Evaluation of placental prostate specific antigen, estradiol and interleukin-6 and their significance in preeclampsia

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OBJECTIVES: The aim of the current study was to evaluate the level of some placental parameters and their probable correlation with increased risk of preeclampsia.
SUBJECTS AND METHODS: The study included a total of fifty women, including twenty five pregnant women with preeclampsia and twenty five normotensive pregnant females. The placental biomarkers studied at delivery included prostate specific antigen (PSA), 17-β estradiol (E2), interleukin-6 (IL-6), lipid peroxidation (MDA), glutathione peroxidase (GPx) and trace elements (zinc, copper and calcium).
RESULTS: A significant difference was recorded in placental PSA ($p<0.002$), E2 ($p<0.012$) and IL-6 ($p<0.05$) levels in preeclamptic women, compared to normotensive pregnant ones. In addition, a significant negative correlation was found between placental PSA with IL-6 and E2.
CONCLUSIONS: Our study demonstrated increased placental PSA and reduced E2 and IL-6 levels as PE-associated biomarkers. Furthermore, the recorded correlation between placental PSA and E2 may represent a new understanding in PE pathogenesis.
Key Words: Preeclampsia; Biomarkers; Placenta; Prostate specific antigen

INTRODUCTION

Preeclampsia (PE), a pregnancy-specific multi-systemic syndrome, is defined by the onset of proteinuric hypertension after 20 weeks of gestation and affects 5-8% of pregnancies worldwide [1]. It is associated with an increased risk of maternal and fetal morbidity/mortality [2]. Although the etiology, pathogenesis, and pathophysiology of PE are poorly understood, its origin is recognized as lying in the placenta rather than the fetus itself [3,4]. It has been postulated that the physiological remodeling of the uterine spiral arteries into dilated uteroplacental vessels observed in normal pregnancies is disrupted in PE, resulting in a poorly perfused and ischemic placenta. In turn, reduced oxygen tension in the placental tissue may lead to the production and secretion of cytotoxic factors that have been suggested to affect the maternal vasculature and exaggerate inflammatory responses [2,5,6]. Currently, delivery is the only cure for preeclampsia [7], either with induction of labor or caesarean section [4]. The understanding of the precise mechanism of PE development could possibly help for planning of appropriate monitoring and for clinical management, following early identification of complications of this disorder. Although no single biomarker has demonstrated a sufficient predictive value for PE to be of clinical use at present, promising strategies for the prediction of PE involve multiparametric approaches, which use a variety of individual biomarkers with other well-recognized clinical parameters in combination [8,9]. To this end, the present study was conducted to investigate the level of some biomarkers and their correlation with PE-associated pregnancies.

SUBJECTS AND METHODS

Subjects

Non-diabetic and non-obese Egyptian pregnant women aged 19-39 years were selected from different maternal hospitals. Preeclampsia was diagnosed when hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg) and proteinuria (≥ +1, random dip-stick) appeared after 20 weeks of the gestational age in previously normotensive women, according to the American College of Obstetricians and Gynecologists Criteria [10].

Study design

The study included 50 women; 25 preeclampsia-diagnosed women (PE) and 25 normotensive women (Control) matched for age and gestational age. All subjects gave written informed consent to participate in the study, which was carried out in accordance with the Helsinki Declaration.

Blood and tissue sampling

Before the caesarean delivery, blood and urine samples were taken from pregnant females from both groups for different biochemical analyses. A central portion was taken from the placenta at delivery and was immediately frozen at -20˚C.

