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Level of CTLA-4 and some other future markers for predicting preeclampsia in pregnant women: a correlation study

R.A. Abdualhay, A.J.M. Al-Fartosy

College of Science, University of Basrah, Basrah, Iraq

Abstract

Background. Preeclampsia (PE) is a dangerous complication of pregnancy and still a major cause of maternal-fetal morbidity and mortality.

Purpose. Evaluate the correlation of CTLA-4 with serum biomarkers of angiogenic factors, oxidative stress, and endothelial dysfunction in preeclamptic pregnant women.

Methodology. The study included 120 pregnant women diagnosed with preeclampsia (PE), as well as 46 healthy pregnant women. We measured their serum levels of NO, eNOS, MDA, ADMA, sENG, sFlt-1, T-AOC, and CTLA-4.

Results. Our findings indicated significant ($p < 0.01$) positive correlations between CTLA-4 and NO, eNOS, and T-AOC ($r = 0.723$ vs. $r = 0.597$; $r = 0.557$ vs. $r = 0.580$; and $r = 0.577$ vs. $r = 0.791$, respectively). On the other hand, CTLA-4 exhibited a significant negative correlation ($r = 0.728$ vs. $r = 0.710$; $r = 0.722$ vs. $r = 0.632$; $r = 0.636$ vs. $r = 0.655$; and $r = 0.790$ vs. $r = 0.717$, respectively) in EOP and LOP patients. According to the area under the receiver operating characteristics (ROC) curve (AUC) findings, CTLA-4 and sFlt-1 may be more effective predictors in early- (AUC = -1.00 and 0.98, respectively) and late-onset preeclampsia (AUC = -1.00 and 1.00, respectively) than other biomarkers and antioxidants such as NO, sENG, eNOS, ADMA, MDA, and T-AOC.

Conclusion. CTLA-4 with biomarkers of angiogenic factors, oxidative stress, and endothelial dysfunction was not significantly different in EOP and LOP patients, which may cast new light on the possible role as an etiological cause of PE and might be a better monitoring index in women with PE.

Keywords: preeclampsia; CTLA-4; oxidative stress; angiogenic factors; endothelial dysfunction

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Introduction

Preeclampsia (PE) affects 5–10% of pregnancies and is a major source of morbidity and mortality for both the mother and the fetus [1]. PE is defined by gestational hypertension in pregnancies with one or more of the following conditions identified after 20 or more weeks of gestation: proteinuria, acute renal injury, liver dysfunction, hematologic abnormalities, neurologic features, and placental dysfunction [2]. In approximately 15% pregnancies, PE results in iatrogenic preterm birth, and the only treatment available for PE is still delivery [3]. The underlying reason for PE remains unknown. The placenta's initial implantation is believed to mark the start of the issue. The placenta's oxidative damage leads to apoptosis, inflammation, and the release of cytokines and anti-angiogenic substances into the mother's bloodstream. The primary cause of PE pathophysiology is endothelial dysfunction, triggered by these chemicals from the placenta that enter the mother's circulation [4].

The physiological oxidative stress that occurs in the early stages of pregnancy, before the start of fetomaternal circulation, has also been shown to promote normal cell differentiation and cytotrophoblast proliferation [5]. Studies assessing the levels of several biomarkers in pregnancy problems, including PE, intrauterine growth retardation, hyperemesis gravidarum, and gestational diabetes, have been published in the literature. Studies on the biochemical markers for the early diagnosis of PE have been ongoing because it is still a significant obstetric problem [6]. In order to have a better knowledge of the pathophysiological mechanisms causing preeclampsia that contribute to the genesis of PE, it may be helpful to investigate serum indicators. This study aims to examine the relationship between CTLA-4 and a group of biochemical indicators, including NO, eNOS, MDA, ADMA, sENG, sFlt-1, and T-AOC, and to compare the levels of these indicators in patients with pregnancy-related complications to those in healthy pregnant women.

