Oxidative stress and risk of polycystic ovarian syndrome in women with epilepsy: implications of malondialdehyde and superoxide dismutase serum levels on female fertility

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Background
Epilepsy is a common neurologic disease and has important implications for women's fertility, as approximately half of the epileptic women have reproductive disorders. Oxidative stress is a key contributor in the pathogenesis of polycystic ovary syndrome (PCOS) and epilepsy. We aimed to investigate the role of malondialdehyde (MDA) and superoxide dismutase (SOD) serum levels as predictors of PCOS in women with epilepsy (WWE) and to evaluate the possible relationship between oxidative stress and epilepsy characteristics as well as clinicomorphological features of PCOS.

Patients and methods
A cross-sectional study enrolled 130 WWE. They were classified to two subgroups; women with PCOS (n=50) and women without PCOS (n=80) according to Rotterdam criteria. Metabolic markers and markers of PCOS were measured. Serum MDA and SOD concentrations were estimated by enzyme-linked immunosorbent assay.

Results
Our results revealed higher levels of MDA and SOD in WWE, in particular, women with PCOS. Moreover, serum MDA and SOD levels were significantly positively correlated with PCOS phenotypes. Linear regression test revealed that Low-density lipoprotein cholesterol (LDL) and luteinizing hormone (LH) were the main predictors of serum MDA levels in PCOS, whereas BMI and LH were the main predictors of serum SOD levels. Regarding antiepileptic medication, there were significantly higher levels of MDA and SOD in patients treated with valproic acid compared with the ones treated with carbamazepine.

Conclusion
The high levels of MDA and SOD among WWE, in particular, women with PCOS, were positively correlated with obesity indices and PCOS phenotypes. Thus, we recommended avoid using valproic acid in women during childbearing period.

Keywords:
malondialdehyde, polycystic ovary syndrome, superoxide dismutase, women with epilepsy

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Introduction

Epilepsy is a common neurologic disease affecting ~50 million in the world, and ~50% of them are women [1]. Epilepsy has important implications for women's health, as approximately half of the epileptic women have reproductive disorders, including menstrual cycle disturbances, hypergonadotrophic and hypogonadotrophic hypogonadism, and polycystic ovary syndrome (PCOS) [2–4].

These fertility disorders have been assumed to be secondary to the direct influence of the epileptogenic lesion, epilepsy, or antiepileptic drugs on the endocrine control centers in the brain (the hypothalamic–pituitary axis); the effects of antiepileptic drugs on peripheral endocrine glands; the effects of antiepileptic drugs on the metabolism of hormones and binding proteins; and secondary endocrine complications of antiepileptic drugs or related weight changes or changes of insulin sensitivity [5,6]. The direct role for epilepsy in the pathogenesis of reproductive endocrine disorders is explained by acute changes in serum prolactin and gonadotropin levels following seizures [7].

PCOS is the commonest endocrinopathy of premenopausal women characterized by both

reproductive and metabolic abnormalities. It affects 5–10% of reproductive-age women. Women with PCOS are characterized by chronic anovulation, hyperandrogenism [8,9], insulin resistance (IR), dyslipidemia and obesity, type 2 diabetes mellitus, low-grade chronic inflammation, and increased oxidative stress [10,11].

The exact pathophysiologic mechanism of PCOS remains still elusive. There is considerable evidence for the involvement of low-grade chronic inflammation and endothelial dysfunction in the pathogenesis of PCOS and its associated features such as IR, dyslipidemia, and atherosclerosis [12]. The prevalence of PCOS in women with epilepsy (WWE) ranges from 3.1 to 26% [13]. Several studies showed an increased incidence of PCOS in women with temporal lobe epilepsy. Moreover, PCOS was also reported to be associated with idiopathic generalized seizures [14].

Oxidative stress is one of the predisposing factors in both epilepsy and PCOS. Lipid peroxidation markers are used as indicators of oxidative stress, as the brain is more vulnerable to injury by lipid peroxidation products than other tissues, and lipid peroxidation is an index of neuronal damage of cell membrane phospholipids [14].

Increasing evidence suggests that OS level is significantly increased in patients with PCOS compared with the normal persons, when oxidative status is evaluated by circulating markers, such as malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase [15].

SOD is a group of metalloenzymes that scavenge superoxide radicals and reduce their toxicity. It is an antioxidant that dismutates the O_2 anion to form O_2 and H_2O_2 [16]. MDA is the end product of lipid peroxidation. If antioxidant enzymes become incapable of dealing with the oxidative damage, oxygen free radicals initiate lipid peroxidation in cell and organelle membranes [17–19].

MDA is a good marker of free radical-mediated damage and oxidative stress (OX) [20]. Thus, the purpose of this study was to investigate the role of MDA and SOD serum levels as predictors of PCOS in WWE, and to evaluate the possible relationship between oxidative stress and epilepsy characteristics and antiepileptic drugs (AEDs) such as valproic acid (VPA) and carbamazepine (CBZ), as well as clinicomorphological features of PCOS.