Biochemical analysis

A 10% placental tissue homogenate was prepared in 10 mM phosphate buffered saline (PBS, pH 7.0) containing 0.5 mM EDTA [11]. The homogenate was centrifuged at 1792 xg at 4˚C for 30 min and the supernatants were further stored at -20˚C. Complete blood picture was performed by the fully automated blood counter Sysmex XE-2100 (Tokyo, Japan). Serum alanine aminotransferase (ALT)/aspartate aminotransferase (AST) activities [12] and glucose level [13], as well as placental malondialdehyde (MDA) concentration [14] and glutathione peroxidase (GPx) activity [15] were determined using kits provided by Biodiagnostic (Giza, Egypt). Placental free prostate specific antigen (PSA), interleukin-6 (IL-6) and 17-β estradiol (E2) levels were determined using ELISA kits purchased from MyBiosource (MyBioSource, Inc., San Diego, CA, USA). Placental tissue copper (Cu), zinc (Zn) and calcium (Ca) concentrations were analyzed in ash by flame atomic absorption spectrophotometry [16] using Savant AA GBC Scientific Equipment (USA).

Statistical analysis

The Kolmogorov-Smirnov test was used to analyse the normal distribution of the variables. The comparison between preeclamptic and control groups was analyzed by chi-square (χ^2) test, Student t-test and Mann Whitney-U test for categorical variables, parametric and non-parametric data, respectively. Spearman's rank correlation coefficient was calculated to

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measure the correlation between markers in total subjects and receiver operating characteristic (ROC) curves were constructed and to assess the diagnostic accuracy of the markers. Cross-tabulation analysis was carried out, and the significance (χ^2) likelihood ratio (LR) were calculated from the chosen cut-off values. The data were analyzed using SPSS statistical software version 21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The difference in age, parity stage, and gender of the baby between the two groups (Control and PE) showed no statistical significance, ensuring the homogeneity of the clinical samples. On the other hand, PE patients at delivery had significantly higher blood pressure (systolic and diastolic) and albuminuria, compared with controls (p<0.001) (Table 1).

Table 1: Demographic and clinical characteristics of all participants at delivery.

		Control (n=25)	PE (n=25)	p<
Age (years)		27.9 ± 4	29.2 ± 4.8	NS [a]
Parity	0	0 (0%)	1 (4%)	NS [b]
	1	9 (36%)	6 (24%)	
	2	5 (20%)	6 (24%)	
	3	1 (4%)	2 (8%)	
	4	10 (40%)	10 (40%)	
Sex	Male	11 (42.3%)	16 (64%)	NS [b]
	Female	15 (57.7%)	9 (36%)	
SBP (mmHg)		110 (110-120)	150 (140-160)	0.001 [c]
DBP (mmHg)		70 (70-80)	90 (90-110)	0.001 [c]
Albuminuria	NIL	25 (100%)	0 (0%)	0.001 [b]
	(+)	0 (0%)	17 (68%)	
	(++)	0 (0%)	4 (16%)	
	(+++)	0 (0%)	4 (16%)	

Values are presented as number (%), mean ± SD, or median (IQR). (a) Student's t-test, (b) Pearson's chi square test, and (c) Mann-Whitney U test. SBP: systolic blood pressure, DBP: diastolic blood pressure, NS: non-significant.

In addition, PE women showed significantly higher blood hemoglobin and placental free PSA levels (p<0.014 and 0.002, respectively), while blood platelets (p<0.049), as well as placental E2 and IL-6 levels (p<0.012 and 0.05, respectively) were significantly decreased, compared to the control group. In contrast, a non-significant change was recorded in serum glucose and liver marker enzymes, as well as placental GPx activity, lipid peroxidation and trace metal concentrations between both groups (Table 2)

Table 2: Statistical significance of blood and placental biochemical parameters at delivery in control and PE pregnancies.