Materials and methods

Study Design and Subjects Recruitment

This study was a case-control clinical trial. Samples were collected from Basrah Hospital for Obstetrics and Children in Basra, Iraq, from October 2024 to April

2025. In addition, some samples were also obtained from a private clinic run by Dr. Fatima Hashem Makhour in Abu-Al-Khaseeb General Hospital. A total of 120 subjects aged between 20 and 40 years were included in the study. The participants were divided into two groups. Group I consisted of 74 patients, who had a blood pressure of 140 mmHg/90 mmHg or higher: 34 with early-onset preeclampsia, occurring between 28 and 32 weeks (EOP), and 40 with late-onset preeclampsia, occurring between 32 and 40 weeks (LOP), all of whom had been diagnosed with PE according to the International Society for the Study of Hypertension in Pregnancy guidelines [7]. Group II (46 normotensive pregnant women) of comparable age were recruited: 20 women at (28-32 weeks) and 26 women at (32.1-40 weeks) of gestation. We did not include pregnant women with a history of twin pregnancies. Additionally excluded were women who appeared to have a history of diabetes, ischemic heart disease, anemia, inflammatory bowel disease, chronic hypertension, smoking, renal disease, arthritis, or any other chronic inflammatory disease.

Samples Collection

A 5 mL fasting venous blood sample was drawn from the vein of the forearm of all the participants into a gel tube, then moved into a centrifuge at 402 x g for 20 minutes to obtain the serum. The collected serum was immediately utilized in the estimation of variables in this study, while the rest were stored in a plain tube deep freezing at -70 °C until used.

Biochemical Quantification

The following procedures were used to analyze blood samples for biochemical parameters: BMI was calculated as the following formula [$\text{BMI (kg/m}^2\text{)} = \text{Wt in kg/Ht in m}^2$] [7]. MDA level was determined using the (Cat No. E1371Hu, BT-Lab, Shanghai, China) kit, which was a solid-phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Serum eNOS was quantified by kit (Cat No. E0908Hu, BT-Lab), NO was estimated by kit (Cat No. E1510Hu, BT-Lab), ADMA was estimated by kit (Cat No. E1887Hu, BT-Lab), and sENG was estimated by kit (Cat No. E0316Hu, BT-Lab). sFlt-1 was assessed by a kit (Cat No. E4049Hu, BT-Lab). CTLA-4 was measured using a kit (Cat No. E0277Hu, BT-Lab). T-AOC was evaluated using a colorimetric antioxidant capacity assay by kit (Cat No. SH0242, BT-Lab).

Statistical Analysis

Statistical analyses were performed using SPSS 26.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism 10.0 (GraphPad Software, San Diego, CA, USA). The data were normally distributed, and Dunnett's t-test was used to determine statistical significance after the analysis of variance was used to compare the groups. Sensitivities and specificities, together with the 95% con-

fidence interval, were computed using the receiver operating characteristics (ROC) curve, which is created by plotting sensitivity (y-axis) against 1-specificity (x-axis) and computing the area under the ROC curve (AUC). Pearson correlation was used to find correlations. An AUC value close to 0 (or 1) indicates a strong diagnostic value; in this case, the values of one group were primarily higher (or lower) than the values of the comparison group. A ($p<0.05$) was deemed statistically significant, a ($p<0.01$) extremely significant.

Results

In both early-onset and late-onset PE, there were no significant differences in age, gestational age, or BMI between PE patients and the healthy control group. In early-onset PE, the mean age of the PE individuals was 31.00 ± 7.14 vs. 29.09 ± 6.33 , and in late-onset PE, it was 27.45 ± 6.20 vs. 26.73 ± 5.5 . (Table 1) summarizes all the participants' and controls' baseline characteristics. (Table 2 & Figure 1) showed that whereas CTLA-4, NO, eNOS, and T-AOC were significantly lower than those of healthy controls, participants with PE had greater levels of SBP, DBP, MDA, ADMA, sENG, and sFlt-1 in early- and late-onset pregnancy ($p<0.01$).

Table 1.

The demographic characteristics of the subjects (n=120)

The characteristics		Preeclampsia Patients		Healthy Control	
		early-onset	late-onset	early-onset	late-onset
Total subject No.		34	40	20	26
Age (Years) (Mean $\pm$ SD)		31.00 ± 7.14	27.45 ± 6.20	29.09 ± 6.33	25.64 ± 5.50
Pregnant age weeks (Mean $\pm$ SD)		30.18 ± 1.50	36.73 ± 1.72	30.14 ± 1.58	36.73 ± 1.72
Duration (Years) (Mean $\pm$ SD)		0.39 ± 0.02	0.59 ± 0.03	-	-
Demographic Area	Urban	25	35	17	22
	Rural	9	5	3	4
Educational Background	Learned	30	37	18	25
	Illiterate	4	3	2	1
Smoking Habits	Positive	0	0	0	0
	Negative	34	40	20	26
Food Habits	Vegetarian	1	2	1	2
	Non -Vegetarian	33	38	19	24

Employment Status	Em- ployed	31	36	18	23
	Non-Em- ployed	3	4	2	3

early-onset: (28-32 weeks) of gestation; **late-onset** : (32-40 weeks) of gestation

Table 2.