Patients and methods

This is a cross-sectional study that included 130 WWE. Epilepsy was diagnosed by an experienced neurologist according to the 2006 International League Against Epilepsy Classification [21]. Diagnosis of partial or generalized temporal lobe epilepsy (TLE) or extra-TLE was based on medical history, seizure semiology, EEG (or video-EEG) recordings, and neuroimaging of the brain. Patients had to have at least one seizure per 2 months and had to be older than 18 years of age. The National Hospital Seizure Severity Scale and seizure frequency obtained from seizure diary was used to evaluate seizure severity. WWE was then stratified into two subgroups: women with PCOS (n=50) and women without PCOS (n=80). The diagnosis of PCOS was based on the 2004 revised Rotterdam criteria [22]. All patients were subjected to thorough history taking, full clinical assessment, and anthropometric measures of obesity. Ovarian volume and antral follicular count (AFC) were evaluated by transvaginal ultrasound. A written informed consent was taken from all of the participants after explaining details and benefits as well as risks to them. The Ethical Committee of the Faculty of Medicine, Zagazig University, has approved the study protocol.

All patients in the study had fulfilled the following exclusion criteria:

(1) Concomitant infectious or systematic inflammatory disease.
(2) Severe psychiatric or neurological diseases.
(3) Any immune modulatory treatment within the past 6 months.
(4) Tumor, surgery, or significant trauma within the past 2 weeks.
(5) Hepatic or renal insufficiency.
(6) Pregnancy and history of hyperandrogenic states (such as nonclassical congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, 21-hydroxylase deficiency, or hyperprolactinemia).
(7) Smoking and alcohol abuse, as both can induce oxidative stress, which were not allowed.
(8) Use of topical or systemic treatment affecting free radical scavenging such as vitamins, antibiotics, and anti-inflammatory drugs for 1 month before blood collection.

All patients in the study were subjected to the following:

(1) Thorough medical history taking, full clinical assessment, and anthropometric measures of obesity.
(2) Neurological assessment, including detailed history of seizure activity [type, frequency, AEDs, EEG, and neuroimaging (computed tomography and/or MRI, when needed)].
(3) Ovarian volume and AFC were evaluated by transvaginal ultrasound.

Sampling of blood

The blood samples of all the study's participants were drawn after an overnight fast and divided into two portions: 1 ml of whole blood was collected into potassium oxalate and sodium fluoride-containing tubes for fasting plasma glucose (FPG). Sera were separated from remaining of the sample part and stored at −20°C until analysis.

Biochemical analysis

We determined FPG using the glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG) levels were measured by routine enzymatic methods (Spinreact). The Low-density lipoprotein cholesterol (LDL) level was calculated using the Friedewald formula [23].

Immunochemical measurements

We estimated luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin concentrations using chemiluminescence immunoassay kit provided by Immunospec Corporation (Canoga Park, California, USA). The fasting serum insulin (FSI), total testosterone, free testosterone, and sex hormone-binding globulin (SHBG) concentrations were measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit provided by DRG International (Springfield Township, New Jersey, USA). We determined IR using the homeostatic model assessment-insulin resistance (HOMA-IR) index, which is defined as fasting insulin value (mU/ml)×FPG value (mg/dl)/405. The β-cell function was calculated using HOMA-β.

Measurement of oxidative stress markers

Quantitative determination of human MDA in serum was done using MyBiosource (Southern California, San Diego, USA) ELISA kit (catalog no.: MBS028063), which applies the quantitative sandwich enzyme immunoassay technique. The MDA concentration in each sample was interpolated from the calibration curve.

Quantitative determination of human SOD in serum was done by Abcam's Human Superoxide Dismutase ELISA kit (catalog no.: ab119520), whose principle is based on quantitative sandwich enzyme immunoassay technique.

Statistical analysis

Data analyses were done with statistical package for the social sciences software (SPSS version 21; Chicago, Illinois, USA). Data were expressed as the mean±SD. The relationships of MDA and SOD serum levels with clinical and laboratory parameters among patients with PCOS were tested with the Pearson correlation. Receiver operating characteristic analysis was performed to assess the diagnostic power of MDA and SOD serum levels. Linear regression analysis was done to detect the main predictors of serum MDA and SOD in the PCOS group. We considered P to be significant at less than 0.05 with a 95% confidence interval (CI).

Results

Clinical and laboratory characteristics of women with epilepsy are shown in Table 1.

Clinical and laboratory characteristics of women with epilepsy groups

Among the WWE group, patients with PCOS had significantly higher values of BMI, diastolic blood pressure, as well as TC, TG, LDL cholesterol, FPG, FSI, and HOMA-IR compared with WWE without PCOS group. In addition, PCOS phenotypes such as ovarian volume, AFC, total testosterone, FAI, FSH, LH, LH/FSH, and dehydroepiandrosterone (DHEA-S) were significantly higher in patients with PCOS compared with WWE without PCOS group. On the contrary, we detected significant lower HDL, quantitative insulin sensitivity check index, HOMA-β, SHBG, and androstenedione in patients with PCOS compared with WWE without PCOS group (Table 2).

