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Oxidative stress indicators in Chinese women with PCOS and correlation with features of metabolic syndrome and dependency on lipid patterns

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Abstract

Objective The aim was to investigate oxidative stress indicators in the blood of women with PCOS without and with metabolic syndrome (MS) and their dependency on lipids, comparing with healthy women. To our knowledge, this is the first study on this topic.

Methods This was a cross-sectional study, and blood tests performed were double-blind. Within 3 months, 205 PCOS patients, from whom 55 also had MS, and 65 healthy women (control) were recruited. Malondialdehyde (MDA) was assessed as an important oxidative indicator, and superoxide dismutase (SOD), total antioxidant activity (TAA), vitamin C (VC), vitamin E (VE) and retinol (RET) as antioxidative indicators. Their correlation with features of MS was analyzed including their dependency on lipid pattern.

Results SOD, TAA, VE and RET in the PCOS group and PCOS + MS group were lower and MDA higher than in the control group ($p < 0.05$). SOD, VE and RET were the lowest in PCOS + MS group ($p < 0.05$). Thus, patients in this group had the highest oxidative stress levels but the lowest antioxidative capacity. SOD and TAA significantly decreased with increase of triglycerides (TG) and LDL-C in the PCOS + MS group ($p < 0.05$), but without dependency on HDL-C. Stepwise multiple linear regression analysis confirmed the different expression of oxidative stress in the three groups and decrease of SOD from control to PCOS group to PCOS + MS group, being associated with an increase of TG.

Conclusions MS can accelerate the oxidative stress process in patients with PCOS and decrease the antioxidative capacity. The decreased antioxidant capacity in PCOS with MS is related to increased TG and LDL-C.

Keywords Oxidative stress · Polycystic ovarian syndrome · Metabolic syndrome · Superoxide dismutase

Introduction

Polycystic ovary syndrome (PCOS) is the most frequent endocrine and metabolic disorder [1]. According to ESHRE/ASRM criteria, the prevalence of PCOS ranges from 6 to 21% [2]. Typical signs of PCOS include oligo- or anovulation, clinical or biochemical hyperandrogenism and/or polycystic ovaries [3]. PCOS is often associated with insulin resistance, obesity, dyslipidemia and other metabolic abnormalities [4] and seriously affects the quality of life, fertility and long-term health of patients.

Signs of metabolic syndrome (MS) include elevated serum triglycerides (TG), decreased high-density lipoprotein cholesterol (HDL-C), abdominal obesity, high blood pressure and increased blood glucose [5]. Worldwide a strong increase in MS has been observed [6–9]. PCOS and MS have several common features which interact with each other [10]. According to a systematic review and meta-analysis, the prevalence of MS is threefold higher in women with PCOS [11].

Although the cause of PCOS still remains largely unclear, increasing evidence suggests that it could be a complex

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multigenetic disorder [1]. Therefore, exploring the pathogenesis of PCOS still remains a challenge for medical scientific research. Oxidative stress is characterized by an excessive formation of reactive oxidants, often combined with insufficient antioxidant defenses [12], which has been recognized in various pathological cardiovascular disorders related to PCOS, MS, insulin resistance, obesity, type 2 diabetes and coronary heart disease, but also for other disorders such as disturbances causing female infertility [13, 14]. PCOS is associated with abnormal circulating markers of oxidative stress [13]. A recent publication also reported that oxidative stress may have a determining role in the developmental process of PCOS [15]. Existing literature already reported an association of circulating oxidative stress indicators with PCOS suggesting the importance of oxidative stress in the pathophysiology of PCOS [13, 15]. However, the specific mechanisms of action are still unknown.

There were many studies on the expression of oxidative stress in PCOS with or without hyperandrogenemia and on the importance of insulin resistance. However, studies investigating oxidative stress in PCOS with MS and the relationship between these two syndromes are very limited. The main aim of our study was to assess the expression of oxidative stress indicators in the blood of women with PCOS and MS (our target group), comparing PCOS patients without MS, and correlation with the typical features of MS. The second aim was to investigate the dependency of the oxidative stress indicators on lipid patterns. In addition, to our knowledge, our study is the first to investigate patients with PCOS who also present MS and to evaluate representative oxidative stress indicators assessed in the blood of these patients. Our results could have consequences not only for further exploring the pathogenesis of the diseases but also for the clinical practice, for example to assess the seriousness of the diseases or to develop antioxidative treatment principles.