The levels of all parameters measured in the serum of preeclampsia patients and healthy control

Variables	(Mean $\pm$ SD)			
	Preeclampsia Patients		Healthy Control	
	early-onset	late-onset	early-onset	late-onset
BMI (kg/m ²)	28.97 $\pm$ 3.48	29.30 $\pm$ 3.93	29.61 $\pm$ 4.08	29.78 $\pm$ 4.16
SBP (mmHg)	154.32 $\pm$ 18.1**	159.32 $\pm$ 18.3**	119.09 $\pm$ 6.5	120.00 $\pm$ 7.8
DBP (mmHg)	88.18 $\pm$ 5.9**	96.14 $\pm$ 8.6**	77.27 $\pm$ 4.8	77.73 $\pm$ 5.5
ADMA (ng/ l)	2630 $\pm$ 1087 **	2278 $\pm$ 943 **	1160 $\pm$ 313	1470 $\pm$ 398
NO (umol/ L)	107.67 $\pm$ 14.21 **	133.37 $\pm$ 10.75 **	163.38 $\pm$ 40.69	181.99 $\pm$ 52.54
eNOS (U/ mL)	25.31 $\pm$ 6.03 **	25.95 $\pm$ 6.77 **	43.54 $\pm$ 12.83	44.87 $\pm$ 16.77
MDA (nmol/mL)	22.14 $\pm$ 10.84 **	24.00 $\pm$ 10.07 **	13.70 $\pm$ 2.5	16.04 $\pm$ 2.90
sENG (ng/ mL)	82.25 $\pm$ 16.43**	81.28 $\pm$ 19.13 **	52.26 $\pm$ 11.12	55.44 $\pm$ 10.32
sftt-1 (mIU/ L)	11.60 $\pm$ 2.77 **	14.03 $\pm$ 5.22 **	3.25 $\pm$ 0.93	3.40 $\pm$ 0.57
T-AOC (mmol/ L)	0.95 $\pm$ 0.26 **	0.95 $\pm$ 0.22 **	1.71 $\pm$ 0.35	1.87 $\pm$ 0.34
CTLA-4 (ng/ mL)	12.62 $\pm$ 3.28 **	12.68 $\pm$ 3.01 **	36.67 $\pm$ 5.69	32.44 $\pm$ 7.33

Data are presented as mean $\pm$ SD, SD: Standard Deviation, p-value: NS: Non-Significant ($p > 0.05$), S: Significant ($*p < 0.05$), and HS: Highly Significant ($**p < 0.01$) indicate the level of significance in comparison with the corresponding control value.

The obtained AUC results indicated that CTLA-4 and sftt-1 could potentially be used as greater predictive biomarkers in early- (AUC = -1.00 and 0.98, respectively) and late-onset preeclampsia (AUC = -1.00 and 1.00, respectively). Also, other biomarkers and antioxidants such as NO, sENG, eNOS, ADMA, MDA, and T-AOC could not be used as predictive biomarkers in pregnant women in the early- (AUC = -0.97, 0.97, -0.92, 0.825, 1.00, and -0.95, respectively) as well as late-onset preeclampsia (AUC = -0.92, 0.91, -0.85, 0.96, 1.00, and -0.98, respectively), as illustrated in (Table 3 & Figure 2).

