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Assessment of 8-hydroxydeoxyguanosine levels in patients with preeclampsia: a prospective study

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Summary

Purpose of investigation: To determine the levels of 8-hydroxydeoxyguanosine (8-OHdG) in preeclampsia (PE) using (enzyme-linked immunosorbent assay (ELISA) method. *Materials and Methods:* Twenty-two pregnant women with severe PE, 18 pregnant women with mild PE, and 40 healthy pregnant women, all between 25 and 41 weeks of gestation, were enrolled in this prospective controlled study. 8-OHdG levels in maternal serum were measured using ELISA method. *Results:* The authors observed no statistically significant difference in 8-OHdG levels between the mild-severe PE and control groups ($p = 0.208$). *Conclusion:* The present results do not support the concept that 8-OHdG has a role in the etiopathogenesis of PE.

Key Words: Oxidative stress; Preeclampsia; 8-hydroxydeoxyguanosine.

Introduction

Preeclampsia (PE) is a disorder associated with the onset of proteinuria and high blood pressure following the 20th week of pregnancy. The incidence of PE is reported as five to seven percent among pregnant women; however, the level of incidence may vary depending on ethnic, geographical, and social differences [1]. Insufficient trophoblastic invasion, placentation problems, widespread endothelial damage, and oxidative stress are the most common pathways that are implicated in PE [2]. Oxidative stress plays a significant role in cellular destruction, cell damage and cell death, and is implicated in the pathogenesis of many disorders, including PE [3]. Insufficient spiral artery conversion [4], leads to discontinuous placental perfusion and a low-level ischemia-reperfusion injury in PE [5]. Accompanying an increase in uterine artery resistance induces vasoconstriction, and thereby results in chronic placental ischemia and oxidative stress. Oxidative stress also induces the release of free oxygen radicals, oxygenated lipids, cytokines, and serum-soluble VEGF-1 (sflt-1) into the maternal circulation, which results in endothelial dysfunction, vascular hyperpermeability, thrombophilia, and hypertension [6]. Although PE is a hypertensive syndrome that is specific to the pregnancy period and affects multiple systems, there is no routine method for the predictive testing of PE. 8-hydroxydeoxyguanosine (8-OHdG) is the best-known sensitive and stable marker of oxidative damage in cellular DNA [6]. It is produced as a result of DNA damage caused by reactive oxygen and hydrogen species, and indicates invariably oxidative stress. 8-OHdG is produced as a result of the oxidation of deoxyguanosine, which has been associated with subsequent mutations. In addition to the aging process, an increase in 8-OHdG levels may occur under many pathological conditions, including cancer, diabetes, and hypertension. Nuclear and mitochondrial DNA are regarded as the most important targets for oxidative attack, induced by free radicals [7-10]. The aim of the present study was to assess the levels of 8-OHdG in mild or severe forms of PE and healthy pregnant women between 25 and 41 weeks of gestation.

Materials and Methods

The study included 22 pregnant women with severe PE, 18 pregnant women with mild PE, and 40 healthy pregnant women, all between 25 and 41 weeks of gestation, who were admitted to the Department of Obstetrics and Gynecology of Gaziantep University Faculty of Medicine, between June 2013 and June 2014. The Hospital Ethics Committee approved the study. A diagnosis of mild PE was based on systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg in two subsequent measurements (after at least ten minutes of rest), with at least a four-hour interval after the 20th week of gestation, and with or without accompanying proteinuria (≥ 0.3 grams/24 hours) in the presence of organ dysfunction (thrombocytopenia, impaired renal dysfunction, impaired liver function, pulmonary edema, headache, blurred vision) in pregnant women who were previously known to be normotensive [11]. The patient group was divided into two groups, as severe PE and mild PE, with a severe PE diagnosis based on ACOG 2013 criteria [11]. The control group comprised healthy pregnant women with no elevation in blood pressure or other systemic diseases. Demographic