Methods

Participants

This study was approved by the Ethics Committee of the Beijing Obstetrics and Gynecology Hospital, Capital Medical University, China. All participants, i.e., PCOS and PCOS + MS patients as well as the control, signed their informed consent before enrolling in the study. Women were included in the study if they met the following criteria: being Chinese, diagnosis of PCOS, aged between 21 and 40 years. The diagnosis of PCOS was made according to the criteria recommended from Chinese Health Authorities which are broadly in line with the Rotterdam criteria and also with the new ESHRE guidelines [3], i.e., at least two of the following three criteria must be present: oligo- or anovulation, clinical and/or biochemical hyperandrogenism and/or polycystic ovaries assessed by ultrasonography (presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume > 10 mL). In addition, other diseases which could present with similar symptoms and/or pathology were excluded such as androgen-secreting neoplasms, congenital adrenal hyperplasia (basal 17-hydroxyprogesterone (17-OHP) concentration ≥ 2 ng/mL (6 nmol/L) [16]), Cushing's syndrome, hyperprolactinemia and thyroid disease.

MS was defined according to the revised NCEP ATP III criteria (2005) [17] with modified cutoff for waist circumference to be more appropriate for an Asian population. MS was diagnosed if three or more of the following criteria were met: (1) blood pressure ≥ 130/85 mmHg or taking antihypertensive medication; (2) waist circumference (WC) ≥ 80 cm; (3) triglyceride level (TG) ≥ 1.69 mmol/L (150 mg/dL); (4) high-density lipoprotein cholesterol HDL-C level) < 1.29 mmol/L (50 mg/dL); (5) fasting plasma glucose level (FBG) ≥ 5.6 mmol/L (100 mg/dL) or taking hypoglycemic medication [17].

Exclusion criteria were a history of taking hormone treatment during the preceding 3 months, heart, liver and kidney disease, pregnancy, history of taking any drug that may affect the oxidative stress state such as hypoglycemic drugs, antihypertensives and antiplatelet aggregation drugs.

A total of 270 women, who attended the Department of Gynecological Endocrinology, Beijing Obstetrics and Gynecology Hospital, Capital Medical University (Beijing, China) were recruited for the study within 3 months. During this enrolling period, we were able to get the patient sample of our target group (PCOS plus MS) according to the sample size calculation (see "Supplementary File"): from the outpatients who come to our department, PCOS patients account for about 25%; most of them are re-visiting patients. Starting with our recruitment in mid-August 2018, we screened 500 women. All women who met the inclusion/exclusion criteria were included in the study. By November 2018, we were able to recruit 205 new patients with the diagnosis PCOS, from whom 55 patients also had metabolic syndrome according to the revised NCEP ATP III criteria (2005) (with modified cutoff for waist circumference). During the same time, we recruited the control group of 65 healthy women, i.e., without PCOS or MS or any cardiovascular diseases, who came to our department for normal physical examination or pre-pregnancy physical examination. The control group did get the same examinations (ultrasound exams and blood tests) like the patients to exclude diseases and meet the above exclusion criteria. We did not match the age between the groups, but to exclude possible confounding factors, we did the stepwise multiple linear regression analysis (see

section "Statistical analysis") to confirm that "age" did not affect the oxidative stress indicators.

Study design

This study was conducted as a cross-sectional study design, but the blood tests were performed double-blind. The total of 270 recruited individuals were divided into three groups: the control group (group "control", $n = 65$), PCOS without MS (group "PCOS", $n = 150$), PCOS with MS (group "PCOS + MS", $n = 55$), which is the target group of our study. According to the sample size calculation (see "Statistical analysis" and "Supplementary file"), this sample size is high enough to meet the aims of the study.

Study measurements

The anthropometric indicators of all participants were measured. Blood samples which were used for the assessment of endocrine, metabolic and oxidative stress indicators were taken after overnight fasting between the second and fourth day of a spontaneous menstrual cycle, or at any time within the menstrual cycle if there were no follicles with a diameter larger than 10 mm as assessed by vaginal ultrasonography. As endocrine indicators, we chose luteinizing hormone (LH), follicle-stimulating hormone (FSH) and total testosterone (TT). According to the definition of MS [17], we assessed its major features which are waist and hip circumference (WC, HC), blood pressure (SBP, DBP), triglyceride (TG), fasting blood glucose (FBG) and high-density lipoprotein cholesterol (HDL-C). To further investigate the dependency on lipid patterns, we also assessed low-density lipoprotein cholesterol (LDL-C). The oxidative stress indicators including malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant activity (TAA), vitamin C (VC) and vitamin E (VE) were determined by spectrophotometry using kits (NanJing Jiancheng Bioengineering Institute, Nan Jing, China). Retinol (RET) was determined using enzyme-linked immunosorbent assay (ELISA) kit (WuHan USCN). The intra- and interassay coefficients of variation for all measurements were less than 5% and 10%, respectively.

