

Oxidative Stress, Neutrophil Elastase and Vascular Endothelial Growth Factor in Obese Pregnant Women with Preeclampsia

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Oxidative stress, inflammation, and vascular endothelial proliferation and obesity are risk factors associated with preeclampsia (PE). The present study aimed to investigate the levels of malondialdehyde (MDA) as a putative circulatory marker of oxidative stress, neutrophil Elastase (NE) as inflammatory marker and vascular endothelial growth factor (VEGF) as marker for vascular permeability-enhancing activities in obese women with preeclampsia PE and compare with normal pregnancy women. The study was carried out on 50 pregnant obese women with PE and 50 normal pregnant women. The preeclampsia women were characterized with high blood pressure 160/110 mmHg and proteinuria. The gestational age ranged from =32 weeks to <37 weeks. Pre pregnancy weight was recorded. Body mass index (BMI) was calculated at delivery. Serum MDA, NE and VEGF were estimated by ELISA. Significant higher levels of serum MDA, NE and VEGF were observed in obese PE patients as compared to normal controls. Our results suggested that obesity; oxidative stress, NE and VEGF biomarkers are risk factors for PE, emphasizing their role as feasible candidate risk markers for cases with high blood pressure in early pregnancy.

Keywords: Malonaldehyde; Neutrophil Elastase; Preeclampsia; VEGF.

Hypertension and proteinuria are pregnancy-specific conditions characterize preeclampsia. Although its precise etiology is unknown, endothelial cell dysfunction might cause maternal symptoms¹. The major cause of maternal and perinatal morbidity and mortality

is preeclampsia, 10% of women had high blood pressure during pregnancy².

Moreover, preeclampsia might lead to problems in the kidneys, liver, clotting system and brain in 2% to 8% of pregnancies, with premature and poor and growth offspring. Although outcome is often good, preeclampsia can be harmful and life threatening.

Generally, preeclampsia and eclampsia are concomitant with direct maternal deaths in 10% to 15%³. The pathophysiology of preeclampsia has been implicated by neutrophils activation which engaged binding and transmigration of neutrophils through the endothelium⁴, through surface receptors on neutrophils and endothelium adhesion molecules. Women with preeclampsia showed stimulated neutrophils which plays an important role in the vascular endothelium pathophysiology of this disorder⁵. Amplified numbers of neutrophils are noted from first to third trimester in the maternal circulation, with elevated neutrophil count. Consequently, unfavorable pregnancy outcome might be linked to excessive inflammatory neutrophil activation and increased concentration of elastase discharged from neutrophil granules⁶. Oxidative stress and oxidative damage in early gestation in early gestation influences the onset of these conditions later in pregnancy^{7,8}. Increased oxidative stress markers perform significant role in the pathophysiology of preeclampsia^{9,10}. Increase in the free radicals causes overproduction of MDA which is the indicator of oxidative stress^{6,7}.

An important angiogenic factor was described to provoke the proliferation and migration of endothelial cells is vascular endothelial growth factor (VEGF), enhances vascular permeability, and modulates thrombogenicity. Consequently, it is implicated in development of normal blood vessel as well as in vascular pathologies¹¹.

The present study aimed to evaluate and compare the biomarker variables of oxidative stress, inflammatory and cytokine VEGF in preeclamptic women and in normal pregnant women.

MATERIAL AND METHODS

The blood samples were collected from 50 preeclamptic and 50 normal pregnant women at the department of obstetrics and gynecology, Cairo University and National Research Centre. The study was approved by the Ethical Committee form of National Research Centre, Egypt (number = 16361), in accordance with the World Medical Association's Declaration of Helsinki. The age of the women ranged from 25-40 years, their mean

age was 34.31 ± 8.83 and pre pregnancy BMI (kg/m²) was 29.9 ± 6.91 .

Circulating markers of oxidative stress MDA, neutrophil elastase and vascular endothelial growth factor (VEGF) were evaluated among women with and without preeclampsia.

A dipstick urine measurement for protein was performed on urine specimen collected from the patient on the clinic days. Testing was performed with multistix reagent strips (Bayer). Proteinuria was classified semi-quantitatively as absent if testing with a dipstick showed no protein or trace levels (level below 300 mg L⁻¹); low grade if the dipstick showed a value of 1+ or 2+ (level between 300 and 3000 mg L⁻¹) and high grade if the dipstick showed a value of 3+ or 4+ (level >3000 mg L⁻¹). Proteinuria was observed with low grade in 46 % and with high grade in 54% of cases.

Statistical analyses

Statistical analyses were performed with SPSS software, version 19.0 (SPSS Inc., Chicago, IL, USA) taking level of significance to be $p < 0.05$. Normal distribution of the data for each parameter was checked by Kolmogorov-Smirnov analysis. Percentage, mean, and standard deviation were used to express normally distributed variables. An unpaired t-test was applied to determine the mean significant difference between normal and preeclamptic groups. Percentage distribution in preeclampsia women was done by using chi-square test.

RESULTS

A total of 100 women were enrolled, 50 obese women with preeclampsia and 50 normotensive controls. The obstetric history shows that the mean gestational age at delivery was 33.54 ± 5.68 in PE. The mean number of pregnancies was 3.69 ± 1.932 . The systolic blood pressure was 172.69 ± 12.85 , diastolic blood pressure was 111.92 ± 9.02 . PE showed significant higher BMI 32.94 ± 8.93 than normal controls. Moreover, the pre-pregnancy BMI was significantly higher in PE women. Proteinuria was observed in PE with low grade in 46 % and with high grade in 54 % and deliveries with previous miscarriages was 92% in PE and 2% in normal controls (table 1). Statistically significant difference was observed between PE and

controls in the mean levels of blood pressure and in frequency of previous miscarriages ($p < 0.05$). Moreover, MDA levels were significantly higher among the preeclampsia patients (5.23 ± 2.58) as compared to normal control group (0.91 ± 0.29) as well as NE in the preeclampsia patients (7.52 ± 2.60) compared to normal control group (3.45 ± 1.23) and VEGF (122.68 ± 16.69) in PE compared to normal pregnancy (82.68 ± 10.14) (table 2) ($P < 0.05$).