Interestingly, regarding epilepsy characteristics, among WWE (n=130), ~38.4% had PCOS, ~40% of patients with PCOS had generalized seizure type, and 60% had partial seizure. Of patients with PCOS, 56% had TLE and 44% had extra-TLE, which was significantly higher than WWE without PCOS (P<0.001).

In patients without PCOS, ~47.5% of patients had generalized seizure type and 52.5% had partial seizure. Of patients with PCOS, 58.7% had TLE. Moreover,

Table 1 Clinical, anthropometric, and laboratory characteristics of studied groups

	Control group (*n*=70) (mean±SD)	WWE group (*n*=130) (mean±SD)	*P*
Age (years)	27.34±4.72	28.54±6.1	0.153
Systolic blood pressure (mmHg)	117.17±4.30	120.44±7.05	<0.001*
Diastolic blood pressure (mmHg)	75.6±7.645	73.72±7.84	0.105
BMI (kg/m^2)	27.48±4.27	30.83±5.71	<0.001*
Seizure type			
Generalized	–	58	
Partial		72	
Epilepsy type			
TLE	–	75	
eTLE		55	
Epilepsy duration (years)	–	7.9±2.7	
Seizure frequency (/month)	–	2.7±2.3	
Total cholesterol (mg/dl)	190.6±20.12	219.29±29.05	<0.001*
Triglycerides (mg/dl)	169.8±20.62	214.03±46.29	<0.001*
LDL cholesterol (mg/dl)	113.36±22.8	136.25±27.7	<0.001*
HDL cholesterol (mg/dl)	43.25±4.55	40.23±5.96	<0.001*
Fasting plasma glucose (mg/dl)	89.7±4.32	88.86±2.1	<0.001*
Fasting serum insulin (U/ml)	8.17±2.42	14.75±6.21	<0.001*
QUICKI	0.35±0.016	0.32±0.019	<0.001*
HOMA-IR	1.81±0.595	3.2±1.337	<0.001*
HOMA-β	110.1±24.40	78.34±93.61	<0.001*
FSH (mIU/ml)	8.9±0.32	8.34±0.4319	<0.001*
LH (mIU/ml)	10.11±1.58	11.1±2.66	<0.001*
LH/FSH	1.13±0.192	1.34±0.377	<0.001*
SHBG (nmol/l)	44.15±17.70	29.42±14.30	<0.001*
DHEA-S (mg/ml)	0.95±0.24	1.43±0.53	<0.001*
Androstenedione (ng/ml)	3.17±1.645	2.4±1.8	<0.001*
Total testosterone (ng/ml)	0.62±0.303	0.92±0.318	<0.001*
Free androgen index	1.46±0.32	3.24±1.88	<0.001*
SOD (ng/ml)	2.01±0.399	4.115±0.785	<0.001*
MDA (nmol/ml)	1.635±0.571	4.35±2.12	<0.001*
AEDs used [*n* (%)]			
No treatment	–	5 (3.8)	
VPA	–	68 (52.3)	
CBZ	–	28 (21.5)	
Others	–	29 (22.3)	

CBZ, carbamazepine; DHEA, dehydroepiandrosterone; ETLE, extratemporal lobe epilepsy; FSH, follicle-stimulating hormone; HOMA-b, homeostasis model assessments of insulin resistance, an index of b-cell function; HOMA-IR, homeostasis model assessments of insulin resistance; LH, luteinizing hormone; MDA, malondialdehyde; QUICKI, quantitative insulin sensitivity check index; SHBG, sex hormone-binding globulin; SOD, superoxide dismutase; TLE, temporal lobe epilepsy; VPA, valproic acid; WWE, women with epilepsy. *P<0.05.

patients with PCOS had significantly higher values of epilepsy duration, as well as seizure frequency compared with WWE without PCOS group (Table 2).

Regarding treatment, 70% of patients with PCOS were treated with VPA and 18% were treated with CBZ. In non-PCOS group, 41.2% of patients with PCOS were treated with VPA and 23.7% were treated with CBZ (Table 2).

Comparison of serum malondialdehyde (nmol/ml) and superoxide dismutase (ng/ml) in the studied groups

Regarding serum MDA level, among WWE group, patients with PCOS (6.52±1.34) had significantly higher levels compared with women without PCOS group (3.11±1.19) (Fig. 1a).

Regarding serum levels of SOD among WWE group, patients with PCOS (4.638±0.16) had significantly higher levels compared with women without PCO (3.78±0.843) (Fig. 1b).