Statistical analysis

Excel 2010 software was used to establish and manage the database, and the Statistical Package for Social Sciences 17.0 for Windows (SPSS) was used for statistical analysis. Continuous variables were verified for normality by the Kolmogorov–Smirnov test. Values were described as mean ± standard deviation (SD) or median (interquartile range) based on normal distribution test. Analysis of the differences between the groups was assessed by the ANOVA or Kruskal–Wallis test for parametric or non-parametric data, respectively. Correlation analysis was used by bivariate analysis (Pearson correlation for normal distribution variables or Spearman correlation for non-normal distribution variables). To compare anthropometric, endocrine and metabolic parameters of PCOS, PCOS + MS with those of control group, the post-hoc analysis of Dunnett test was used to compare those indicators of the former groups with control groups. Since the above analysis was based on simple statistical analysis without controlling for confounding variables including age, TG and HDL-C, etc. (except for group variables representing PCOS and MS status), linear regression was used to control for confounding factors with a stepwise method, entry significance level was set to 0.05 and removal significance level was set to 0.1. SOD, MDA, TAA and VE were regarded as dependent variables, and the factors which showed statistically significant differences were set as independent variables including age, TG, group, HDL-C and the others. In the linear regression analysis model, SOD, MDA, TAA and VE were taken as dependent variables. "Group" (defined as allocation to one of the three groups) was set as an ordinal category variable, representing the status with PCOS and MS or not.

The results of the sample size estimation for the target group, i.e., patients with PCOS and MS, suggest that a sample of 55 women can meet the requirements of statistical power (see supplementary file). Significance level was set as $p < 0.05$.

Results

Baseline anthropometric, endocrine and metabolic parameters and between-group analysis

As shown in Table 1, we analyzed anthropometric, endocrine and metabolic parameters of the control group ("control", $n = 65$), PCOS without MS group (group "PCOS", $n = 150$) and PCOS with MS group (group "PCOS + MS", $n = 55$). Comparing the three groups, there was a statistically significant difference of all parameters ($p < 0.05$), except for FSH. Compared with the control, anthropometric characteristics including SBP, DBP, BMI, WC, HC, endocrine parameters including LH, TT, and metabolic parameters including FBG, TG, LDL-C were higher, and HDL-C lower in group PCOS + MS ($p < 0.05$). In group "PCOS" (i.e., patients with PCOS without additional MS), only LH and TT were higher ($p < 0.05$). The other parameters were similar to the control group. In addition, compared with group PCOS, SBP, DBP, BMI, WC, HC, TT, FBG, TG, and LDL-C were higher, but LH and HDL-C were lower in group PCOS + MS ($p < 0.05$).

Table 1 Anthropometric, endocrine and metabolic parameters: between-group analysis

Characteristic	Control ($n=65$)	PCOS ($n=150$)	PCOS + MS ($n=55$)	p
Age (years)	31.11 ± 4.76	26.93 ± 5.34*	29.40 ± 4.60*,§	< 0.001
SBP (mmHg)	116.00 (19.00)	116.50 (10.00)	131.00 (20.00)*,§	< 0.001
DBP (mmHg)	75.00 (12.00)	78.00 (12.00)	84.50 (15.00)*,§	< 0.001
BMI (kg/m^2)	20.94 (4.00)	21.74 (5.00)	29.16 (5.00)*,§	< 0.001
WC (cm)	75.00 (12.00)	75.00 (14.63)	94.00 (16.50)*,§	< 0.001
HC (cm)	92.00 (8.50)	92.00 (11.00)	104.5 (13.5)*,§	< 0.001
LH (IU/L)	4.52 (4.27)	10.08 (10.27)*	7.41 (7.76)*,§	< 0.001
FSH (IU/L)	5.78 (3.91)	6.41 (3.59)	5.86 (1.95)	0.884
TT (nmol/L)	37.66 (15.17)	47.71 (28.60)*	48.35 (26.59)*	< 0.001
FBG (mmol/L)	5.02 (0.40)	4.92 (0.55)	5.37 (0.85)*,§	< 0.001
TG (mmol/L)	0.85 (0.58)	0.91 (0.55)	1.92 (1.18)*,§	< 0.001
HDL-C (mmol/L)	1.28 (0.44)	1.27 (0.30)	1.04 (0.22)*,§	< 0.001
LDL-C (mmol/L)	2.46 (0.65)	2.58 (0.75)	3.14 (1.10)*,§	< 0.001

