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Original Article

Serum ischemia modified albumin and dynamic thiol/disulfide homeostasis in early- and late-onset preeclampsia

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Abstract

Aim: In the present study, we aimed to evaluate serum IMA, IMA/albumin ratio, and DTDH levels in patients with early- and late-onset PE compared to healthy controls.Impaired homeostasis between oxidant and antioxidant mechanisms, inflammatory processes, and endothelial dysfunction play a key role in the pathogenesis of preeclampsia (PE). Serum ischemia modified albumin (IMA) and dynamic thiol/disulfide homeostasis (DTDH) levels are elevated in the presence of inflammation, oxidative stress, and endothelial dysfunction.

Material and Methods: A total of 24 patients with early-onset PE and 62 patients with late-onset PE were included.The control group consisted of 46 healthy controls with similar gestational weeks. Serum samples were collected and IMA, albumin, and native, total, and disulfide thiol levels analyzed. Corrected IMA/albumin ratios were also calculated.

Results: Disulfide levels, disulfide/native and disulfide/total thiol levels were higher in patients with early-onset PE compared to late-onset patients(p=0.008, p=0.022, and p=0.021, respectively). However, there was no significant difference between the late-onset PE patients and late-onset PE controls. Although there was no significant difference in the IMA levels between the patient and control groups, the IMA/albumin ratio was higher in the early-onset and late-onset PE patients, compared to the control group. However, there was no significant difference between the early-onset and late-onset PE patients.

Conclusion: Our study results showed increased disulfide levels, disulfide/native thiol, disulfide/total thiol and IMA/albumin ratio in the early-onset PE patients, indicating increased oxidative stress in the pathogenesis of PE. In the late-onset PE patients, there was an increase only in the IMA/albumin ratio. However, further large-scale, prospective studies are needed to confirm the diagnostic value of these markers in the clinical practice

Keywords: ischemia modified albumin; preeclampsia; thiol

INTRODUCTION

Preeclampsia (PE) is one of the major causes of maternal, fetal and neonatal mortality and morbidity, accounting for 3 to 5% of all pregnancies (1). In the literature, PE is classified as early- and late-onset (2). Early-onset PE is defined as the onset before 34 weeks of pregnancy, while late-onset PE is defined as the onset after 34 weeks of pregnancy. Although initial manifestations of both early- and late-onset PE are similar, they have distinct biochemical biomarkers, genetic risk factors, prognosis, and clinical characteristics (3).

Preeclampsia is a multi-systemic disease and impaired homeostasis between oxidant and antioxidant mechanisms, inflammatory processes, and endothelial dysfunction have been proposed to play a key role in its pathogenesis (4).

The thiol group, which is also known as mercaptans, contain a sulfhydryl group (-SH), establishes a covalent bond with a disulfide bridge in case of oxidative stress. The disulfide bonds are re-reduced to thiol group to maintain the continuity of dynamic thiol-disulfide homeostasis (DTDH) (4). The DTDH plays a central role in antioxidant defense mechanism, detoxification, and apoptosis (5).The majority of thiols in plasma are associated with albumin and SH groups are oxidized in the presence of oxidative stress, thereby, leading to structural changes in albumin (6). Albumin plays a major role in the regulation of oncotic pressure with antioxidant, anti-inflammatory, and anti-thrombotic effects (7). In case of oxidative stress, certain alterations occur in the N terminal portion of albumin, leading to reduced binding to heavy metals such as copper and cobalt (8). This new chemical structure of albumin is termed as ischemia modified albumin (IMA) (8).

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Increased IMA concentrations have been shown to be associated with inflammation, oxidative stress, and endothelial dysfunction (9). As placental hypoxia and oxidative stress are involved in the pathogenesis of PE, IMA has been suggested to be a useful biomarker in the diagnosis of PE.

Despite similar initial symptoms of early- and late-onset PE, the former type of PE has been suggested to be more frequently associated with incomplete spiral artery remodeling, inadequate trophoblast invasion, improper immune adaptation, and endothelial dysfunction (9). Compared to late-onset PE, early-onset PE is a more severe phenotype associated with a higher rate of fetal and neonatal morbidity and mortality (9).

Although the exact pathophysiology of PE has not been well understood yet, oxidative stress has been suggested to play a role.In a systemic review and meta-analysis, IMA was shown to be a useful biomarker for oxidative stress, hypoxia, and endothelial dysfunction in PE patients (10). In addition, DTDH has been thought to play a role in the antioxidant defense mechanism and pathophysiology of PE.In the present study, we aimed to evaluate serum IMA, IMA/albumin ratio, and DTDH levels in patients with early- and late-onset PE, compared to healthy controls.