Effect of AEDs (valproic acid and carbamazepine) on OS markers: malondialdehyde and superoxide dismutase

WWE treated by VPA (5.93±1.53) had significantly higher levels of MDA compared with WWE treated by CBZ (3.121±1.3) (Fig. 2a). Furthermore, WWE treated by VPA (4.43±0.392) had significantly higher

Table 2 Clinical, anthropometric, and laboratory characteristics of women with epilepsy groups

	Without PCO group (*n*=80) (mean±SD)	With PCO group (*n*=50) (mean±SD)	*P*
Age (years)	28.46±6.34	28.6±5.99	0.899
Systolic blood pressure (mmHg)	118.94±7.15	121.38±6.87	0.054
Diastolic blood pressure (mmHg)	71.44±6.44	75.15±8.322	<0.001*
Hirsutism score	4.66±2.69	18.4±61.19	<0.001*
BMI (kg/m^2)	28.26±6.75	32.43±4.274	<0.001*
Ovarian volume	5.46±1.97	9.26±3.98	<0.001*
AFC	4.46±1.97	11.26±4.68	<0.001*
Seizure type [*n* (%)]			<0.001*
Generalized	38 (47.5)	20 (40)	
Partial	42 (52.5)	30 (60)	
Epilepsy type [*n* (%)]			<0.001*
TLE	47 (58.75)	28 (56)	
eTLE	33 (41.25)	22 (44)	
Epilepsy duration (years)	2.5±2.4	5.4±2.7	<0.001*
Seizure frequency (/month)	2.1±1.1	3.7±1.8	<0.001*
Total cholesterol (mg/dl)	202.88±32.94	229.55±20.68	<0.001*
Triglycerides (mg/dl)	193.84±27.50	226.65±51.09	<0.001*
LDL cholesterol (mg/dl)	121.05±33.3	145.75±18.01	<0.001*
HDL cholesterol (mg/dl)	43.06±5.96	38.46±5.281	<0.001*
Fasting plasma glucose (mg/dl)	88.82±1.92	88.88±2.22	<0.001*
Fasting serum insulin (μU/ml)	12.05±5.1	16.44±6.26	<0.001*
QUICKI	0.33±0.016	0.319±0.018	<0.001*
HOMA-IR	2.64±1.141	3.59±1.32	<0.001*
HOMA-β	88.87±27.54	57±16.87	<0.001*
FSH (mIU/ml)	8.65±0.27	8.153±0.39	<0.001*
LH (mIU/ml)	9.15±0.27	9.853±0.99	<0.001*
LH/FSH	1.11±0.1012	1.49±0.41	<0.001*
SHBG (nmol/l)	33.48±13.13	26.88±14.5	<0.001*
DHEA-S (mg/ml)	1.16±0.49	1.60±0.48	<0.001*
Androstenedione (ng/ml)	4.12±1.9	1.38±0.40	<0.001*
Total testosterone (ng/ml)	0.848±0.36	0.97±0.27	<0.001*
Free androgen index	2.36±0.73	3.80±2.16	<0.001*
AEDs used [*n* (%)]			
No treatment	3 (3.7)	2 (4)	0.611
VPA	33 (41.2)	35 (70)	0.875
CBZ	19 (23.7)	9 (18)	0.655
Others	25 (31)	4 (8)	<0.001*

AFC, antral follicular count; CBZ, carbamazepine; FSH, follicle-stimulating hormone; LH, luteinizing hormone; QUICKI, quantitative insulin sensitivity check index; SHBG, sex hormone-binding globulin; VPA, valproic acid.

levels of SOD compared with WWE treated by CBZ (4.14±0.726) (Fig. 2b).

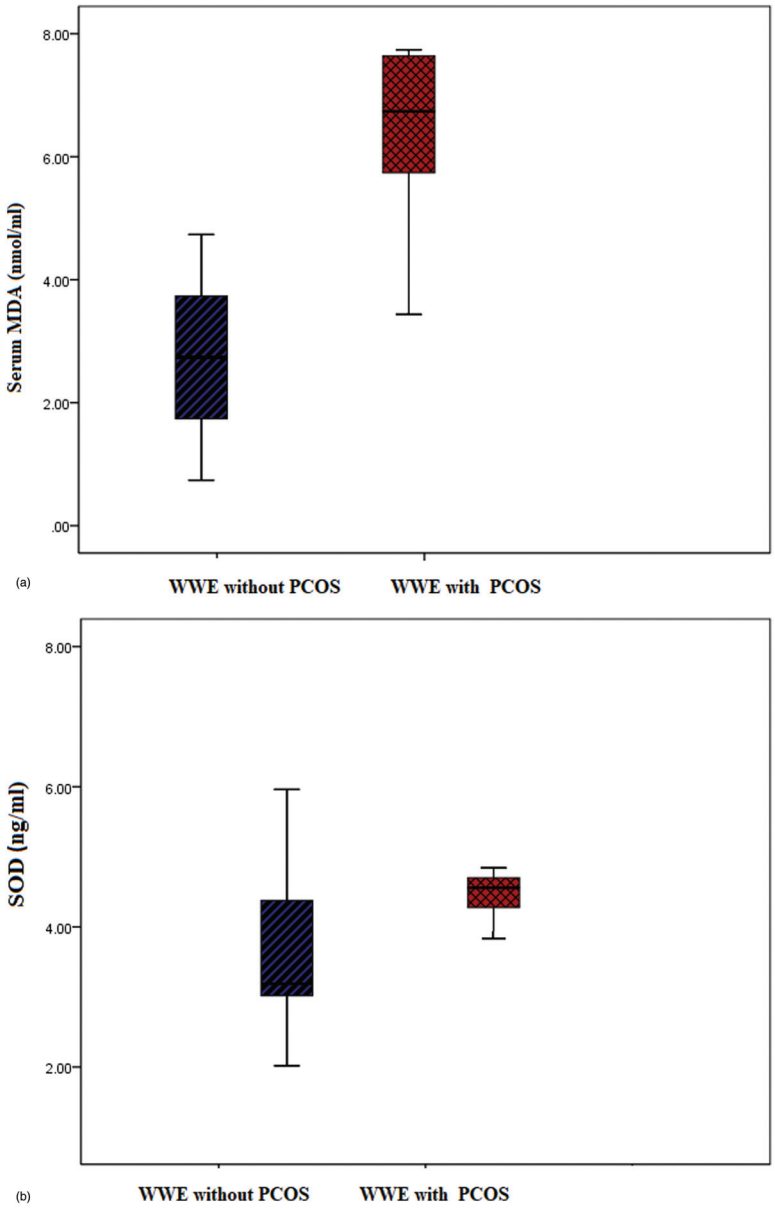
Correlation between both malondialdehyde (nmol/ml) and superoxide dismutase (ng/ml) and clinical and biochemical parameters of patients with polycystic ovary syndrome