Values are expressed as mean ± SD or median (interquartile range) for parametric or non-parametric data, respectively. The p in the table means the statistical significances between the three groups

SBP systolic blood pressure, *DBP* diastolic blood pressure, *BMI* body mass index, *WC* waist circumference, *HC* hip circumference, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *TT* total testosterone, *FBG* fasting blood glucose, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

*$p < 0.05$ compared with control group

§$p < 0.05$ compared with PCOS group

Oxidative stress indicators: between-group analysis

In Fig. 1, the results are shown in form of "box plots". Most of the oxidative stress indicators show statistically significant differences among the three groups ($p < 0.05$) except VC. Compared with the control group, MDA levels were higher and SOD, TAA, VE, and RET levels were lower in group PCOS and group PCOS + MS ($p < 0.05$). Compared

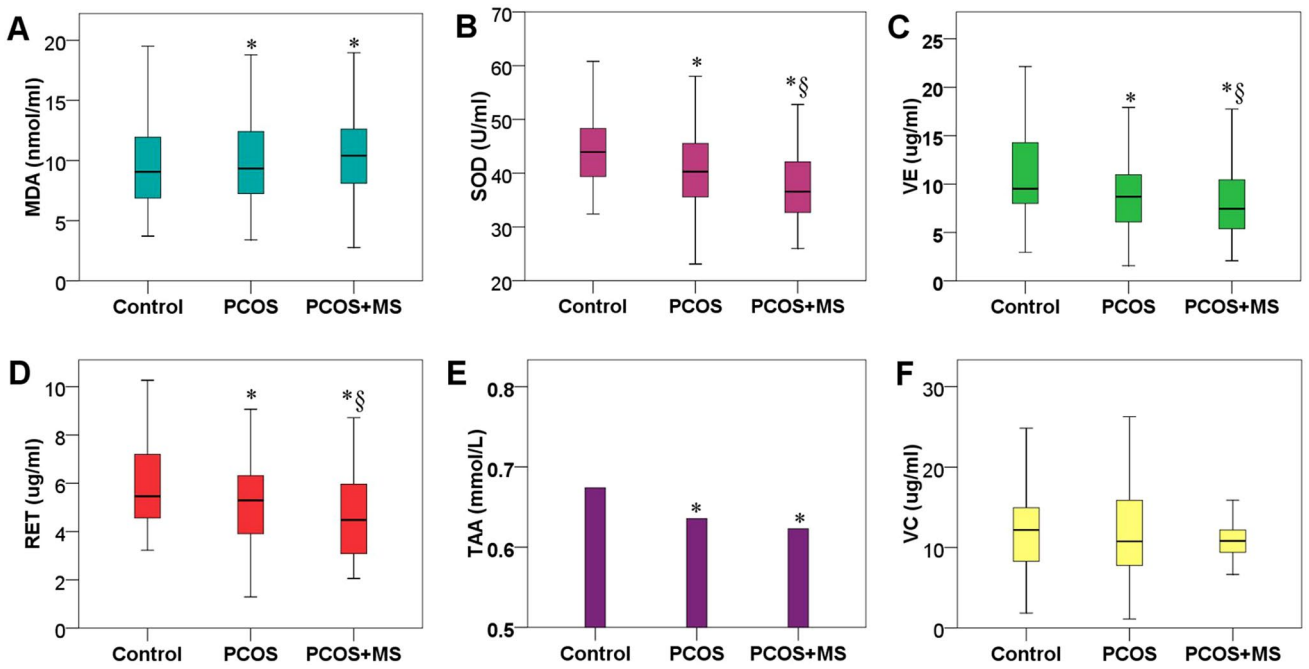

Fig. 1 Oxidative stress indicators: between group analysis. *MDA* malondialdehyde, *SOD* superoxide dismutase, *VE* vitamin E, *RET* retinol, *TAA* total antioxidant activity, *VC* vitamin C; *$p < 0.05$ compared with control group; §$p < 0.05$ compared with PCOS group

with group, PCOS SOD, VE, and RET levels were lower in group PCOS + MS ($p < 0.05$). Therefore, patients in the group PCOS with MS had the highest oxidative stress level and the lowest antioxidative capacity, respectively, compared with the control group and the patients who only had PCOS, but not MS.