MATERIAL and METHODS

Study population

This case-control study was carried out at the Department of Obstetrics and Gynecology of Bursa Yuksek Ihtisas Training and Research Hospital between July 2019 and November2019. A total of 132 participants including 86 patients with PE and 46 healthy controls were included. The patient group was classified as early-onset (n=24) and late-onset PE (n=62). A written informed consent was obtained from each participant. The study protocol was approved by the Ethics Committee of Bursa Yuksek Ihtisas Training and Research Hospital (2011-KAEK-25 2019/07-07). The study was conducted in accordance with the principles of the Declaration of Helsinki.

The control group consisted of healthy women with singleton pregnancy with similar gestational weeks that were under follow-up in our outpatient clinics without any fetal abnormality. Of the control group, 12 were in the <34th week of pregnancy (early-onset PE controls), while 34 were in the ≥34th week of pregnancy (late-onset PE controls). Patients with chronic hypertension, thyroid dysfunction, renal or cardiovascular disease, and multiple pregnancy were excluded from the study. Those with gestational diabetes, preterm premature rupture of the membranes, and women preterm delivery were also excluded. In the control group, no PE or other adverse pregnancy outcomes were observed during pregnancy follow-up after collecting blood samples.

The PE diagnosis was made based on at least two high systolic (≥140 mmHg) or diastolic (≥90 mmHg) blood pressure measurements (at 4-hour intervals) in a previously normotensive pregnant woman, and also the presence of one or more of the following findings: (1) proteinuria (≥300 mg/24 h;≥30 mg/moLprotein:creatinine ratio; or a dipstick-test result ≥ 2+); (2) other maternal organ dysfunction, including acute kidney injury(creatinine ≥90 µmol/L; 1 mg/dL); liver involvement (elevated transaminases, e.g., alanine aminotransferase [ALT] or aspartate aminotransferase [AST] >40 IU/L) with or without right upper quadrant or epigastric abdominal pain; neurological complications (e.g., eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, and persistent visual scotomata); or hematological complications (thrombocytopenia with a platelet count <150,000/µL,disseminated intravascular coagulation, hemolysis); and (3) uteroplacental dysfunction (such as fetal growth restriction (FGR),abnormal umbilical artery Doppler waveform analysis, or stillbirth)(11).Early-onset PE is defined as the onset before 34 weeks of pregnancy, while late-onset PE is defined as the onset after 34 weeks of pregnancy.

Biochemical analyses

A 5-mL venous blood sample was drawn from each patient during ward stay and from each healthy control during outpatient visit. The samples were centrifuged at 3,500 rpm for 10 min and kept at -80º until analysis. The samples were, then, sent to Biochemistry Lab of YildirimBeyazit University, Ankara, Turkey and thiol and IMA levels were analyzed.

The IMA levels were determined using the rapid colorimetric method developed by Bar-Or et al. (12). A known amount of cobalt was added to the serum sample and unbound cobalt was measured from absorbance of colored complex through the addition of dithiothreitol. The IMA levels were given in absorbance unit (ABSU). Albumin-adjusted IMA levels were calculated using the following formula described by Lippi et al. (13):(individual serum albumin concentration/median albumin concentration of the population) x IMA value.

Thiol/disulfide homeostasis was determined using a novel spectrophotometric method developed by Erel and Neselioglu (5). Accordingly, reducible disulfide bonds were reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native thiol groups were determined after the reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid. Half of the difference between the total thiols and the native thiols was recorded as the dynamic disulfide amount. Once the native thiols and total thiols were determined, disulfide amount, disulfide/totalthiol ratio, disulfide/native thiol ratio, and nativethiol/total thiol ratio were calculated. All measurements were made using the Cobas 501 automated clinical chemistry analyzer (Roche Diagnostics, Basel, Switzerland) and the results were given in µmol/L.

Statistical Analysis

For determining the minimum required sample size, Type-I error probability was 5%, Type-II error probability was 20% (80% power), and the effect size was taken as 0.13 (16).

Based on these results, minimum required total sample size was calculated as 86 subjects.

Statistical analysis was performed using the SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), quartile (25th, median, and 75th), and number and frequency. The Kolmogorov-Smirnov test was used to test normal distribution of continuous variables. The Kruskal-Wallis test was used to analyze significant differences between non-normally distributed continuous variables. The *post-hoc* Dunn test was performed to determine groups with significant differences. The Fisher-Freeman-Halton exact test was used to examine the distribution of categorical variables.The receiver operating characteristic (ROC) curve was used to establish the predictive value of IMA/albumin ratio for early- and late-onset PE between the patient and control groups.Ap value of <0.05 was considered statistically significant.