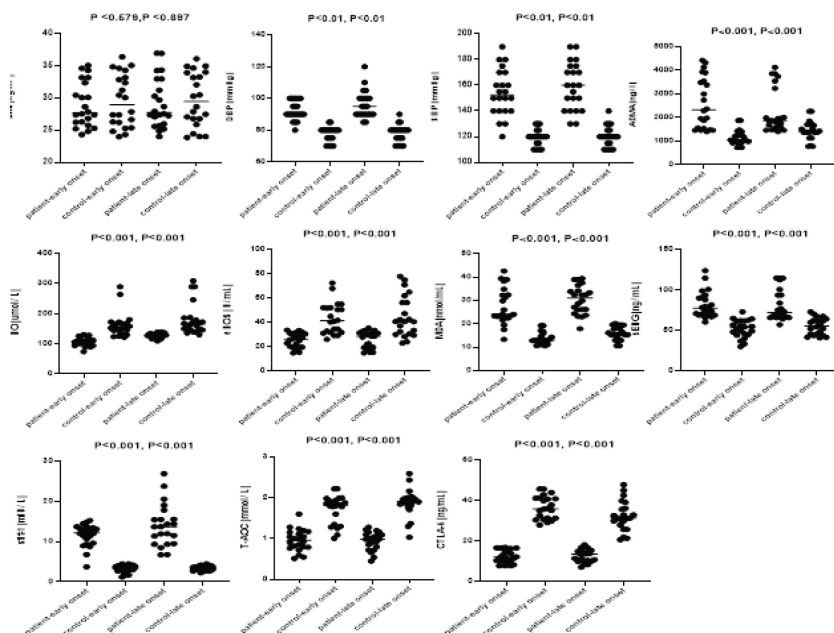
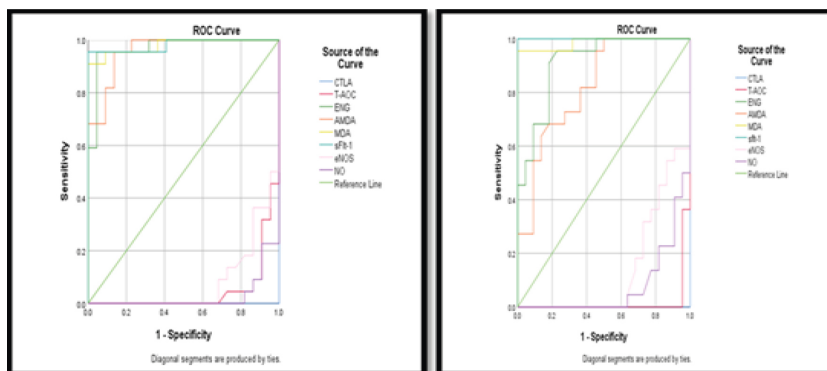


Fig. 1. Comparison of BMI, DBP, SBP, ADMA, NO, eNOS, MDA, sENG, sFlt-1, T-AOC, and CTLA-4 levels between early-onset (28-32 weeks) and late-onset (32-40 weeks) pre-eclampsia patients and healthy control



A: early-onset PE (28-32 weeks) **B:** late-onset PE (32-40 weeks)

Fig. 2. The ROC curve for serum CTLA-4, NO, eNOS, MDA, ADMA, sENG, sFlt-1, and T-AOC levels in early- and late-onset preeclampsia patients. A: early-onset PE (28-32 weeks); B: late-onset PE (32-40 weeks)

Table 3.

**ROC and AUC analysis of the measured biomarkers for the diagnosis
of preeclampsia patients in early- and late-onset PE**

Variables	PE	Cut-off concentration	Sensitivity %	Specificity %	AUC	95% CI of AUC	P-value
CTLA-4 (ng/mL)	early-onset PE	16.79	100	95.5	-1.00	1.00-1.00	<0.01
	late-onset PE	19.46	100	100	-1.00	1.00-1.00	<0.01
sflt-1 (mIU/l)	early-onset PE	6.67	95.5	100	0.98	0.95-1.00	<0.01
	late-onset PE	5.63	100	100	1.00	1.00-1.00	<0.01
MDA (nmol/mL)	early-onset PE	19.49	90.9	100	0.98	0.95-1.00	<0.01
	late-onset PE	21.64	95	100	0.99	0.95-1.00	<0.01
T-AOC (mmol/l)	early-onset PE	1.24	90.9	90.9	-0.95	0.90-1.00	<0.01
	late-onset PE	1.29	95.5	100	-0.98	0.95-1.00	<0.01
NO (umol/l)	early-onset PE	126.28	90.9	90.9	-0.97	0.94-1.00	<0.01
	late-onset PE	148.88	95.9	73.0	-0.92	0.85-1.00	<0.01
sENG (ng/mL)	early-onset PE	65.60	95.5	95.5	0.97	0.92-1.00	<0.01
	late-onset PE	63.51	95.5	77.3	0.91	0.82-0.99	<0.01
ADMA (ng/l)	early-onset PE	1726.87	68.2	81.8	0.825	0.70-0.94	<0.01
	late-onset PE	1448.75	95.5	86.4	0.96	0.91-1.00	<0.01
eNOS (U/mL)	early-onset PE	31.21	86.4	81.8	-0.92	0.84-0.99	<0.01
	late-onset PE	36.34	65	100	-0.85	0.75-0.97	<0.01