		Control	PE	p<
Blood	Glucose (mg/dL)	90.5±19.0	105.1±33.2	NS (a)
	ALT (U/L)	15.4±9.0	17.8±8.0	NS (a)
	AST (U/L)	16.9±9.8	19.0±7.7	NS (a)
	Hemoglobi n (g%)	10.9 (10.2-11.4)	11.6 (10.9-12.3)	0.014 (b)
	Platelets (x103/ mm3)	243 (207-281)	199 (178-246)	0.049 (b)
Placenta	GPx (U/g)	505.8 (311.3-641.9)	615.2 (447.4-938.6)	NS (b)
	MDA (nmol/g)	566.4 (360.5-640)	482.8 (325.7-758.6)	NS (b)
	Free PSA (ng/g)	8.01 (6.07-10.16)	15.78 (10.5-18.4)	0.002 (b)
	Estradiol (ng/g)	3.66 (1.7-5.2)	1.92 (1.5-2.6)	0.012 (b)
	IL-6 (pg/g)	175.3 (82.2-219.6)	96.4 (79.4-155.9)	0.05 (b)
	Ca* (mg/kg)	1161.9 ± 700	1471.6 ± 1100.5	NS (a)
	Zn*	54.4 ± 17.1	61.2 ± 19.4	NS (a)
	Cu*	3.6 ± 1.0	3.2 ± 1.3	NS (a)

Values are presented as mean ± SD or median (IQR). (a) Student's t-test, (b) Mann-Whitney U test. (*n=8).

Placental free PSA was negatively correlated with E2 and IL-6, as well as blood platelets, and positively correlated with PE markers (SBP, DBP and albuminuria). On the other hand, placental E2 was positively correlated with IL-6 and blood platelets, and negatively correlated with DBP and albuminuria. Placental IL-6 was significantly correlated with PE markers (SBP, DBP and albuminuria), and the increase in albuminuria was accompanied by a decrease in blood platelets count. Placental calcium was positively correlated with copper level and blood platelets count (Table 3).

Table 3: Significant correlations between chosen parameters for all participants.

Parameters		r*	p<
PSA	Estradiol	-0.476	0.001
	IL-6	-0.394	0.005
	Platelets	-0.325	0.025
	SBP	0.416	0.003
	DBP	0.466	0.001
	Albuminuria	0.5	0.001
Estradiol	IL-6	0.399	0.004
	Platelets	0.325	0.023
	DBP	-0.314	0.028
	Albuminuria	-0.349	0.014
IL-6	SBP	-0.312	0.03
	DBP	-0.317	0.026
	Albuminuria	-0.31	0.003
Platelets	Albuminuria	-0.352	0.011
	Ca	0.575	0.02
Ca	Cu	0.505	0.046

*Spearman's correlation coefficient.

Receiving operating curves (ROC) were generated for the studied parameters and the sensitivity, specificity, area under the curve (AUC), a well as the best cut-off value that discriminates between PE patients and healthy women were detected (Table 4).

Cross-tabulation revealed that all PE patients (100%) had blood SBP ≥ 125 mmHg, DBP ≥ 85 mmHg and albuminuria ≥ +1 (Table 5).

Table 4: Sensitivity, specificity and cut-off values of different biochemical parameters at delivery in control and PE pregnancies.

	AUC	Sensitivity%	Specificity%	Cut-off value	p<
Free PSA (ng/g)	0.756	75	80	10.05	0.002
Estradiol (ng/g)	0.71	67	68	2.26	0.012
Hemoglobin (g%)	0.7	65	64	11.25	0.014
Platelets (10^3/mm^3)	0.661	62	64	221.5	0.049
IL-6 (pg/g)	0.663	58	76	148.1	NS

Table 5: Cross-tabulation and regression analysis showing the reliability of markers for preeclampsia.

Parameter	Cut-off values	Counts	Control	PE	χ2	LR	Wilks' Lambda	F	p<
SBP (mmHg)	<125	n	24	0	0.001	67.9	0.21	179.68	0.001
		%	100.00%	0.00%					
	≥ 125	n	0	25					
		%	0.00%	100.00%					
DBP (mmHg)	<85	n	24	0	0.001	67.9	0.26	135.37	0.001
		%	100.00%	0.00%					
	≥ 85	n	0	25					
		%	0.00%	100.00%					
Albuminuria	<+1	n	24	0	0.001	67.9	0.35	88.53	0.001
		%	100.00%	0.00%					
	≥ +1	n	0	25					
		%	0.00%	100.00%					
Free PSA (ng/g tissue)	<10.05	n	18	5	0.001	15.7	0.81	11.13	0.002
		%	75.00%	20.00%					
	≥ 10.05	n	6	20					
		%	25.00%	80.00%					
Estradiol (ng/g tissue)	>2.26	n	16	8	0.015	6	0.81	10.83	0.002
		%	66.70%	32.00%					
	≤ 2.26	n	8	17					
		%	33.30%	68.00%					

χ^2: Pearson chi-square; LR: Likelihood ratio.