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Table 1. — *Demographic and clinical characteristics of preeclamptic pregnant and healthy pregnant patients.*

Variables	Control (n=40) (mean±sd)	Mild preeclampsia (n=18) (mean±sd)	Severe preeclampsia (n=22) (mean±sd)	*p*
Age (years)	29.35±5.97	34.22±5.57 †	31.36±7.34	0.027*
BMI (kg/m^2)	27.72±0.90	28.11±0.81	28.61±1.20†	0.004*
Blood pressure				
Systolic (mm/Hg)	113.63±10.50	145.28±5.55	174.09±17.84	0.001*
Diastolic (mm/Hg)	70.00±0.01	91.67±3.84	107.05±11.49	0.001*
Gravidity				
Nulliparous, n (%)	6 (15.0)	6 (33.3)	11 (55.0)	0.013*
Multiparous, n (%)	34 (85.0)	12 (66.7)	11 (50.0)	
Gravidity, median [min-max]	3 [1-8]	4 [1-11]	1.5 [1-10]	0.100*
Parity, median [min-max]	2 [0-5]	2 [0-8]	0 [0-8]	0.091

* Significant at $p < 0.05$, † Significant *vs.* control group at $p < 0.05$, ‡ Significant *vs.* mild preeclampsia group at $p < 0.05$.

Table 2. — *Biochemical characteristics of preeclamptic pregnant and healthy pregnant.*

Variables	Control (n=40) (mean± sd)	Mild preeclampsia (n=18) (mean± sd)	Severe preeclampsia (n=22) (mean± sd)	*p*
Leukocytes (10^3/μL)	10.53±3.06	9.89±1.69	10.51±3.61	0.735
Hemoglobin (g/dL)	11.68±1.27	11.34±1.24	11.51±1.75	0.694
Platelets (10^3/μL)	245.40±65.36	226.78±67.61	224.59±96.01	0.502
AST (IU/L)	15.73±4.05	18.11±7.25	58.14±106.64†‡	0.001*
ALT (IU/L)	10.05±4.34	13.06±5.25	41.55±62.501†	0.001*
BUN (mg/dL)	14.00±5.58	21.11±9.44†	30.05±13.40†	0.001*
Creatinine (mg/dL)	0.48±0.11	0.65±0.31†	1.10±1.77†	0.001*
Albumin (g/dL)	3.49±0.35	3.05±0.29†	3.078±0.59†	0.001*
Urinary protein (mg/dL)	——	1.61±0.69	2.64±0.58	0.001

* Significant at $p < 0.05$, † Significant *vs.* control group at $p < 0.05$, ‡ Significant *vs.* mild preeclampsia group at $p < 0.05$.

Table 3. — *8-OHdG levels of the three groups.*

Variables	Control (n=40) (mean±sd)	Mild preeclampsia (n=18)	Severe preeclampsia (n=22)	*p*
8-OHdG ng/ml (mean sd)	5.11±1.46	6.17±3.50	5.84±2.29	0.208

data of the groups (age, gravida, parity) and gestational age, calculated based on the last menstrual period, were recorded. The laboratory tests requested from the participants included a complete blood count (CBC), a complete urine analysis (CUA), AST, ALT, serum albumin, urea, and creatinine. Also venous blood samples were collected from the participants without labor for analysis of serum 8-OHdG levels at the time of PE diagnosis. Blood samples of three ml were obtained from antecubital veins and serums were obtained by centrifugation at 3,000 rpm for ten minutes and were finally placed into Eppendorf tubes within an hour and stored at -80°C. The exclusion criteria were multiple pregnancies, pre-pregnancy or newly diagnosed systemic disease, pregnancy complications other than PE, alcohol, smoking or any drug use.