Relationship between oxidative stress indicators and the features of MS

As shown in Table 2, the bivariate analysis regarding the correlation between MDA, RET, SOD, TAA and various features of MS (waistline, SBP, DBP, HDL-C, TG and FBG) show that SOD and TAA were significantly negatively correlated with TG in the group PCOS + MS ($p < 0.05$), especially SOD ($r = -0.520$), i.e., with increasing TG there is a decrease in antioxidative capacity. In contrast, there was no significant correlation with HDL-C.

We added LDL-C into Table 2 although it has not been included in the definition of MS [17]. As with TG, the increase in this lipid-marker is correlated with a decrease in antioxidant capacity.

Association of important independent variables with serum oxidative stress indicators by stepwise multiple linear regression analysis

Stepwise multiple linear regression analysis was conducted to check for confounding factors as assessed from Table 1. The significant effects are listed in Table 3. None of the oxidative stress indicators was affected by possible confounding factors (e.g., age), with the exception that TG and allocation to one of the three groups (in the table named as "Group"), which had a significant effect on SOD, gradually decreasing from control group, to PCOS group, to PCOS + MS group ($p < 0.05$). TG had a significant positive effect on SOD ($\beta = -0.352$, $p < 0.05$), which implies that with increase of 1 mmol/L of TG, SOD would decrease 0.352 times.

Group-allocation had a significant effect on the antioxidative markers TAA and VE ($p < 0.05$) and on the oxidative stress marker MDA ($p < 0.05$), but without impact on TG. These results show that the expression of oxidative stress has been reflected differently in the three groups.

Discussion

In our study, we measured oxidative stress indicators and found that PCOS with or without MS had lower antioxidant capacity compared with the control group. In addition, women in our target group PCOS + MS presented higher oxidative stress level, but lower antioxidant capacity, compared to the two other groups. In this group (PCOS + MS),

Table 2 Main oxidative stress indicators and features of metabolic syndrome: correlation analysis

Characteristic	MDA			RET			SOD			TAA		
	Control	PCOS	PCOS + MS	Control	PCOS	PCOS + MS	Control	PCOS	PCOS + MS	Control	PCOS	PCOS + MS
Waistline	0.113	0.083	−0.056	−0.090	−0.199*	0.254	0.015	−0.055	−0.261	−0.179	−0.089	−0.128
SBP	−0.083	−0.060	−0.001	−0.314*	−0.024	−0.036	0.196	0.072	−0.186	−0.286*	0.117	−0170
DBP	0.048	−0.025	0.056	−0.238	0.016	0.078	0.220	0.079	−0.130	0.171	0.136	−0.211
HDL-C	−0.164	0.072	0.110	−0.043	−0.024	−0.265	−0.057	−0.038	0.256	−0.129	0.094	−0.036
TG	0.111	−0.012	0.089	−0.228	−0.158	0.121	−0.171	−0.144	−0.520*	0.112	−0.099	−0.328*
FBG	0.013	−0.028	0.008	−0.110	−0.054	0.089	−0.068	−0.015	−0.125	−0.044	0.156	−0.169
LDL-C	0.028	0.067	0.074	−0.030	−0.144	−0.130	−0.221	−0.165*	−0.169*	−0.039	−0.112	−0.299*

MDA malondialdehyde, *SOD* superoxide dismutase, *RET* retinol, *TAA* total antioxidant activity, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *FBG* fasting blood glucose

*$p < 0.05$

Table 3 Association of important independent variables with serum oxidative stress indicators by stepwise multiple linear regression analysis