Similarly, cutoff values of 10.05 and 2.26 ng/g for placental free PSA and E2, respectively, differentiated significantly between PE patients and normotensive pregnant controls (80% and 68% of PE patients had placental free PSA and E2 levels ≥ 10.05 and 2.26 ng/g, respectively). The strength of the studied parameters (according to the likelihood ratio) in differentiating between PE and normal pregnancy is in the following descending order; blood pressure (systolic and diastolic, respectively), albuminuria, placental free PSA and E2. These parameters were also taken into a regression analysis to descript their predictive values qualitatively. The PSA and E2 have relatively high PE predictive values.

DISCUSSION

The prostate specific antigen (PSA), a serine protease, is a biological marker for prostate cancer and benign prostatic diseases. It has been shown to be expressed in many female organs, including the breast, ovary and in amniotic fluid. Previous studies reported that the placental syncytiotrophoblast is the site of PSA synthesis and secretion in both maternal serum and amniotic fluid during pregnancy [17,18].

In the present study, the placental free PSA level was significantly increased in preeclamptic women (64%), compared with normal pregnant ones. In agreement with our finding, Can et al. [17] demonstrated that the placental PSA content was significantly elevated in preeclamptic women (43%, $p<0.05$), with respect to normotensive pregnancies. PSA is an initiator of the protease cascade, which is an important biological mechanism for tissue remodeling in the uterus. Also, the proteolytic activity of PSA on different biological substrates could in part explain the potential role of the elevated level of placental PSA in preeclampsia [17,19]. In line with the recorded positive correlation between placental PSA and blood pressure in the current study, Can et al. [17] observed a positive relationship between systolic or diastolic blood pressure and placental PSA content in preeclamptic women.

Placental E2 level was significantly decreased (47%) in preeclamptic women compared to normal pregnant women in the current study. Our finding is in a good agreement with that of Açikgöz et al. [5] who showed that estriol and E2 levels were reduced significantly in the placental tissue of preeclamptic patients compared to normal pregnant women. Similarly, Hertig et al. [20] and Yin et al. [21] demonstrated an inverse correlation between circulating estrogen (E2 and estrone) levels and the severity of PE.

Androgens derived from the maternal and fetal adrenal glands in the human placenta are converted into estrogens by the enzymatic action of placental aromatase (androstenedione is converted into estrone and testosterone into E2). Perez-Sepulveda et al. [2,22] demonstrated that preeclamptic patients have lower placental aromatase mRNA and protein levels, as well as circulating E2/testosterone and estrone/androstenedione ratios, in comparison with normotensive controls, indicating the loss of both aromatase expression and activity, and this impairment may be due to chronic placental ischemia. Accordingly, it is conceivable that decreased placental estradiol is likely due to an alteration in the aromatase pathway, which is reduced in PE due to placental hypoxia (a pathologic hallmark of

PE). Furthermore, the decrease in estradiol is associated with a decrease in 2-methoxyestradiol, a naturally occurring metabolite of estradiol that helps maintain placental homeostasis, and its reduction could possibly lead to PE.

We recorded a negative correlation between placental free PSA and E2 ($p<0.001$). It was postulated that PSA may be regulated by androgens and represents an additional biochemical marker of androgen action in women [23]. The lower estrogen concentrations in PE, probably resulted from reduced aromatase activity, could explain the higher circulating total and free testosterone levels and the involvement of androgens in the pathophysiology of preeclampsia [24-26].