Measurement of serum 8-OHdG levels

The specific kit was used to measure serum 8-OHdG levels. As a competitive enzyme-linked immunosorbent assay (ELISA) kit, the specific one used in the present study is considered appropriate for the measurement of oxidative damage in the tissue, serum, and plasma. The ELISA tests were carried out by the Department of Biochemistry in the Gaziantep University Faculty of Medicine.

Statistical methods

A Kolmogorov-Smirnov test was applied to check if the continuous variables were normally distributed. Furthermore, the Student's *t*-test was used to compare normally distributed variables between two independent groups, while a Mann Whitney U-test was used to compare abnormally distributed variables. ANOVA and LSD multivariate analysis tests were used to compare the normally distributed variables between more than two independent groups, the Kruskal Wallis *t*-test was used to compare the abnormally distributed variables and the Dunn test was used for subgroup comparisons. The relationship between numeric variables was analyzed using Spearman's rank correlation coefficient, and a chi-square test was used for the non-numeric variables. Multiple linear regression analysis was performed to adjust age and BMI effect on 8-OHdG. The statistical analysis was made using SPSS for Windows 22.0 software package, and a p-value < 0.05 was deemed statistically significant.

Results

The comparison of demographic and clinical features of the controls and the patients with severe and mild PE are presented in Table 1. The number of nulliparous pregnant women was higher in both the mild and severe PE groups when compared to the control group ($p = 0.013$) (Table 1). Furthermore, a comparison of the blood pressure values revealed significantly higher systolic and diastolic blood pressures in both PE groups when compared to the control group as expected ($p < 0.05$). Hemoglobin values and leukocyte and platelet counts did not differ significantly between the patient and control groups ($p = 0.735$, $p = 0.694$, and $p = 0.502$, respectively). In contrast, significant differences were recorded between the groups in terms of ALT, AST, BUN, creatinine, and albumin values (Table 2), with AST, ALT, BUN, and creatinine values being significantly higher, and albumin values significantly lower in the severe PE group when compared to other two groups, with a significance of $p = 0.001$. 8-OHdG levels also did not differ significantly between the mild-severe PE groups and the control group ($p = 0.208$). The levels were 6.17 ± 3.50, 5.84 ± 2.29, 5.11 ± 1.46 ng/ml, respectively (Table 3, Figure 1). There was still no significant difference between the groups after adjustment for age and BMI ($p = 0.19$) (Table 4).

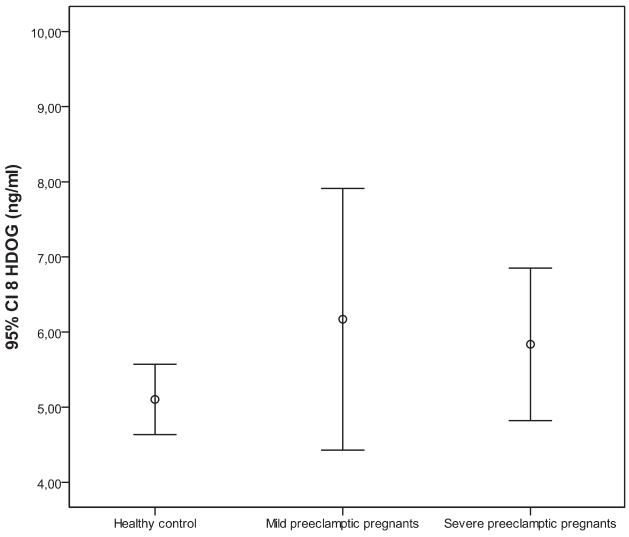

Figure 1. — Comparison of the three groups according to 8-OHDdG.