RESULTS

A total of 132 participants including 86 patients with PE and 46 healthy controls were included in this study. Of the patients, 24 had early-onset PE and 62 had late-onset PE. Of the healthy controls, 12 were early-onset PE controls and 34 were late-onset PE controls.The mean age was similar between the early- and late-onset PE patients; however, it was statistically significantly higher than the control group (p=0.014). In addition, there was a statistically significant difference in the body weight between the early- and late-onset PE patients with the highest body weight in the late-onset PE compared to the other groups (p<0.001). Also, the systolic and diastolic blood pressures were statistically significantly higher in the early-onset PE patients (p<0.001). Demographic characteristics are shown in Table 1.

Table 1. Demographic and clinical characteristics of early- and late-onset preeclampsia patients and controls

							Percentile			
	Group	n	min	max	Mean*	SD	25th	Median	75th	p
Systolic blood pressure	EO-PE patients	24	140	200	163.33[a]	17.11	150.00	160.00	170.00	**<0.001**
(mmHg)	LO-PE patients	62	140	220	155.32[b]	17.44	140.00	150.00	160.00	
	LO-PE controls	34	100	120	109.12[c]	10.26	100.00	105.00	120.00	
	EO-PE controls	12	100	130	112.50[c]	8.66	102.50	115.00	120.00	
Diastolic blood pressure	EO-PE patients	24	100	130	105.42[a]	8.33	100.00	100.00	110.00	**<0.001**
(mmHg)	LO-PE patients	62	80	130	96.77[b]	8.64	90.00	100.00	100.00	
	LO-PE controls	34	60	80	64.71[c]	7.48	60.00	65.00	70.00	
	EO-PE controls	12	50	80	70.00[c]	8.53	60.00	70.00	80.00	
Gravida	EO-PE patients	24	1	8	2.54	1.69	1.00	2.00	3.00	0.515
	LO-PE patients	62	1	8	2.63	1.54	1.00	2.50	3.25	
	LO-PE controls	34	1	4	2.18	.90	1.75	2.00	3.00	
	EO-PE controls	12	1	4	2.00	1.13	1.00	1.50	3.00	
Parity	EO-PE patients	24	0	4	1.21	1.28	0	1.00	2.00	0.862
	LO-PE patients	62	0	5	1.15	1.21	0	1.00	2.00	
	LO-PE controls	34	0	3	1.06	.85	0	1.00	2.00	
	EO-PE controls	12	0	3	1.42	1.24	0	1.50	2.75	
Age (year)	EO-PE patients	24	17	41	30.63[ab]	7.25	24.00	32.50	37.00	**0.014**
	LO-PE patients	62	16	45	31.03[b]	6.61	27.00	31.00	36.00	
	LO-PE controls	34	19	39	27.24[c]	6.79	22.75	25.50	31.25	
	EO-PE controls	12	17	44	26.00[c]	7.20	20.25	22.00	34.25	
Gestational age (week)	EO-PE patients	24	26	33	30.50[a]	2.21	28.25	31.50	32.00	**<0.001**
	LO-PE patients	62	30	42	36.15[c]	1.62	35.00	36.00	37.00	
	LO-PE controls	34	32	38	37.03[d]	1.47	36.00	37.00	38.00	
	EO-PE controls	12	34	40	33.00[b]	1.71	32.00	32.50	33.00	
Body weight (kg)	EO-PE patients	24	59	117	83.63[b]	12.92	76.00	82.50	87.25	**<0.001**
	LO-PE patients	62	57	140	92.90[c]	17.78	80.00	92.50	100.00	
	LO-PE controls	34	55	89	76.26[b]	10.76	67.75	79.00	85.00	
	EO-PE controls	12	55	98	69.25[a]	8.79	65.25	68.00	73.50	
Height (cm)	EO-PE patients	24	155.00	180.00	162.50	6.14	158.50	162.00	165.00	0.486
	LO-PE patients	62	151.00	175.00	163.87	4.47	160.00	165.00	166.25	
	LO-PE controls	34	145.00	168.00	162.44	6.47	158.00	163.00	167.00	
	EO-PE controls	12	145.00	175.00	162.58	6.36	160.25	165.00	167.00	

*Superscript letters next to the mean values indicate groups with significant differences. Separate superscript letters (i.e., a. b. c) indicate statistically significant differences in the mean values between the groups. The Kruskal-Wallis test was used for all statistical analyses
SD, standard deviation; EO, early-onset; LO, late-onset; PE, preeclampsia

A total of 12 patients (54.5%) in the early-onset PE group and 11 patients (17.7%) in the late-onset PE group and four women (11.8%) in the control group had FGR. There was no statistically significant difference in the FGR rates between the late-onset PE group and late-onset PE controls; however, a statistically significant difference was observed between the early-onset PE and the other two groups (p=0.002).