Placental IL-6 was significantly decreased (28%) in preeclamptic women compared to normal pregnant ones and its level could be a marker for PE, as it was negatively correlated with blood pressure, albuminuria and PSA, and positively correlated with estradiol. Previous studies have shown that amniotic fluid IL-6 was decreased in pregnancies complicated by preeclampsia, and the production of IL-6 was lower in preeclamptic placental explants cultured in vitro, compared with normal placentas [27]. In contrast, Benyo et al. [6] found that placental levels of inflammatory cytokines (TNF- α, IL-1α, IL-1β and IL-6) were not significantly different in PE compared to normal pregnancy. Similarly, the literature contains controversial reports regarding circulating levels of IL-6 in PE, recording either an increase [28,29], or a marked decrease [30,31] in IL-6 level. Benyo et al. [6] suggested that sources other than the placenta contribute to the elevated concentrations of TNF-α and IL-6 found in the circulation of preeclamptic women. According to Udenze et al. [32], the disparity in the reports of the aforementioned studies may be from differences in time of sampling, studies not distinguishing between mild or severe forms of preeclampsia, and differences in study design; prospective or cross sectional as opposed to case control studies.

There is an association between adverse pregnancy outcome with increasing Hb levels (and thus whole blood viscosity) and a strong correlation exists between the prevalence of preeclampsia and plasma volume restriction and hemorheological disorders [1,33]. Likewise, the present result revealed a significant increase in Hb level in preeclamptic women compared to healthy pregnant ones. However, this increase was not correlated with any of the studied parameters. According to Centlow et al. [34], blood hemoglobin is normally elevated in response to low oxygen levels and it has been argued that the placenta in PE suffers from poor perfusion and hypoxia. Furthermore, Hansson et al. [35] suggested that hemoglobin and its degradation products are toxic and can cause oxidative stress, hemolysis, vasoconstriction, kidney, and vascular endothelial damage in preeclampsia.

In PE patients, the coagulation-fibrinolytic system is thought to be one of the most seriously affected systems by maternal inflammatory reactions and immune dysfunction [36]. The super-hypercoagulable state of women with PE may lead to systematic disorders of metabolism as well as multiple organ dysfunctions and may even threaten maternal and fetal lives. Therefore, the dysfunction of the blood coagulation-fibrinolysis system is a salient characteristic of PE that varies in severity, and is a good predictor for the onset and clinical degree of PE [37]. In the current study, platelets count in preeclamptic women was lower by 12%, compared with normal pregnant ones. This decrease was correlated with the severity of PE, namely the decrease in E2 ($p<0.023$) and calcium ($p<0.02$), and with the increase in PSA ($p<0.025$) and albuminuria ($p<0.011$). These results are in line with previous studies [37-39]. Conversely, Makuyana et al. [40] and Ceyhan et al. [41] observed no significant difference in platelet count in preeclampsia. Therefore, elevated hemoglobin and decreased platelet count emerge as good candidates for preeclampsia diagnosis, since they are simple and habitually done methods, with lower cost and greater accessibility in the clinical laboratory.

Although placental levels of Zn, Cu, and Ca are considered by some authors as factors having a role in the etiopathogenesis of preeclampsia and as severity indicators in preeclamptic women [42], the present result revealed no significant changes in these three elements in the placenta of PE women, compared with normotensive pregnant ones. One potential explanation may arise from the analytical method used to monitor the elemental concentrations, and/or the portion of placenta analyzed. Similarly, we did not record any significant change in placental lipid peroxidation and GPx activity of PE women, compared with normotensive pregnant ones that could probably have been elevated during the gestational age but returned to baseline levels at delivery.

In conclusion, we demonstrated increased placental PSA content in PE patients that is negatively correlated with E2 and IL-6 levels. The decreased placental E2 is likely due to an alteration in the aromatase pathway as a result of placenta-induced hypoxia, which leads to an increase in the level of androgen, with a subsequent increase in PSA production. This may explain in part the relation between low placental E2 level and the increase in PSA. Further studies are necessary to define the role of PSA and E2 and to examine their effects on the pathophysiology of preeclampsia.

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