4-13.3)	5.2 (4.3-10.3)	0.159
FSH (U/L)	7.2 ± 2.2	7.3 ± 3.1	0.909
E2 (pg/mL) ^a	38.5 (20-54.5)	35.3 (23-42.5)	0.311
DHEA-S (μg/dL) ^a	346 (220.9-455.8)	269.8 (195.7-326.5)	0.029
170H-P (ng/dL) ^a	1.2 (1-1.5)	1.1 (0.8–1.5)	0.224
f-T (pg/mL) ^a	2.7 (1.1-3.2)	1.5 (1.0-1.9)	< 0.001

PCOS, Polycystic ovary syndrome; BMI, body mass index; WHR, waist-hip ratio; FGS, Ferriman-Gallwey score; HOMA-IR, homeostatic model assessment-insulin resistance; TG, triglyceride; LDL-C, low density lipoprotein; HDL-C, high density lipoprotein; T-C, total cholesterol; LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, estradiol; DHEA-S, dehydroepiandrosterone sulfate; 170H-P, 17 hydroxyprogesterone; f-T, free testosterone. Data are presented as mean ± standard deviation and median (25th-75th inter Quartile Range); *Mann-Whitney-U test; *bthe Student's t-test; p < 0.05 is statistically significant.

fasting glucose, fasting insulin, and HOMA-IR values. The two groups were similar in terms of serum levels of FSH, estradiol, and 170H-P (p > 0.05).

Table 2 displays the serum levels of CRP, NLR and IMA as markers of chronic inflammation and OS in both of the groups. The serum IMA levels of adolescents with PCOS was detected significantly higher than the control subjects (0.44 ± 0.12 ABSU versus 0.35 ± 0.10 ABSU) as a determinant of OS in adolescents and young girls with PCOS (p<0.001). The markers of chronic inflammation were within the normal ranges in both of the groups and at the same time the comparison of NLR and CRP between the two groups revealed insignificant results (p=0.686and p = 0.469, respectively). At the optimum cut off point of 0.35 ABSU, the sensitivity and specificity of serum IMA levels for detection of PCOS were found to be as 85.37% (70.83-94.43%, 95% confidence interval, CI and 46.34% (30.66–62.58%, 95% CI), respectively. The negative predictive value was 76% (58.50-87.68, 95% CI) and the positive predictive value was 61.40% (53.81-68.48, 95% CI). And also, the correlation analyzes revealed that there was a

Table 2: Markers of chronic inflammation and oxidative stress.

Variables/groups	PCOS (n=41)	Control (n=41)	p-Value
C-RP (mg/L) ^a	2.6 (1.6-4.3)	2.3 (1.6-4.7)	0.686
NLRa	2.0 (1.4-2.4)	1.9 (1.4-2.3)	0.469
IMA (ABSU) ^b	0.44 ± 0.12	$\textbf{0.35} \pm \textbf{0.10}$	< 0.001

PCOS, Polycystic ovarian syndrome; C-RP, C-reactive protein; NLR, neutrophil-lymphocyte ratio; IMA, ischemia-modified albumin; ABSU, absorption unit. aMann-Whitney-U test; bthe Student's t-test. p < 0.05 is statistically significant.

Table 3: Spearman's correlation test between IMA with other hormonal and metabolic parameters.

Variables/groups	PC	OS (n=41)	To	tal (n=82)
	r	p-Value	r	p-Value
Age	0.134	0.411	0.161	0.151
BMI	0.016	0.924	0.274	0.013
WHR	0.195	0.351	0.006	0.964
FGS	0.373	0.066	0.036	0.783
NLR	0.347	0.097	0.118	0.373
CRP	0.079	0.733	0.040	0.769
TG	-0.016	0.944	0.053	0.696
LDL-C	0.382	0.056	0.264	0.052
HDL-C	0.180	0.424	-0.075	0.583
f-T	0.107	0.617	0.057	0.667
DHEA-S	-0.033	0.883	-0.074	0.669
HOMA-IR	-0.213	0.193	0.061	0.591

r, Spearman's correlation coefficient. p < 0.05 is statistically significant.

significant positive correlation between serum IMA and BMI in all groups (r = 0.274, p = 0.013) (Table 3).