The laboratory test results showed similar AST, ALT, creatinine, and urea levels between the control groups; however, these levels were statistically significantly higher in the early-onset PE patients (p<0.001). The mean platelet count was also similar between the control groups, but was statistically significantly lower in the early-onset PE group than the other groups (p<0.001). In addition, the mean white blood cell count was statistically significantly higher in the early-onset PE patients than the other groups (p=0.01). The mean hemoglobin level was comparable between the early- and late-onset PE patients, while it was statistically significantly higher in both control groups (p=0.006) (Table 2).

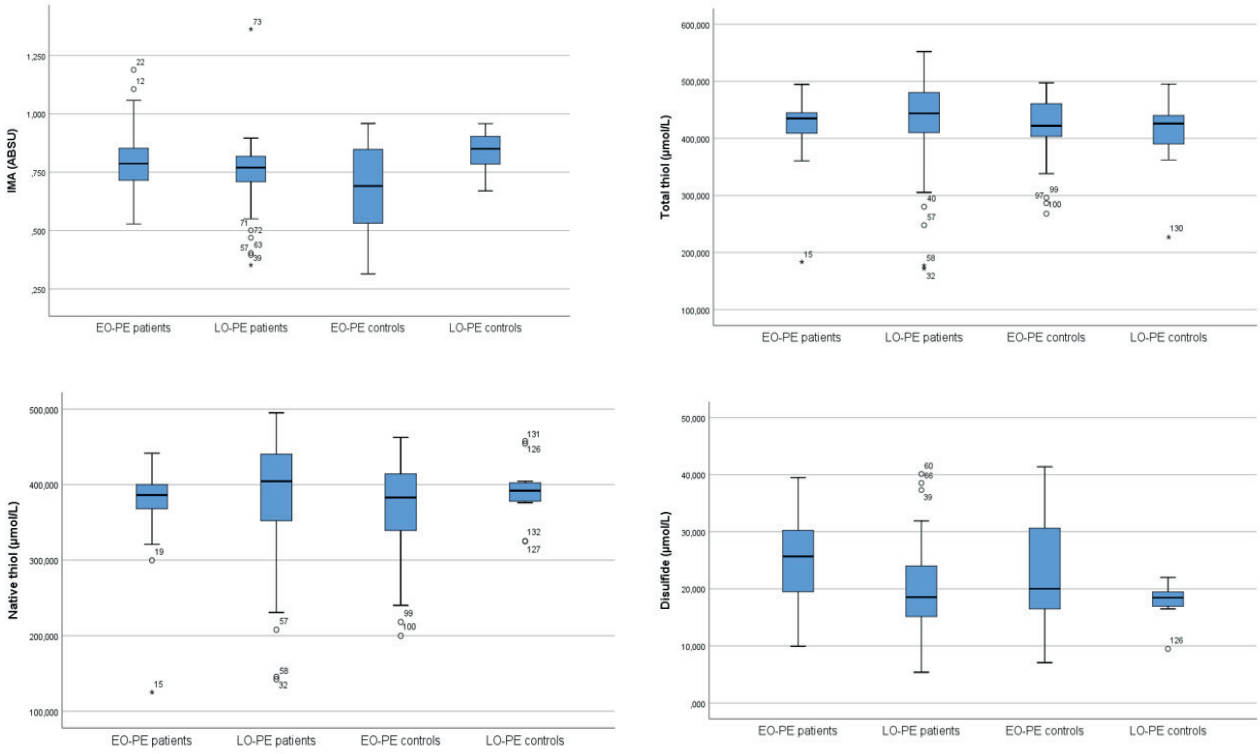

Figure 1. IMA, Disulfide and thiol data for each group on the box-plot chart

There was no significant difference in the native thiol and total thiol ratios between the groups (p=0.204 and p=0.303, respectively). However, the disulfide levels, disulfide/native thiol ratio (Index 1) and disulfide/total thiol ratio (Index 2) were significantly higher in the patients with early-onset PE, compared to late-onset PE (p=0.008, p=0.022, and p=0.021, respectively). These levels were also higher in the early-onset PE controls, while there was no significant difference between the patients and controls with late-onset PE. In addition, native/total thiol ratio (Index 3) was lower in the patients with early-onset PE compared to the patients with late-onset PE and early-onset PE controls, while there was no significant difference between the patients and late-onset PE controls (p=0.022) (Table 3).