Table 4. — *Multiple linear regression model for 8-OHdG level estimations.*

Variables	Unstandardized Coefficients		*p*
	B	Std. Error	
Constant	7.716	7.473	0.305
Groups	0.434	0.328	0.190
Age (years)	0.028	0.041	0.501
BMI (kg/m^2)	-0.120	0.273	0.661

Discussion

Despite advances in the methods for its diagnosis and treatment, PE continues to be associated with an increased risk of maternal mortality [12]. To date, although many theories have been put forward, the pathogenesis of PE has yet to be elucidated, although oxidative stress and inflammatory response are two of the leading theories. Placenta is the main source of free radicals, with maternal leukocytes and endothelium being contributory factors [13]. 8-OHdG is produced as a result of DNA destruction by reactive oxygen species, which can be detected in the urine, serum, and tissue samples of both humans and animals, and is the most sensitive marker of oxidative stress. Furthermore, 8-OHdG levels in tissue reflect oxidative stress in the organs [14]. Takagi *et al.* [15] evaluated the tissue 8-OHdG, 4-hydroxynonenal, thioredoxin, and redox factor-1 levels in healthy pregnant women and pregnant women with PE, IUGR, and PE plus IUGR. Their findings revealed higher values in patients with PE, IUGR, and PE plus IUGR when compared to healthy pregnant women. In another study, DNA was isolated from placental tissue, and 8-OHdG levels were compared between healthy pregnant women and pregnant women with severe PE and IUGR plus severe PE. No difference was identified between the healthy pregnant women and those with severe PE; however, the 8-OHdG levels were higher in pregnant women with IUGR plus severe PE [16]. Immunohistochemical staining for 8-OHdG in the nuclei of placental trophoblasts in healthy pregnant women, and in those with PE or IUGR plus PE had been evaluated. A higher rate of staining for 8-OHdG in the PE and IUGR plus PE groups when compared to the healthy subjects were reported [17]. Kimura *et al.* [18] investigated the rate of immunohistochemical staining for 8-OHdG in the nuclei of placental trophoblasts in patients with early-onset PE plus IUGR, and in those with late onset PE plus IUGR. The rates of immunohistochemical staining for 8-OHdG in the nuclei of placental trophoblastic cells in the two PE groups were higher when compared to those seen in healthy pregnant women, with the staining in the early-onset group being more prominent. Higher 8-OHdG levels were reported in umbilical cord blood obtained from the cord elements of babies born to mothers with PE/eclampsia when compared to healthy pregnant women [19]. Decreased placental activity of indoleamin 2,3-dioxygenase (IDO) was found to be related to oxidative damage and negatively correlated to IDO activity and placental 8-OHdG levels in PE women, while 8-OHdG was markedly higher in PE pregnant women when compared to normotensive pregnant women [20]. In the present study, the authors evaluated serum 8-OHdG levels in healthy and PE women, with the intention being to reveal its relationship with disease severity. As a potential biomarker in the etiopathogenesis of PE, the levels of 8-OHdG were measured in maternal serum using the ELISA method. There was no statistically significant difference between the results of 8-OHdG levels in the control, mild PE, and severe PE groups. Although there are studies suggesting that placental 8-OHdG levels associated with oxidative damage may play a role in the etiopathogenesis of the disease and in the development of IUGR in PE pregnant women; however, as far as it is known, the present study is the first to evaluate 8-OHdG levels in maternal serum in PE patients.

Conclusion

In conclusion, the measurement of 8-OHdG levels in maternal serum of PE women does not seem to be an appropriate method for the early detection of PE, although previous studies have suggested that 8-OHdG levels in the placenta and umbilical cord blood may play a role in the etiopathogenesis of PE. Women with PE had higher BMI than controls. The pathophysiological mechanism behind the increased BMI in PE is not fully understood although increased insulin resistance and a state of inflammation associated with obesity are likely important contributing factors. However, there was still no significant difference between the 8-OHdG levels of the groups after adjustment

for age and BMI (Table 4). Further studies of larger numbers of patients are required in order to evaluate the value of 8-OHdG measurements in samples other than the serum (i.e. urine) in the early diagnosis of PE. Future development of better and more sensitive tests for determining 8-OHdG levels in maternal serum may help to come up with evidence for its role in PE.

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