(Table 4) and (Figure 2) showed that the correlations between CTLA-4 and NO, eNOS, and T-AOC were positive ($p<0.01$) and highly significant ($r=0.723$ vs. $r=0.597$; $r=0.557$ vs. $r=0.580$; and $r=0.577$ vs. $r=0.791$, respectively). Additionally, CTLA-4 showed a highly significant negative ($p<0.01$) correlation with MDA, ADMA, sENG, sFlt-1 ($r=0.728$ vs. $r=0.710$; $r=0.722$ vs. $r=0.632$; $r=0.636$ vs. $r=0.655$; and $r=0.790$ vs. $r=0.717$, respectively), in early- and late-onset preeclampsia.

Table 4.

**Correlation coefficient of CTLA-4 vs. other clinical parameters
in early-onset and late-onset preeclampsia patients**

The correlation of CTLA-4 vs. other variables	Correlation coefficient (r)	
	early-onset PE (28-32 weeks)	late-onset PE (32-40 weeks)
BMI (kg/m²)	-0.066	-0.006
SBP (mmHg)	-0.744**	-0.700**
DBP (mmHg)	-0.766**	-0.743**
ADMA (ng/ L)	-0.722**	-0.632**
NO (umol/ L)	0.732**	0.597**
eNOS (U/ mL)	0.557**	0.580**

MDA (nmol/mL)	-0.728**	-0.710**
sENG (ng/ mL)	-0.636**	-0.655**
sflt-1 (mIU/ L)	-0.790**	-0.717**
T-AOC (mmol/ L)	0.577**	0.791**

Spearman's correlation test, r = strength of correlation or correlation coefficient, (-) inverse correlation, (+) proportional correlation, significant ($p < 0.05$), highly significant ($p^{**} \leq 0.01$)

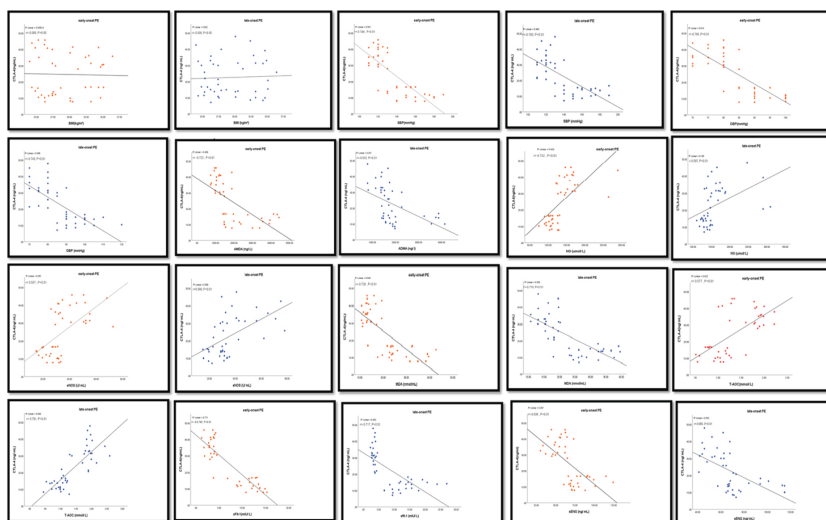


Fig. 3. Correlation coefficient of CTLA-4 vs. other clinical parameters (BMI, DBP, SBP, ADMA, NO, eNOS, MDA, T-AOC, sFlt-1, sENG) in early-onset and late-onset pre-eclampsia patients