Although there was no significant difference in the IMA levels between the patient and control groups, the IMA/albumin ratio was higher in the early-onset and late-onset PE patients, compared to the control groups (p=0.001). However, there was no significant difference between the early-onset and late-onset PE patients (Table 3).The IMA, disulfide and thiol data for each group on the box-plot chart are shown in Figure 1.

According to the ROC curve to identify the predictive value of IMA/albumin ratio for early- and late-onset PE between the patient and control groups, the IMA/albumin ratio had a statistically significant discriminatory ability (p<0.001). Based on a cut-off value of 0.8034, the sensitivity and specificity of IMA/albumin ratio were calculated as 75% and 91.2%, respectively for the diagnosis of early-onset PE (Figure 2). In addition, the IMA/albumin ratio had a statistically significant discriminatory ability with regards to late-onset PE and control group (p=0.008). Using a cut-off value of 0.7644, the sensitivity and specificity of IMA/albumin ratio were calculated as 75.8% and 75%, respectively (Figure 3).

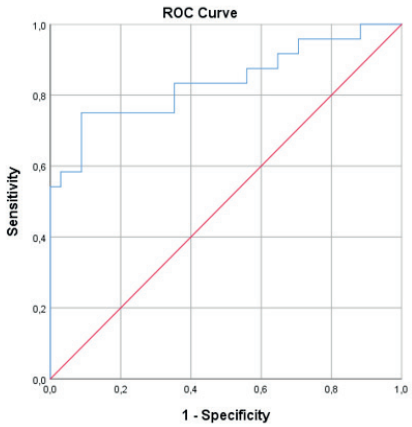

Figure 2. ROC curve for IMA/albumin ratio in the early-onset PE group

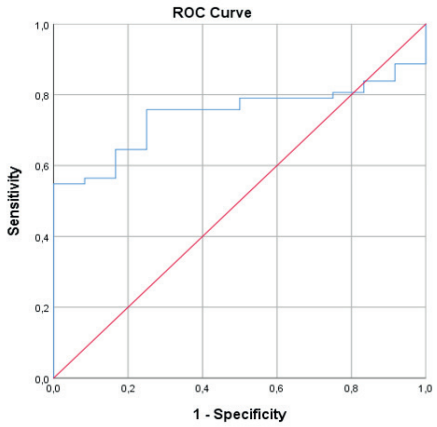

Figure 3. ROC curve for IMA/albumin ratio in the early-onset PE group

Table 2. Biochemical analyses of early- and late-onset preeclampsia patients and controls

							Percentile			
	Group	n	min	max	Mean*	SD	25th	Median	75th	p
Platelets (10_3/mL)	EO-PE patients	24	48	380	127.88[a]	74.14	86.00	103.50	158.00	**<0.001**
	LO-PE patients	62	68	365	167.48[b]	69.28	119.25	149.50	196.25	
	LO-PE controls	34	216	364	262.71[c]	69.72	197.00	275.00	322.50	
	EO-PE controls	12	117	382	272.00[c]	38.66	248.25	263.00	282.50	
WBC (10^3/mL)	EO-PE patients	24	7.70	22.30	15.78[a]	4.47	11.23	17.25	18.75	
	LO-PE patients	62	6.40	23.00	13.11[b]	4.05	10.35	12.20	16.10	**0.010**
	LO-PE controls	33	8.60	14.30	12.62[b]	3.52	9.70	11.60	15.50	
	EO-PE controls	12	7.40	20.00	10.95[b]	1.52	9.53	11.20	11.60	
Hemoglobin (g/dL)	EO-PE patients	24	7.70	13.90	10.95[b]	1.52	9.53	11.20	11.60	**0.006**
	LO-PE patients	62	7.40	13.90	10.72[b]	1.53	9.73	10.90	11.65	
	LO-PE controls	34	9.80	13.60	10.70[b]	1.29	9.80	10.90	11.43	
	EO-PE controls	12	8.40	13.70	11.55[a]	1.22	10.93	11.80	12.50	
AST (IU/L)	EO-PE patients	24	17.00	260.00	109.67[a]	65.47	42.25	120.00	144.00	**<0.001**
	LO-PE patients	62	15.00	135.00	47.81[b]	34.54	25.00	34.50	49.75	
	LO-PE controls	34	14.00	28.00	21.68[c]	8.80	14.75	19.50	26.25	
	EO-PE controls	12	10.00	41.00	19.42[c]	4.01	16.25	19.00	22.00	
ALT (U/L)	EO-PE patients	24	12.00	294.00	102.79[a]	76.20	22.50	114.50	129.75	**<0.001**
	LO-PE patients	62	7.00	210.00	41.44[b]	41.23	16.50	24.50	43.00	
	LO-PE controls	34	6.00	18.00	17.44[c]	10.35	10.75	14.00	23.00	
	EO-PE controls	12	6.00	48.00	12.67[c]	3.65	10.25	12.00	15.75	
Urea (mg/dL)	EO-PE patients	24	7.40	31.90	20.65[a]	6.75	14.35	21.60	26.10	**<0.001**
	LO-PE patients	62	5.00	28.80	13.52[b]	5.39	9.75	11.85	16.95	
	LO-PE controls	34	3.60	11.07	8.52[c]	2.34	7.18	8.45	9.80	
	EO-PE controls	12	3.70	13.60	6.43[d]	2.46	4.60	6.10	8.00	
Creatinine (mg/dL)	EO-PE patients	24	.53	1.70	1.04[a]	.33	.72	1.10	1.24	**<0.001**
	LO-PE patients	62	.52	1.70	.85[b]	.26	.67	.80	.93	
	LO-PE controls	34	.40	.80	.65[c]	.13	.58	.61	.70	
	EO-PE controls	12	.47	1.10	.66[c]	.11	.60	.68	.72	
Albumin (g/dL)	EO-PE patients	24	2.109	6.965	4.59[a]	1.17	3.84	4.66	5.13	**<0.001**
	LO-PE patients	62	1.493	7.084	4.70[a]	1.11	4.25	4.69	5.26	
	LO-PE controls	34	3.360	4.020	3.52[b]	.39	3.36	3.59	3.73	
	EO-PE controls	12	1.944	4.008	3.73[b]	.25	3.50	3.78	3.98	