Discussion

The current study designed to assess the association of PCOS and IMA in non-insulin resistant and non-obese adolescents and young women. We showed that serum levels of IMA were significantly increased in PCOS patients respect to healthy controls. And also we demonstrated a significant positive correlation between serum IMA and BMI in all groups.

To date there have been conflicting data gathered in the literature regarding the association between OS markers and metabolic parameters. Among those metabolic parameters, hyperinsulinemia and IR were demonstrated to be in a cause-consequence relationship with low grade inflammation, OS and increased CVD [19]. Furthermore, presence of obesity and increased BMI were demonstrated to be in strong association with magnitude and frequency of IR and also obesity comes up with an intrinsic potential to increase the risk of CVD development [20]. Evaluation of the same topic from an inflammation stand point revealed that inflammation encountered in adipose tissue was a critical regulator of the overall low-level systemic inflammatory response in diet or genetic induced obesity models [21]. According to us, the age of the evaluated cohort should be another additional important issue in studies evaluating metabolic parameters, especially the markers of OS. The decreased mitochondrial function by the influence of normal aging leads to reduced production of adenosine triphosphate and increased accumulation of reactive oxygen species [22] which in turn resulted in increased overall OS. So, it was important for us to optimize and standardize the level of chronic inflammation that might occur by the effect of normal aging in evaluating the risk of CVD. And also, according to us the studies conducted to determine the presence of increased baseline risk of diseases known as age-related diseases such as atherosclerosis and T2DM might be conducted on certain cohorts at the smallest possible age to feel free from the effects of aging and aging related metabolic disturbances. So we were confident about the results that they were free from additional metabolic disturbing factors like ageing, obesity and IR.

In the current study, we did not encounter the evidence of chronic low-grade inflammation in our cohort of women with PCOS. We were expecting to reach this finding because of lean body features of our study subjects. It was

Table 4: Data from previous studies of serum IMA levels in PCOS patients.

Studies	Number of patients with PCOS	Number of Mean age of patients patient cohort with PCOS	Presence of obesity Evidence BMI (kg/m²)	Presence of IR Evidence HOMA-IR values	Hyperandrogenemia Evidence Serum levels of androgens	Presence of oxidative stress Evidence Serum IMA levels	Presence of chronic inflammation Evidence Markers of chronic lowgrade inflammation	The correlation analyzes Evidence Statistically significant r=correlation coefficient
Cakır et al. [30]	52	23.6±6.2 years	No 24.19±4.0	No Yes Increased HOMA-IR Increased f-T levels (3.82 ± 4.28) (2.53 ± 1.11 pg/mL)	Yes Increased f-T levels (2.53±1.11 pg/mL)	No (0.28±0.058 ABSU)	No hs-CRP (1.72±1.88 mg/L)	No No correlation with any of the analyzed parameters
Ozturk et al. [31]	53	25.6±4.6 years	No 26.8±2.4	A S	Yes Increased f-T levels (8.1±2.9 pg/mL)	No (0.221±1.7 ABSU)	No hs-CRP (2.1±1.7 mg/L)	NA S
Guven et al. [32]	41	22.1/±4.4 years No 24.	NO 24.17±4.01	NO 2.04±9.54	yes Increased Total Testosteron (0.89±0.56 ng/dL)	Yes 0.63±0.26	NA NA	yes total testosterone (r=0.357) body mass index (r=0.3751)
Caglar et al. [33]	61	22.6±4.0 years	No 22.7±3.9	Yes [2.1 (0.5–14.03)]	Yes Increased f-T levels [2.4 (0.8–5.5) pg/mL]	Yes No [0.94 (0.6–1.2) ABSU] hs-CRP [1.3 (0.2)	No hs-CRP [1.3 (0.2–18.6) mg/L]	NA
Beyazit et al. [34]	46	28.1±4.7 years	No 25.5±4.0	Yes Yes Increased HOMA-IR Increased FT levels (3.6 ± 2.2) (2.48 ± 1.13 pg/mL)	Yes Increased f-T levels (2.48±1.13 pg/mL)	Yes [0.52 (0.21–1.12) ABSU]	No hs-CRP (2.2±1.8 mg/L)	Yes f-T (r=0.430)

IMA, Ischemia-modified albumin; PCOS, polycystic ovarian syndrome; IR, insulin resistance; BMI, body mass index; HOMA-IR, homeostatic model assessment-insulin resistance; f-T, free testosterone; ABSU, absorbance units; hs-CRP, high-sensitivity C-reactive protein; NA, not available.