Independents	Unstandardized coefficients		Standardized coefficients	*t*	*p*
	β	SE			
SOD as dependent					
Constant	44.941	5.275		8.519	< 0.001
TG (mmol/L)	− 3.839	0.704	− 0.352	− 5.453	< 0.001
Group	− 3.055	0.998	− 0.198	− 3.060	0.002
MDA as dependent					
Constant	7.785	1.597		4.874	< 0.001
Group	2.184	0.770	0.174	2.837	0.005
TAA as dependent					
Constant	0.689	0.015		45.690	< 0.001
Group	− 0.024	0.007	− 0.202	− 3.327	0.001
VE as dependent					
Constant	12.292	0.863		14.247	< 0.001
Group	− 1.451	0.416	− 0.212	− 3.488	0.001

In the linear regression analysis model, SOD, MDA, TAA and VE were taken as dependent variables. "Group" (defined as allocation to one of the three groups) was set as an ordinal category variable, representing the status with PCOS and MS or not, in which control group was numbered 1, PCOS without MS group numbered 2, PCOS with MS group numbered 3

the significant decrease of SOD and TAA (antioxidative indicators) was significantly associated with an increase in triglycerides (TG) and LDL-C ($p < 0.05$), but without dependency on HDL-C. Therefore, impairment of lipid patterns could contribute to a decrease of antioxidant capacity in patients with PCOS + MS.

It is already well known that negative changes in lipids are often observed in patients with PCOS [18, 19]. Women with PCOS also often exhibit dysfunctional lipid metabolism and increased oxidative stress indicator levels [20]. However, we found that studies are lacking which investigate oxidative stress indicators and lipids in women with PCOS and also having MS.

Oxidative stress indicators which we assessed in our study include oxidation capacity indicators which are represented by MDA and antioxidant capacity indicators (SOD, TAA, RET, VC, VE). These indicators are involved in the balance of oxidation and anti-oxidation in the body. There can be pathophysiological situations or other conditions (e.g., environment, smokers, certain other diseases, etc.) where excessive oxidative cell stress is developed which could be detrimental for patients who have decreased or lacking antioxidative capacity. With respect to PCOS and metabolic syndrome, both with high prevalence and incidence in reproductive women, it seems to be clear that oxidative stress elicits a decisive role in the pathophysiology of these diseases.

Regarding the existing literature on oxidative stress indicators, one study found that PCOS was associated with an increase in oxidative stress (assessed by measurement of reactive oxygen species, ROS), followed by decreased serum total antioxidant levels. However, the oxidative stress indicators which we assessed were not investigated in this study [21]. Our study compared the expression of oxidative stress indicators (MDA, SOD, TAA, RET, VC, VE) in three groups. MDA, which we found to be increased in our group PCOS and group PCOS + MS, is a common marker of lipid peroxidation, which is thought to be one of the best representative indicators of oxidative stress in PCOS [14]. A systematic review and meta-analysis indicated a 47% increase in mean MDA levels in PCOS women compared to controls [14]. Likewise in our study, MDA was also observed to be higher in PCOS women compared to our control group, but the highest expression was in the women with PCOS + MS. In our department, patients with PCOS mostly attend because of infertility. Indeed, PCOS is the most common cause of anovulatory infertility in women of reproductive age, and the intrinsic characteristic of the ovary is an increase in the number of follicles failing in the selection of dominant follicles. Oxidative stress can affect the normal structure and function of follicles and obviously in PCOS patients with or without MS, this is often represented by an increase in MDA levels.

Compared to our control group, antioxidant capacity indicators including TAA and SOD were lower in PCOS with or without MS, and the expression of SOD in PCOS with MS were the lowest. TAA has been described as an antioxidant marker representing the potency to destroy free radicals. The superoxide anion is dismutated to hydrogen peroxide by SOD, which is very powerful to protect against reactive oxygen species [22]. Likewise we found the lowest expression of non-enzymatic antioxidants such as RET and VE in PCOS patients with MS.

One study found a highly significant decrease in SOD activity in PCOS patients compared to the control group, suggesting that SOD could be a valid clinical indicator for determining oxidative stress in PCOS [22]. Another study found that TAA was decreased in women with PCOS [23]. In one study, it was observed that there was no difference in VE in infertile PCOS patients compared to the control group [24], but in another study it was reported that VC and VE were lower in patients with PCOS compared to controls [25]. In our study, VE in PCOS patients was also lower than in the control group and VE was the lowest in PCOS with MS, but there was no significant difference in VC among the three groups.