Discussion

Preeclampsia is marked by systemic inflammation, endothelial dysfunction, and a disruption in the balance of immune regulation; research has shown a significant decline in CTLA-4 expression, primarily in its soluble and/or membrane-bound forms. Several immunopathological mechanisms can explain this reduction, primarily due to regulatory T cells expressing CTLA-4 [8]. In a normal pregnancy, Tregs have a vital function in ensuring the mother's immune system accepts the fetus. In preeclampsia, the number of Tregs and their suppressive function are significantly reduced, leading to decreased CTLA-4 expression. Approximately 30-45% of T-cells in human decidua are CD4⁺ T-cells, and 45-75% are CD8⁺ T-cells, which are cytotoxic T lymphocytes. CD8⁺ T-cells

are more common in human decidua in terms, but less common in peripheral blood. These cells can recognize allogeneic MHC molecules but do not attack fetal cells during pregnancy. It is thought that the condition arises from decreased MHC class I expression in fetal trophoblast cells [4; 9]. CTLA-4 is usually present at low levels on the surface of effector T cells and regulatory T cells, thereby affecting the strength of initial T-cell activation. When CTLA-4 is suppressed, cytotoxic T-cell activation increases, and Treg cells are prevented from suppressing the immune system. Establishing maternal-fetal tolerance and completing placentation effectively are crucial for a successful pregnancy. When the immune balance is disturbed, spontaneous abortions, preeclampsia, and intrauterine growth restriction of the fetus may occur due to inadequate placental perfusion. Extra villous trophoblasts instruct decidual immune cells to regulate fetal tolerance and promote placental development [10].

Preeclampsia is characterised by chronic placental hypoxia, which disrupts immune signalling and may reduce CTLA-4 expression through epigenetic mechanisms. Recent research indicates that DNA methylation and histone modifications of immune checkpoint genes - such as CTLA-4 - can dampen their expression in low-oxygen and inflammatory environments. Reduced CTLA-4 expression leads to an unchecked immune response, increased T-cell activation, and a pro-inflammatory state, which are all characteristic features of preeclampsia's underlying pathology. This implies that CTLA-4 could serve as both a marker of immune dysregulation and a potential therapeutic target [8].

The results showed that patients with pre-eclampsia had significantly higher levels of ADMA in their serum than healthy individuals. Some studies have identified ADMA as a natural inhibitor of the enzyme eNOS in the endothelium, thereby reducing NO production and contributing to endothelial dysfunction. Researchers Savvidou *et al.*, also reported high ADMA levels in early pregnancy as a potential predictor of the development of preeclampsia. High levels of ADMA may thus play a crucial role in vasoconstriction, hypertension, and decreased placental blood flow observed in patients with pre-eclampsia [11].

The lower levels of endothelial nitric oxide synthase (eNOS), nitric oxide (NO) in pregnant women with preeclampsia compared to healthy pregnant women may be attributed to Endothelial Dysfunction and Oxidative Stress. eNOS is crucial for producing NO in vascular endothelial cells, playing a key role in vasodilation and maintaining vascular balance. In preeclampsia, endothelial dysfunction leads to reduced eNOS expression or activity, subsequently lowering NO production. It was demonstrated that significantly decreased eNOS levels in women with preeclampsia, suggesting impaired endothelial

function as a primary contributor. Preeclampsia is characterized by elevated oxidative stress, which increases the production of reactive oxygen species (ROS), particularly superoxide anions [9; 12]. These anions react with nitric oxide to form peroxynitrite (ONOO^-), a cytotoxic compound that not only reduces nitric oxide bioavailability but also exacerbates endothelial damage. Some scientific reports indicated that oxidative stress in the placenta and vasculature plays a major role in reducing nitric oxide levels and contributing to vasoconstriction and hypertension. Asymmetric dimethylarginine (ADMA) is a well-known endogenous inhibitor of eNOS [13].

The study revealed a statistically significant decrease in T-AOC levels among PE patients, caused by heightened oxidative stress and an overproduction of ROS. Preeclampsia is associated with an excessive production of reactive oxygen species (ROS), typically resulting from placental hypoxia-reperfusion and mitochondrial dysfunction, which overpowers antioxidant defenses, thereby depleting T-AOC. Consumption of Antioxidants may be one reason for reduced T-AOC in preeclampsia. With high ROS levels, antioxidants (enzymatic and non-enzymatic) are consumed rapidly to neutralize free radicals. A significant drop in circulating T-AOC levels is made worse by lower levels of antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase, and essential micronutrients (for instance, vitamins A, C, E, zinc, selenium) in women with preeclampsia. Inflammation Impairs Antioxidant Defense [14]. Preeclampsia is associated with systemic inflammation, which suppresses antioxidant enzyme expression and activity, compounding the deficiency in T-AOC despite the high oxidative burden. A recent Turkish clinical study confirmed significantly lower serum T-AOC in early-onset preeclampsia compared to controls, alongside elevated total oxidant capacity and malondialdehyde (MDA), a marker of lipid peroxidation.