DISCUSSION

PE is clinically detected after 20 weeks' gestation and potentially life-threatening. It affects 5% of pregnancies and is characterized by hypertension and proteinuria in mild cases, and even seizures, organ damage and maternal death in severe cases. In our study we found that the mean age was 34.31 ± 8.835 , not all of our cases were primigravida with the mean number of pregnancies 3.69 ± 1.932 . Similarly, other study

12 described preeclamptic women with maternal age 31.0 ± 4.6 . This report stated that the gestational age in their patients was 33.54 ± 5.68 and the mean BMI was 32.94 ± 8.93 this coincides with the findings of Sharma *et al.*, 2006 and Wang *et al.*, 2017 who reported preeclamptic women with gestational age 35.2 ± 3.6 and BMI 25.86 ± 2.82 . Moreover, Sohlberg *et al.*, 2012 elucidated that the high maternal body mass index increases the risk of preeclampsia. Likewise, in previous studies done on extremely obese teenagers they detected that they were almost four times likely to develop preeclampsia and eclampsia compared with non-obese women. However, obesity elevated the risk for preeclampsia and eclampsia among all women in the study, teenagers were most at risk because of the compound effect of young age and obesity^{8,9}.

Magee *et al.*, 2014 reported that ISSHP-definition of pre-eclampsia occur when the systolic blood pressure is > 140 mmHg and diastolic blood pressure is > 90 mmHg developing after 20 weeks gestational age.

Table 1. Clinical characteristic of normal controls and women with preeclampsia

	Normal Control Pregnancies Mean \pm SD	Preeclampsia Mean \pm SD
Maternal age (years)	36.31 ± 8.835	34.31 ± 8.835
Pre-pregnancy BMI (kg/m ²)	22.9 ± 4.93	$30.9 \pm 6.91^*$
Gestational age at delivery (weeks)	35.54 ± 2.61	33.54 ± 5.68
Number of pregnancies	3.69 ± 1.932	3.69 ± 1.932
Systolic blood pressure (mmHg)	114.9 ± 6.7	$172.69 \pm 12.84^*$
Diastolic blood pressure (mmHg)	73.67 ± 5.5	$111.92 \pm 9.02^*$
Body Mass Index (kg/m ²)	23.22 ± 6.91	$32.94 \pm 8.93^*$
Deliveries with previous miscarriages [n (%)]	1 (2%)	46 (92%)*
Cases presenting with proteinuria 1+ or 2+ [n (%)]	-	23 (46%)
Cases presenting with proteinuria 3+ or 4+ [n (%)]	-	27 (54%)

* $P \leq 0.05$: PE vs. healthy pregnant women

Table 2. The levels of malonaldehyde, neutrophil elastase and VEGF in preeclampsia

	Malonaldehyde (μ mole/L)	Neutrophil elastase (μ mole/L)	VEGF (pg/ml)
Women with preeclampsia	$5.23 \pm 2.58^*$	$7.52 \pm 2.60^*$	$122.68 \pm 16.69^*$
Normal Control Pregnancies	0.91 ± 0.29	3.45 ± 1.23	82.68 ± 10.14

* $P \leq 0.05$: PE vs. healthy pregnant women

In our study the mean systolic blood pressure was 172.69 ± 12.84 and the mean diastolic blood pressure 111.92 ± 9.02 in women with preeclampsia which is higher than the normal control pregnancy. Proteinuria (> 300 mg/day) occurs in preeclampsia and classified as low grade +1 or +2 which occurred in 46% of our cases and moderate and high grade in 54% of cases.

In PE, OS induced in the ischemic placenta produces the release of cytotoxic factors into the maternal circulation, stimulating the inflammatory response and activating maternal endothelial cells^{6,15}. During normal pregnancy, placental oxidative stress (OS) is present during all three trimesters and is necessary to obtain normal cell function. However, if OS reaches a certain level, pregnancy complications might develop^{3,15}. PE is related to an impaired endothelial function that accounts for activation of the coagulation cascade, altered vascular reactivity and loss of vascular integrity. Impaired endothelial malondialdehyde levels were significantly higher as shown in the study done by^{16,17}. Preeclampsia is a chronic inflammatory disease in pregnancy, which is associated with damaged maternal endothelium a potent stimulator for neutrophil activation¹⁸. Neutrophils are increased due to augmented physiologic stress and impaired neutrophilic apoptosis during pregnancy¹⁹. Elevated levels of VEGF in preeclamptic women compared to normal pregnant was reported in some studies^{11,20}. However, other studies delineated decreased level of serum VEGF in preeclamptic women²¹. The decrease in VEGF level becomes more significant with increasing PE severity, proposing that the decrease in VEGF synthesis and release may be an important factor in PE pathogenesis²². The association between of preeclampsia and high BMI reported in our study is consistent with other studies^{23,24}. Pre-pregnancy obesity was observed in our study in agreement with previous studies reported association between re-pregnancy BMI and preeclampsia^{25,26}

CONCLUSION

Combination of obesity, elevated MDA, NE and VEGF biomarkers in the early third trimester may be important risk factors in PE

pathogenesis and could predict cases at risk of high-normal blood pressure in early pregnancy.

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