All these results show an increase in the oxidation capacity and a decrease in antioxidant capacity in PCOS with MS, thus further reflecting that MS can accelerate the oxidative stress process in patients with PCOS. This can have

consequences for the further prognosis of women presenting PCOS and also MS, like further development of cardiovascular diseases because oxidative stress plays an important role in the pathogenesis of vascular changes and can influence biochemical processes in MS [26]. According also to our results, oxidative stress can be increased by antioxidant deficiency in patients with MS. This can lead to oxidative changes from intracellular to extracellular spaces and from confined to distant sites, all together to an oxidative stress situation [27, 28]. From these studies, it was concluded that PCOS patients with MS have relatively inadequate antioxidant functions, which contribute to increased oxidative stress in these patients.

Regarding the dependency on lipids, our bivariate analysis investigating the relationship between oxidative stress indicators and various features of metabolic syndrome found that both SOD and TAA were significantly negatively correlated with TG and LDL-C. According to our stepwise linear regression analysis, especially an increase in TG had a significant effect on SOD, gradually decreasing from the control group, to the PCOS group, to PCOS + MS group. Likewise, a recent publication in PCOS patients also reported that the increasing oxidative stress related to dysfunctional lipid metabolism with relative shortage of antioxidant capacity can contribute to the pathogenesis of PCOS [29]. Another study found that there was impairment of antioxidant function of HDL-C in women with PCOS [30], which contrasts with our results. One explanation can be that changes in HDL-C are often small (smaller than changes of TG and LDL-C), which could lead to different results in the statistical evaluation of different studies.

Not all women with PCOS will develop also MS despite women with MS often present with reproductive or endocrine characteristics of PCOS [10, 11]. In our study, we found that MS components are essential features of oxidative stress indicators in PCOS patients. MS components especially are found in overweight and obese patients and often are associated with indicators of oxidative stress [31]. It was suggested that particularly the same metabolic disturbances found in MS might be responsible for oxidative stress which are found also in PCOS patients [32]. Therefore, treatment of metabolic disturbances should be important within the management concept to avoid aggravation of symptoms of PCOS caused by development also of MS. In addition, perhaps treatment with metformin can avoid that PCOS patients develop MS since oxidative stress has been clearly demonstrated to be implicated in the pathogenesis of insulin resistance [15]. However, to prove this concept of prevention and treatment, more research is needed.

In terms of the *strengths of our study*, first should be considered that PCOS is the most common endocrine and metabolic disorder, and the prevalence and incidence of MS are very high in women with PCOS. At present, there are only few studies on oxidative stress in PCOS, and to our knowledge, our study is the first one investigating patients with PCOS who also present MS and to evaluate oxidative stress indicators assessed in blood. Our study shows that MS can accelerate the oxidative stress process in patients with PCOS, and the oxidative stress indicators which we have assessed in our study could even have a causal relationship within the etiology of the diseases. Used as "surrogate markers" assessed in blood, they could point on the clinical severeness of the diseases predicting further prognosis. Our findings also can provide a basis for the development of antioxidant therapy principles in PCOS patients with MS in clinic.

The main *limitation* is that randomized prospective studies with clinical endpoints are needed to prove this concept of the importance of oxidative stress indicators, whereby our study can be used for planning and designing those future studies. Our study is a cross-sectional study with experimental endpoints, i.e., laboratory markers indicating oxidative stress. Another limitation is that there are still open questions in terms of the precise mechanisms by which MS affects oxidative stress in PCOS. Thus, also further basic research such as cellular experiments in vitro is needed.

Conclusions

By assessing certain already well-defined oxidative stress indicators in the blood, we (to our knowledge for the first time) were able to demonstrate that PCOS patients with MS have the highest oxidative stress levels and lowest antioxidant capacity, compared to patients with PCOS (without MS) and compared to healthy women. From the comparison of the patients with and without PCOS, we can conclude that MS can accelerate the oxidative stress process in patients with PCOS. The decreased antioxidant capacity in PCOS with MS is related to increased levels of TG and LDL-C which supports results from other studies that disturbed lipid metabolism can contribute to the pathogenesis of these diseases.

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Author contribution HW: conceptualization: equal, data curation: lead, writing—original draft: lead; XR: conceptualization: lead, data curation: lead, funding acquisition: lead, project administration: lead, supervision: lead, writing—review and editing: lead; YL: data curation: equal; JC: data curation: supporting; AOM: conceptualization: equal, methodology: supporting, supervision: equal, writing—review and editing: lead.

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Compliance with ethical standards

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

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