*Superscript letters next to the mean values indicate groups with significant differences. Separate superscript letters (i.e., a. b. c) indicate statistically significant differences in the mean values between the groups. The Kruskal-Wallis test was used for all statistical analyses.
SD, standard deviation; EO, early-onset; LO, late-onset; PE, preeclampsia; WBC, white blood count; AST, aspartate aminotransaminase; ALT, alanine aminotransferase

Table 3. Oxidative stress biomarkers of early- and late-onset preeclampsia patients and controls

							Percentile			
	Group	n	min	max	Mean*	SD	25th	Median	75th	p
Native thiol (µmol/L)	EO-PE patients	24	124.992	441.595	371.36	61.74	366.50	386.14	400.00	0.204
	LO-PE patients	62	142.135	495.000	386.94	77.08	351.05	404.40	440.42	
	LO-PE controls	34	325.120	457.600	373.08	63.73	338.88	382.96	416.20	
	EO-PE controls	12	199.840	462.400	391.24	40.20	377.11	392.08	403.36	
Total thiol (µmol/L)	EO-PE patients	24	183.540	494.570	421.20	61.40	407.50	434.99	444.83	0.303
	LO-PE patients	62	171.760	552.000	426.45	77.40	408.00	443.76	480.71	
	LO-PE controls	34	227.000	495.075	417.68	55.95	403.39	422.10	462.16	
	EO-PE controls	12	268.065	497.375	410.43	69.26	376.13	426.00	441.92	
Disulfide (µmol/L)	EO-PE patients	24	9.931	39.500	24.92[a]	6.76	19.25	25.67	30.35	**0.008**
	LO-PE patients	62	5.386	40.123	19.76[b]	7.50	14.84	18.53	24.03	
	LO-PE controls	34	9.480	21.990	22.60[ab]	9.39	16.49	20.00	30.82	
	EO-PE controls	12	7.082	41.385	17.92[b]	3.08	16.94	18.45	19.59	
IMA (ABSU)	EO-PE patients	24	.528	1.189	.80[ab]	.16	.71	.79	.85	**0.009**
	LO-PE patients	62	.352	1.363	.75[b]	.15	.71	.77	.82	
	LO-PE controls	34	.670	.958	.68[b]	.18	.52	.69	.85	
	EO-PE controls	12	.314	.959	.84[a]	.08	.78	.85	.91	
IMA/Albumin	EO-PE patients	24	.41	1.67	.90[a]	.30	.70	.86	1.04	**<0.001**
	LO-PE patients	62	.15	1.50	.85[a]	.24	.77	.86	.98	
	LO-PE controls	34	.64	.85	.58[c]	.17	.43	.60	.74	
	EO-PE controls	12	.27	.86	.75[b]	.06	.71	.75	.80	
Index 1	EO-PE patients	24	2.445	23.421	7.24[a]	3.93	5.11	6.52	8.22	**0.022**
	LO-PE patients	62	1.554	16.183	5.46[b]	2.74	3.54	5.09	6.61	
	LO-PE controls	34	2.072	5.846	6.59[ab]	3.88	4.21	5.29	8.79	
	EO-PE controls	12	1.752	17.867	4.65[b]	1.00	4.29	4.68	5.52	
Index 2	EO-PE patients	24	2.331	15.950	6.15[a]	2.55	4.63	5.76	7.06	**0.021**
	LO-PE patients	62	1.507	12.226	4.82[b]	2.11	3.31	4.62	5.84	
	LO-PE controls	34	1.989	5.234	5.65[ab]	2.87	3.88	4.78	7.48	
	EO-PE controls	12	1.692	13.163	4.24[b]	.86	3.95	4.28	4.97	
Index 3	EO-PE patients	24	68.101	95.337	87.69[a]	5.11	85.89	88.47	90.74	**0.022**
	LO-PE patients	62	75.548	96.987	90.37[b]	4.23	88.32	90.77	93.38	
	LO-PE controls	34	89.532	96.021	88.87[ab]	5.59	85.56	90.43	92.23	
	EO-PE controls	12	73.673	96.615	91.51[b]	1.71	90.06	91.44	92.10	