In PCOS group (*n*=50), serum MDA and SOD levels were significantly positively correlated with systolic and diastolic blood pressure as well as PCOS phenotypes such as ovarian volume AFC, total testosterone, LH, and DHEA-S. Even more interestingly, serum MDA and SOD levels were significantly positively correlated with cardiometabolic factors such as FPG, FSI, HOMA-IR, TC, TG, and LDL cholesterol. On the contrary, serum MDA and SOD levels were negatively correlated with HDL, quantitative insulin sensitivity check index, HOMA-β, SHBG, and androstenedione (Table 3).

Linear regression analysis with serum malondialdehyde (nmol/ml) and superoxide dismutase (ng/ml) levels as dependent variables in women with epilepsy groups

In the WWE group, linear regression analysis revealed that only LDL and LH were the main predictors of serum MDA levels among other clinical and laboratory biomarkers of PCOS, whereas linear regression analysis revealed that only BMI and LH were the main predictors of serum SOD levels among other clinical and laboratory biomarkers of PCOS (Table 4).

Figure 1



(a) Comparison of serum MDA (nmol/ml) levels in studied groups. (b) Comparison of serum SOD (ng/ml) levels in studied groups. MDA, malondialdehyde; SOD, superoxide dismutase.

Accuracy of serum malondialdehyde (nmol/ml) and superoxide dismutase (ng/ml) for discriminating polycystic ovary syndrome from nonpolycystic ovary syndrome among women with epilepsy by receiver operating characteristic analysis

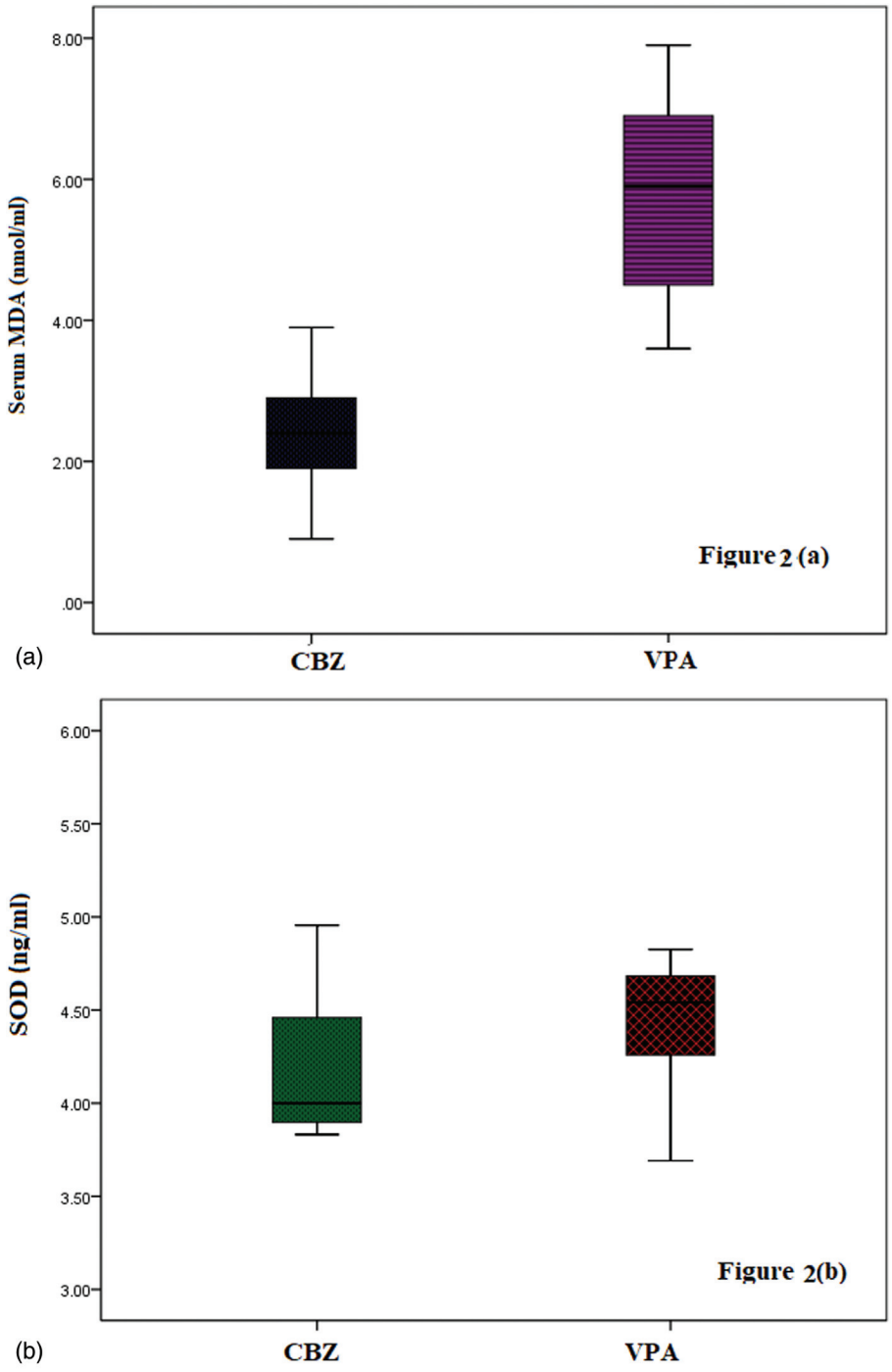
Among WWE, regarding the power of serum MDA levels in discriminating PCOS from non-PCOS, at a cutoff value of 3.75, the AUC was 0.997 (95% CI: 0.990–1.000), with sensitivity of 90% and specificity of 98% (Fig. 3a).