previously demonstrated that chronic low-grade inflammation status of women with PCOS was mainly caused by necrosis of adipocytes [23, 24]. Detection of OS without the evidence of chronic inflammation in adolescents and young girls with PCOS made us think that OS or oxidative damage would be an early pathophysiological developmental event or state emerged prior to weight gain and chronic inflammation. On the other hand, there were some other researchers advocating that chronic inflammation encountered in women with PCOS was not related with adipose tissue or obesity [25]. But Samy et al. showed that BMI was an important determinant of CRP levels and chronic inflammation in women with PCOS [26].

Even though our cohort was consisted of lean subjects, our results revealed a statistically significant relationship between serum levels of IMA and BMI values. When we extrapolated this finding into clinical practice, we reached to a conclusion that gaining weight might results in exacerbation of OS and development of increased risk of CVD. The addition of other unfavorable metabolic features like obesity, increased WHR, dyslipidemia to the patient clinic would also exacerbate the OS and chronic low-grade inflammation, leading to formation of a state like metabolic syndrome together with increased risk of CVD. This finding was also let us thought once more that development of OS might be an early pathophysiological state emerged before formation of other features of PCOS and/ or other clinical entities that were linked well with PCOS like obesity, IR, T2DM and metabolic syndrome. Additionally, Valle Gottlieb et al. showed higher IMA levels in patients with metabolic syndrome and advocated a possible association between microvascular dysfunction and increased IMA levels [7].

So in the light of those discussions, we advocated that those findings were favoring the notion considering PCOS as an oxidative state because it was previously associated with decreased antioxidant concentrations due to determined reduced glutathione and decreased haptoglobulin levels [27, 28]. Total antioxidant capacity of the body was reported to be lower respect to control subjects in women with PCOS [29]. On the other hand, the literature review shows that there is conflicting data with regard to serum IMA levels in PCOS patients. Some studies found a higher IMA level in PCOS patients compared to controls [30, 31] whereas others did not observe a significantly different IMA level between PCOS and control groups [32–34]. Considering the evidence of increased OS and its association with the development of potential complications in PCOS patients, it would be of importance to study whether serum IMA could be studied as a simple marker of increased OS in PCOS patients. Similarly, it is not clearly known that

hyperandrogenemia has any effect on oxidant status of women with PCOS. While some of the studies did not show significant correlation between serum levels of IMA and serum androgens [30, 31], the others showed statistical correlation between serum fT levels and IMA [32–34].

At this point it would be better to underline that the current study was the first study reporting alterations of serum levels of IMA in adolescent PCOS cohort. Due to the unique feature of our patient cohort, we are not favoring to make this kind of an assessment as the presence of clinical and laboratory hyperandrogenism is a must for having diagnosis of PCOS in patients at their adolescence. According to us, it would also be wrong indeed to assess a correlation between serum levels of IMA and features leading to build the diagnosis at least for the adolescent PCOS cases. We thought that the different conclusions reported by those studies might be derived from differences encountered in the study cohorts like differences encountered in age intervals, status and level of IR of the observed cohorts, BMI and WHR values of the assessed subjects and most importantly from the numbers of included subjects (Table 4). Although our sample size comparable with the others, the main limitation of our study is the limited sample size. Also, we did not evaluated serum albumin levels which were known to be strongly associated to IMA levels [35]. IMA/albumin ratio may be more valuable clinically. Lastly, it would be better to analyze other OS parameters.

The high IMA levels indicative of chronic hypoxia might mean that PCOS might be a clinical entity intrinsically predisposing to development of metabolic complications and CVD. Furthermore the detected correlation between IMA and BMI might mean that future weight gain have a potential to deteriorate the balance between ROS and the antioxidant defense system in women with PCOS. So, herein we are postulating that OS was one of the earliest pathophysiological entities encountered in PCOS. In the light of current literature, avoidance from obesity and having regular physician visits would be offered to adolescents and young women with PCOS as a precaution against the possible development of metabolic complications. However, further longitudinal studies are still needed to clarify the precise risk for CVD in PCOS cases.

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Authors' contributions

Concept: AK, TT Design: AT, RTA, CT

Data Acquisition: AT, RTA, SB Data analysis: AT, SB, YT

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