Elevated malondialdehyde (MDA) levels in pregnant women with preeclampsia may be higher than in those who are healthy. Chronic placental hypoxia-reperfusion and ischemia in preeclampsia trigger the overproduction of reactive oxygen species, which then attack membrane polyunsaturated fatty acids. Lipid peroxidation results in the formation of high levels of lipid peroxides and increases MDA levels as a byproduct [15; 16]. A meta-analysis of oxidative stress biomarkers in preeclampsia showed that women with the condition have significantly elevated MDA, making MDA a reliable marker of lipid peroxidation in preeclampsia. A recent in vitro and clinical study reported that serum MDA levels significantly increased in women with preeclampsia versus healthy controls, preeclamptic patients, and correlated positively with blood pressure

severity [17]. Elevated MDA reflects uncontrolled lipid peroxidation, which damages endothelial cell membranes, promotes vasoconstriction, reduces prostacyclin synthesis, and impairs vascular permeability, key pathophysiological features of preeclampsia [18].

The elevated levels of soluble fms-like tyrosine kinase-1 (sFlt-1) in pregnant women with preeclampsia as opposed to those with a healthy pregnancy might be attributed to placental hypoxia and ischemia. A key factor driving the rise in sFlt-1 in preeclampsia is placental hypoxia resulting from shallow trophoblastic invasion and the inadequate remodelling of the spiral arteries. In response to hypoxic stress, the placenta releases high levels of sFlt-1, which is an anti-angiogenic factor that binds to vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), and as a result, neutralizes their activity and prevents angiogenesis from occurring. Increased sFlt-1 serves as a decoy receptor by reducing the availability of VEGF and PlGF in the maternal bloodstream. This results in widespread maternal endothelial dysfunction, which contributes to hypertension, proteinuria, and other systemic features of preeclampsia. In a hypoxic placenta, oxidative stress and inflammatory signals cause an increase in the production of anti-angiogenic factors, such as sFlt-1 [19; 20].

The results of our research indicated a considerable rise in serum sEng concentrations among PE patients relative to healthy pregnant women. In preeclampsia, inadequate placental blood flow causes hypoxic stress within the trophoblast cells. The process stimulates the excessive production of soluble endoglin (sEng), a circulating anti-angiogenic protein that suppresses the signalling of transforming growth factor-beta (TGF- β), a factor which is vital for endothelial cell function and vascular stability. TGF- β 1 bound by sEng decoy receptor prevents its activation of the signaling pathway in endothelial cells [21]. This disrupts the production of NO, angiogenesis, and endothelial repair, which contributes to vascular damage and clinical manifestations of preeclampsia. In preeclampsia, the damaged syncytiotrophoblast layer in the placenta experiences heightened rates of apoptosis and necrosis, resulting in the release of microparticles and anti-angiogenic factors such as soluble endoglin into the maternal bloodstream [22].

The inverse relationship between CTLA-4 and ADMA levels in pre-eclamptic patients could be attributed to CTLA-4 acting as a key immune checkpoint, regulating T-cell activation and promoting maternal immune tolerance during pregnancy [23]. Studies have found that reduced CTLA-4 expression in women with preeclampsia contributes to immune system imbalance and increased systemic inflammation. The accumulation of ADMA, which inhibits endothelial

nitric oxide synthase (eNOS), leads to impaired nitric oxide (NO) production, causing endothelial dysfunction and increased vascular resistance, characteristics of preeclampsia [18; 24]. Elevated ADMA levels have been consistently reported in preeclamptic women. Reduced expression of CTLA-4 is linked to higher levels of pro-inflammatory cytokines and oxidative stress, which could in turn worsen endothelial damage and, as a result, contribute to higher ADMA levels. Consequently, the existing research supports a logical inverse connection between CTLA-4 and ADMA in preeclampsia. Lower CTLA-4 expressions may promote immune-mediated vascular damage and oxidative imbalance, facilitate ADMA elevation, and worsen endothelial dysfunction [25].