Regarding SOD, at a cutoff value of 3.619, the AUC to diagnose PCOS among studied participants was 0.728 (95% CI: 0.630–0.827), with sensitivity of 94.3% and specificity of 52% (Fig. 3b).

Discussion

Epilepsy is a chronic neurological disorder characterized by recurrent seizures and is often accompanied by cognitive deficits and mood disorders [24–26]. It affects ~1% of the population in the world including one million women in the childbearing period [6]. There are intriguing reports suggesting that WWE has many reproductive disorders and menstrual cycle dysfunction as well, which are considered the main contributing factors of fertility disorders in WWE [7]. These effects are partially explained by the effect of seizures on prolactin, gonadotropin, and sex steroid hormone levels.

Figure 2



(a) Effect of AEDs (VPA and CBZ) on serum MDA (nmol/ml) levels. (b) Effect of AEDs (VPA and CBZ) and serum SOD (ng/ml) levels. CBZ, carbamazepine; MDA, malondialdehyde; SOD, superoxide dismutase; VPA, valproic acid.

Moreover, AEDs and psychosocial as well as socioeconomic factors may have a contributing role [5].

OS occurs owing to an imbalance between oxidative and antioxidative mechanisms. A growing body of evidence supports the hypothesis that epilepsy is mediated by oxidative stress, leading to abnormal structural alterations of cellular proteins, membrane lipids, DNA, and RNA [12,27,28].

Increasing evidence suggests that OS is also intimately involved in PCOS pathogenesis as patients with PCOS show more serious OS compared with the normal. There is a cross-link between OS and physiological and clinical characteristics of PCOS [15].

The mechanisms underlying oxidative stress in PCOS and epilepsy are not fully understood, but recent studies strongly suggest that ~50% of WWE have

Table 3 Pearson's correlation of serum malondialdehyde (nmol/ml) and superoxide dismutase (ng/ml) with clinical, anthropometric, as well as biochemical characteristics of polycystic ovary syndrome

Characteristics	MDA (nmol/ml)		SOD (ng/ml)	
	R	*P*	*R*	*P*
Systolic blood pressure (mmHg)	0.320	<0.001*	0.337	<0.001*
Diastolic blood pressure (mmHg)	0.270	<0.001*	0.415	<0.001*
Hirsutism score	0.365	<0.001*	0.618	<0.001*
BMI (kg/m^2)	0.212	<0.001*	0.517	<0.001*
Ovarian volume	0.363	<0.001*	0.243	<0.001*
AFC	0.469	<0.001*	0.299	<0.001*
Fasting plasma glucose (mg/dl)	0.282	<0.001*	0.333	<0.001*
Fasting serum insulin (μU/ml)	0285	<0.001*	0.328	<0.001*
QUICKI	–0.104	0.394	–0.032	0.584
HOMA-IR	0.103	0.061	0.234	0.061
HOMA-β	–0.566	0.650	–0.113	0.393
Total cholesterol (mg/dl)	0.284	<0.001*	0.483	<0.001*
Triglycerides (mg/dl)	0.277	<0.001*	0.349	<0.001*
LDL cholesterol (mg/dl)	0.263	<0.001*	0.487	<0.001*
HDL cholesterol (mg/dl)	–0.269	<0.001*	–0.456	<0.001*
LH (mIU/ml)	0.551	<0.001*	0.538	<0.001*
SHBG (nmol/l)	–0.500	<0.001*	–0.354	<0.001*
DHEA-S (mg/ml)	0.602	<0.001*	0.523	<0.001*
Androstenedione (ng/ml)	–0.168	<0.001*	–0.453	<0.001*
Total testosterone (ng/ml)	0.252	<0.001*	0.247	<0.001*

AFC, antral follicle cells; DHEA-S, dehydroepiandrosterone sulfate; HOMA-b, homeostasis model assessment, an index of b-cell function; HOMA-IR, homeostasis model assessments of insulin resistance; MDA, malondialdehyde; QUICKI, quantitative insulin sensitivity check index; SHBG, sex hormone-binding globulin; SOD, superoxide dismutase. *$P<0.05$.