*Superscript letters next to the mean values indicate groups with significant differences. Separate superscript letters (i.e., a. b. c) indicate statistically significant differences in the mean values between the groups. The Kruskal-Wallis test was used for all statistical analyses.
Index 1=disulfide/native thiol*100; Index 2=disulfide/total thiol*100; Index 3=native thiol/total thiol*100
SD, standard deviation; EO, early-onset; LO, late-onset; PE, preeclampsia; IMA, ischemia-modified albumin

Table 4. Correlation analysis results

		INDEX 1			INDEX 2			INDEX 3		
		r	P	N	r	P	N	r	P	N
Early-onset PE	IMA	.277	.189	24	.277	.189	24	-.277	.189	24
	IMA/albumin	.107	.619	24	.107	.619	24	-.107	.619	24
Late-onset PE	IMA	-.095	.464	62	-.095	.464	62	.095	.464	62
	IMA/albumin	-.203	.114	62	-.203	.114	62	.203	.114	62
Early-onset PE controls	IMA	-.107	.548	34	-.107	.548	34	.104	.557	34
	IMA/albumin	-.266	.128	34	-.266	.128	34	.270	.123	34
Late-onset PE controls	IMA	.168	.601	12	.175	.586	12	-.168	.601	12
	IMA/albumin	-.413	.183	12	-.406	.191	12	.413	.183	12

Index 1=disulfide/native thiol*100; Index 2=disulfide/total thiol*100; Index 3=native thiol/total thiol*100
SD, standard deviation; EO, early-onset; LO, late-onset; PE, preeclampsia; IMA, ischemia-modified albumin

The correlation analysis results of IMA and IMA/albumin ratio and relevant indices between the early- and late-onset patient and control groups are summarized in Table 4. There was no statistically significant correlation between the IMA and IMA/albumin ratio and relevant indices.

DISCUSSION

The use of early- and late-onset PE concepts has become widely accepted as a more optimal indicator of disease significance which helps us to gain a better understanding of the complex etiopathogenesis of this medical riddle. Early-onset PE has been shown to be associated with abnormal placental development, while late-onset PE is more frequently associated with maternal microvascular disease such as chronic hypertension or genetic predisposition (2,9). Thus, early- and late-onset PE have different etiologies and should be considered as different entities (2). In the present study, therefore, we evaluated serum IMA, IMA/albumin ratio, and DTDH levels in patients with early- and late-onset PE compared to the control group. In our study, the disulfide levels, disulfide/native thiol ratio (Index 1) and disulfide/total thiol ratio (Index 2) were found to be significantly higher in the patients with early-onset PE, compared to late-onset PE and early-onset PE controls. However, there was no significant difference in the IMA levels between the patient and control groups, while the IMA/albumin ratio was higher in the early-onset and late-onset PE patients, compared to the control groups.These findings indicated no significant difference between the early-onset and late-onset PE patients.

Albumin which has antioxidant effects accounts for 70% of serum total antioxidant capacity (7).Recent studies have demonstrated that IMA levels increase in healthy pregnancies, remain high during pregnancy, and pregnancy-related pathologies (14,15). Therefore, IMA has been considered a predictive biomarker for PE (24). In a systemic review and meta-analysis, IMA was shown to be a useful biomarker for oxidative stress, hypoxia, and endothelial dysfunction in PE patients (10).