In the context of preeclampsia, both nitric oxide (NO) and endothelial nitric oxide synthase (eNOS) levels are significantly reduced, contributing to vascular endothelial dysfunction and increased blood pressure. Similarly, CTLA-4 expression is also found to be decreased in preeclamptic patients, suggesting a possible immunological disruption. A positive correlation appears to exist between CTLA-4 and both NO and eNOS levels. The concurrent decrease in these markers may indicate an integrated process in which immune dysregulation, characterised by reduced CTLA-4, may impair endothelial function through a decrease in nitric oxide availability [26]. The discovery implies that CTLA-4 could impact vascular homeostasis not only by promoting immune tolerance but also by affecting the NO signalling pathway. Lower levels of CTLA-4 in preeclamptic women have been linked with increased systemic inflammation and oxidative stress. MDA, a byproduct of lipid peroxidation, is a well-established marker of oxidative stress and is found to be significantly elevated in women with preeclampsia. Increased oxidative stress may be a consequence of an exaggerated immune response due to insufficient immune regulation [27].

Research indicates that there is a correlation between the expression of CTLA-4 and T-AOC levels in cases of preeclampsia in women, with both markers significantly lower than those in normal pregnancies; CTLA-4 operates as a vital checkpoint molecule that inhibits T-cell activity to facilitate immune tolerance. Reduced expression of CTLA-4 has been linked to stronger pro-inflammatory immune responses that often occur in preeclamptic situations. T-AOC, by contrast, offers a comprehensive indicator of the body's antioxidant defence systems. A decrease in T-AOC suggests a compromised ability to neutralize oxidative stress, which is a hallmark of the pathophysiology of preeclampsia [21; 28]. The parallel decline in CTLA-4 and T-AOC levels suggests a possible functional link between immune dysregulation and oxidative imbalance. Reduced immune regulation via CTLA-4 could potentially intensify oxidative stress or

vice versa, thereby creating a cycle that worsens the severity of the disease. Preeclampsia is characterized by significantly elevated expression levels of anti-angiogenic markers, namely soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sENG). These factors contribute to endothelial dysfunction and impaired placental vascular development by antagonizing pro-angiogenic factors such as VEGF and PlGF [29]. Conversely, CTLA-4 levels are significantly decreased in pre-eclamptic patients. Emerging evidence suggests an inverse correlation between CTLA-4 and both sFlt-1 and sENG. The relationship between reduced immune regulation and endothelial dysfunction implies that low CTLA-4 expression may be connected to increased anti-angiogenic factors. The inverse correlation supports the hypothesis that disruptions to immune system controls, like less CTLA-4, could lead to the development of preeclampsia via the disordered regulation of blood vessel formation and immune system interactions with the lining of blood vessels [30].

Conclusion

Our results indicated that the correlation of CTLA-4 with biomarkers of angiogenic factors, oxidative stress, and endothelial dysfunction was not significantly different in EOP and LOP patients, which may cast new light on the possible role as an etiological cause of PE and might be a better monitoring index in women with PE. Our results support the use of CTLA-4 and studied biomarkers in early first- and third-trimester antenatal screening for pre-eclampsia.

Ethics committee conclusion. The study received ethical approval from Basrah University (11/45/3729), and informed consent was obtained from each participant after explanation of the procedures in full detail. The informed consent and ethical guidelines were followed, based on the Declaration of Helsinki for the year 2000.

Data availability. All the findings of this study are presented within the paper.

Conflict of interest information. The authors declare no conflict of interest.

Author contributions. R.A. Abdualhay contributed to the planning and performance of the research experiments, and A.J.M. Al-Fartosy contributed to the analysis of the results and the writing of the manuscript.

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DATA ABOUT THE AUTHORS

Raghda Abdulsamad Abdualhay, PhD, Prof.

College of Science, University of Basrah

Basrah, Iraq

husseinalihasan526@gmail.com

Adnan Jassim Mohammed Al-Fartosy, PhD, Prof.

College of Science, University of Basrah

Basrah, Iraq

adnan.jassim@uobasrah.edu.iq

<https://orcid.org/0000-0002-0681-6452>ORCID:

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