Table 4 Linear regression analyses in women with epilepsy to test the influence of the main independent variables against serum malondialdehyde (nmol/ml) and superoxide dismutase (ng/ml) levels (dependent variable)

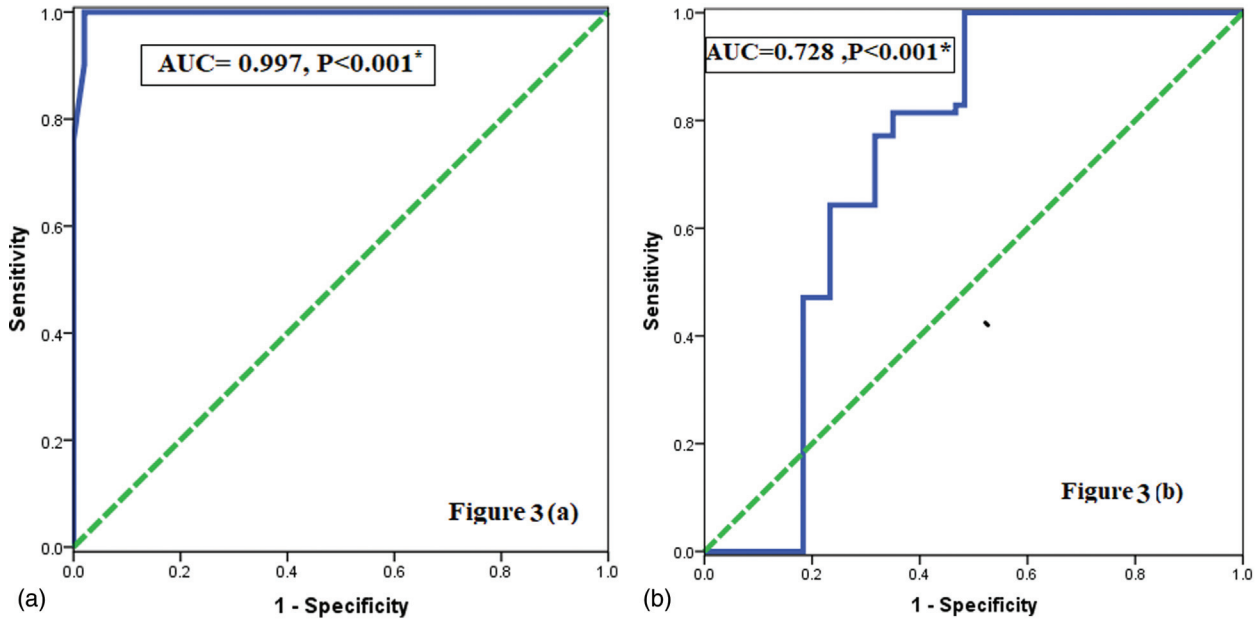
Model	Unstandardized coefficients		Standardized coefficients	*t*	*P* value	95% CI	
	B	SE	β			Lower bound	Upper bound
MDA							
Constant	03.347	0.896	–	3.738	<0.001*	5.120	1.574
LDL	0.018	0.008	0.241	2.318	<0.001*	0.003	0.034
FSI	0.373	0.544	1.093	0.686	0.494	0.704	1.451
HOMA-IR	1.598	2.545	1.008	0.628	0.531	6.635	3.439
BMI	0.046	0.044	0.125	1.064	0.289	0.040	0.133
LH	0.305	0.068	0.384	4.460	<0.001*	0.170	0.440
SOD							
Constant	247.837	64.280	–	3.856	<0.001*	120.59	375.076
LDL	1.051	0.571	0.202	1.842	0.068	0.079	2.181
FSI	41.151	39.073	1.768	1.053	0.294	36.191	118.493
HOMA-IR	174.497	182.662	–1.615	0.955	0.341	536.06	187.071
BMI	6.757	3.132	0.267	2.157	<0.001*	12.957	0.557
LH	31.228	4.909	0.576	6.362	<0.001*	21.512	40.945
Total testosterone	1.873	36.866	0.004	0.051	0.960	–71.101	74.847

CI, confidence interval; HOMA-IR, homeostasis model assessments of insulin resistance; LH, luteinizing hormone; MDA, malondialdehyde; SOD, superoxide dismutase. *$P<0.05$.

reproductive disorders. Early assessment of levels of OS could potentially help in early diagnosis and prevention of PCOS. Thus, the purpose of this study was to investigate the role of MDA and SOD serum levels as predictors of PCOS in WWE, and to evaluate the possible relationship between oxidative stress and epilepsy characteristics, antiepileptic drugs, such as VPA and OXC, as well as clinicomorphological features of PCOS.