Furthermore, previous studies have shown an inverse correlation between the IMA and albumin levels (17). Plasma albumin levels affect the detection of IMA levels via albumin cobalt binding assay (18). Therefore, correction of IMA levels through albumin concentrations is critical. In previous studies, serum IMA levels increased in healthy pregnant women, compared to healthy non-pregnant controls(14,19). Similarly, the IMA/albumin ratio were higher compared to healthy controls (14,19). Of note, the IMA levels were found to significantly increase in patients with PE compared to healthy pregnant women (14,16,17,20). Similarly, the IMA/albumin ratio were higher in this patient population (14,17,20). In a meta-analysis investigating the diagnostic accuracy of IMA in the diagnosis of PE, the sensitivity and specificity of IMA were found to be 0.80 and 0.76, respectively (21).

In a study, Bahinipati et al. (20) reported that malondialdehyde (MDA), IMA, IMA/albumin ratio were found to be significantly higher in healthy pregnant women than healthy non-pregnant controls and found a correlation between IMA and MDA levels. Therefore, the authors concluded that IMA was a useful biomarker for oxidative stress. Gafsou et al. (20) found higher IMA levels and IMA/albumin ratio in PE patients compared to healthy pregnant and non-pregnant women. When the patients were further divided into subgroups as <30, 30 to 35, and >35 weeks of pregnancy, the authors found no significant correlation between the gestational age and IMA levels. In the present study, we found no significant difference in the IMA levels between early- and late-onset PE patients and healthy controls. However, there was a significant difference in the IMA/albumin ratio between the early-onset PE patients and controls and late-onset PE patients and controls. Based on a cut-off value of 0.8034, the sensitivity and specificity of IMA/albumin ratio were calculated as 75% and 91.2%, respectively for the diagnosis of early-onset PE. In addition, the IMA/albumin ratio had a statistically significant discriminatory ability with regards to late-onset PE and control group (p=0.008). Using a cut-off value of 0.7644, the sensitivity and specificity of IMA/albumin ratio were calculated as 75.8% and 75%, respectively.

It has been well-established that DTDH is involved in preserving the antioxidant defense mechanism and apoptotic and enzymatic activities and cellular mechanisms (5). As oxidative stress plays a role in the pathophysiology of PE, DTDH is considered to be involved in PE. In a study, Ozler et al. (22) found higher disulfide thiol levels and disulfide/native thiol and disulfide/total thiol ratios with lower native and total thiol ratios in PE patients, compared to healthy controls. When PE patients were further divided into subgroups as <32 weeks of pregnancy and ≥32 weeks of pregnancy, no significant difference was seen in the disulfide levels and disulfide/total ratios. However, the sample size was very small (n=6) in the <32 weeks of pregnancy subgroup. In another study, Korkmaz et al. (23) reported the lowest native, total, and disulfide levels in severe PE patients compared to mild PE patients and controls. Similarly, Yuvaci et al. (24) found lower native, total, and disulfide thiol levels in severe PE patients than mild PE and control subjects. However, the aforementioned authors reported higher disulfide/native and disulfide/total thiol ratios in severe PE patients. This finding indicates thiol-disulfide balance changes in favor of disulfide. Of note, increased disulfide/native thiol ratio suggests increased oxidative stress. In the present study, we found higher disulfide levels, disulfide/native thiol ratio, and disulfide/total thiol ratio in patients with early-onset PE than late-onset PE patients. These ratios were also higher in early-onset PE patients than early-onset PE controls, although there was no significant difference between the late-onset PE patients and controls.

The main strength of the present study is that the patients were classified as early- and late-onset PE patients and healthy controls with similar gestational weeks were included. The fact that the present study did not use a similar classification to previous studies investigating the relationship between IMA and thiol and PE contributes to the literature. Nonetheless, small sample size is the main limitation of the present study, although it is consistent with the previous studies.In addition, we included PE patients as well as those with obesity and FGR, which are known risk factors for oxidative stress. In our study, we observed a statistically significant difference in the body weight between the early- and late-onset PE patients and controls.

CONCLUSION

In conclusion, our study results showed increased disulfide levels, disulfide/native thiol, disulfide/total thiol and IMA/albumin ratio in the early-onset PE patients, indicating increased oxidative stress in the pathogenesis of PE. In the late-onset PE patients, there was an increase only in the IMA/albumin ratio. However, further large-scale, prospective studies are needed to confirm the diagnostic value of these markers in the clinical practice.

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