The interesting result of this study was the association of AEDs, VPA and CBZ, with MDA

Figure 3



(a) ROC curve of serum MDA (nmol/ml) for discriminating PCOS among WWE. (b): ROC curve of serum SOD (ng/ml) for discriminating PCOS among WWE. NDA, malondialdehyde; PCOS, polycystic ovary syndrome; ROC, receiver operating characteristic; SOD, superoxide dismutase; WWE, women with epilepsy.

and SOD. The results of this study reported significantly higher levels of MDA and SOD in patients treated with VPA compared with the ones treated with CBZ.

In agreement with our results, Luef *et al.* [29] suggested that the endocrine changes observed in epileptic patients treated with VPA are secondary to obesity, because, generally, these changes are found only in those epileptic patients who gained weight. Moreover, it has been demonstrated that VPA can directly stimulate pancreatic β cells.

In explanation for the relation between VPA treatment and obesity in WWE, Pylvänen *et al.* [30] noticed that VPA was considered a strong antiepileptic drug which is able to interact with gene expression of many proteins, including insulin, leptin, and adiponectin. In that context, weight gain during VPA treatment is related to an increase in insulin levels. This hyperinsulinemia and the consequent IR are strictly related to the degree of obesity [31].

Additionally, omics studies have indeed demonstrated metabolic and endocrine abnormalities in epileptic patients who gained weight after therapy with VPA. As a matter of fact, it is known that weight gain is associated with pathologic consequences related to obesity such as reproductive disorders, dyslipidemia, hypertension, IR, diabetes mellitus, and atherosclerosis and its related vascular implications [32].

This study revealed that regarding epilepsy characteristics, among WWE, ~44.6% had generalized seizure type and 55.4% had partial seizure; moreover, 57.6% had TLE. Interestingly, among WWE (n=130), ~38.4% had PCOS, ~40% of patients with PCOS had generalized seizure type and 60% had partial seizure. Of the patients with PCOS, 56% had TLE.

In accordance with the results, there are intriguing reports suggesting that epilepsy influences the reproductive endocrine system by affected the hypothalamic–pituitary axis as the discharges from the medial temporal lobe can stimulate the secretion of LH and gonadotropin-releasing hormone [33].

This study attempted to pierce out the prevalence of PCOS among WWE. Our results revealed that among 130 WWE, ~50 patients were diagnosed with PCOS according to Rotterdam criteria. Of patients with PCOS, 70% were treated with VPA and 18% were treated with CBZ. In non-PCOS group, 41.2% were treated with VPA and 23.7% were treated with CBZ.

Similar to this results, Stephen *et al.* [34] observed that women taking VPA had significantly higher testosterone levels. This was reflected in their elevated FAI, which is a particularly sensitive marker of high testosterone, a finding which is also supported by reports of Hopkinson *et al.* [35] who noticed that WWE treated with VPA had high risk of PCOS.

The main objective of this study was to evaluate the association between PCOS clinical and laboratory markers and OS. We studied MDA and SOD as a marker of OS. We found that MDA and SOD were significantly higher in patients with PCOS compared with the control group. Moreover, this study revealed a significantly positive correlation between both MDA and SOD and BMI, IR, and PCOS phenotypes.

Our findings are in concordance with the study by de Melo *et al.* [36]. They detected higher levels of MDA as a marker of OS in infertile women with PCOS.

Similar results were confirmed by Turan *et al.* [37]. They found high values of OS in infertile women compared with fertile women, suggesting that infertility is associated with OS in these women.

Supporting our results, the study by Desai *et al.* [38] revealed MDA levels were found to be significantly high in PCOS group compared with the control group.

In contrast to this study, a cross-sectional investigation conducted in Iran reported an increase in serum and free fluid levels of MDA in obese patients with PCOS compared with lean patients with PCOS, proving obesity not PCOS was associated with OS [39].

To better elucidate the association of serum levels of MDA and SOD, linear regression test was done, and we observed among clinical and laboratory markers of PCOS, only LDL and LH were the main predictors of serum MDA levels in PCOS, whereas BMI and LH were the main predictors of serum SOD levels.

It would be of considerable practical importance to reveal that obese patients are expected to have high OS levels [40], and significant correlations of OS markers with obesity indexes, such as BMI and WC, are discovered [40,41].

As a matter of fact, IR appears to encourage OS because hyperglycemia and higher levels of free fatty acid lead to reactive oxygen species production [42,43].

In an attempt to assess the diagnostic power of expression and serum levels of MDA and SOD, we evaluated our results using receiver operating characteristic analysis. We observed that the AUC of MDA levels was higher than serum levels of SOD in the diagnosis of patients with PCOS from those without PCOS.

Conclusion

There were higher levels of OS markers, MDA and SOD, in both WWE and PCOS. Moreover, they were significantly positively correlated with obesity indices and PCOS phenotypes. We recommended that obese women and/or women with a history of menstrual irregularities may benefit from the screening of metabolic and reproductive test before starting AED treatment. Those with abnormal results should be advised about weight reduction and should not be prescribed VPA.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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