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Research Article
Evaluation of Some Cytokines and Gene Expressions in Pre-eclampsia

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Abstract

Background and Objective: Preeclampsia (PE) is a disorder characterized by hypertension and proteinuria. There is accumulating evidence that this is a disease of the endothelium. Angiogenic factors may be responsible for the regulation of placental vascular development. Clinicians cannot predict pre-eclampsia prior to the onset symptoms. An ideal bio-marker for pre-eclampsia prediction is during the first trimester. This study investigated the serum levels of tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) and the gene expressions of vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS) and p53 in PE trying to find out potential bio-markers for prediction and diagnosis of PE. **Materials and Methods:** A total of 100 female volunteers were involved in this study and their ages were ranged from 25-35 years. They were divided into three groups: Group (1) was 20 healthy non-pregnant women, group (2) was 20 pregnant women normal pregnancies and group (3) was 60 preeclamptic patients. The study participants were enrolled at the Department of Obstetrics and Gynaecology at Mansoura University Hospital, Mansoura, Egypt. The study was approved by the Research Ethics Committee (Faculty of Science, Al Azhar University, Egypt) approved on the March 15, 2014) all women gave written informed consent. Serum levels of CRP, IL-10 and TNF-α were evaluated, in addition to the gene expression of VEGF, eNOS and p53. **Results:** Significant elevations in the serum levels of blood pressure, TNF-α and CRP were observed in PE patients. Additionally, the gene expression of VEGF, eNOS and P53 were down-regulated in preeclampsia. **Conclusion:** Elevated serum levels of TNFα and CRP, in addition to the down-regulation of eNOS may be used as good predictors for preeclampsia. The TNF-α and VEGF gene were recommended used as markers for PE to be added to routine testes of pregnant women.

Key words: Cytokines, pre-eclampsia, VEGF, P53 and eNOS

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Preeclampsia (PE) is one of the major causes of morbidity and mortality for both mother and her fetus. Preeclampsia affects 1-10% of pregnant women worldwide and is characterized by hypertension and proteinuria which developing after 20 weeks of gestation[1,2]. Pregnancy which terminated early by caesarean delivery, the fetus will be with prematurity, low birth weight and respiratory distress syndrome. Women with pre-eclampsia are characterized by an increased risk of cardiovascular and renal disease in future years[3]. The PE is an endothelial damage disease[4]. Which may be altered vascular endothelial growth factor (VEGF) signaling in endothelial cells[5,6]. However, in the high-risk population, the diagnosis of PE may not be straightforward and therefore it may be important to study bio-markers to improve the predictive value of ultrasound screening[7,8]. Evidence of placental dysfunction in PE has associated endoplasmic reticulum (ER) stress, unfolded protein response (UPR), increased autophagy and apoptosis. Placental apoptosis and expression of p53 downstream cell cycle regulators in trophoblast cells[9]. It is now time, to demonstrate the angiogenic markers that could directly affect obstetrician's management decisions, improve health outcomes and reduce costs to the healthcare. So, this study was aimed to investigate the serum levels of IL-10, the pro-angiogenic cytokine TNF-α and C-reactive protein (CRP), in addition to the gene expression of VEGF, endothelial nitric oxide synthase (eNOS) and p53 in pre-eclampsia with special highlighting on finding a predicting bio-marker for that disorder. This study was designed to investigate the serum levels of IL-10, the pro-angiogenic cytokine TNF-α and C-reactive protein (CRP) in addition to the gene expression of VEGF, endothelial nitric oxide synthase (eNOS) and p53 to predict pre-eclampsia bio-marker's to improve placentation.

MATERIALS AND METHODS

Study design: The study was designed using a case-controlled approach. A total of 100 women were involved in this study, their ages were ranged between 25-35 years. Participants were categorized into three groups. Group 1:20 healthy non-pregnant women, group 2:20 healthy pregnant women and group 3:60 pre-eclamptic patients. Pre-eclampsia was defined by increased blood pressure ($\geq$140 mm Hg systolic or $\geq$90 mm Hg diastolic on $\geq$2 occasions at least 6 h apart) that occurred following 20 weeks of gestation in a woman with previously normal blood pressure, accompanied by proteinuria ($\geq$0. 3 g/24 h or $\geq$1+ on dipstick in the absence of urinary tract infection). All participants were enrolled at the Department of Obstetrics and Gynaecology at Mansoura University Hospital, Mansoura, Egypt. The study was approved by the Research Ethics Committee (Faculty of Science, Al Azhar University, Egypt) approved on the March 15, 2014), all women gave written informed consent. Multi foetal gestation, foetal infection and rupture of membranes were excluded. All participants were subjected to full history and complete clinical examination within the study. Fasting blood samples were drawn and a part of the sample was taken on EDTA as whole blood sample (for gene expression assessment) and another part was taken on without anti-coagulant for separation of serum by centrifugation at 3000 rpm for 10 min (for cytokines determination).

Immunological parameters: Serum levels of interleukin-10 (IL10) and tumor necrosis factor- α (TNFα) were estimated using immunoassay kits (Quantikine ELISA, R and D Systems Inc. , USA).

C-reactive protein (CRP) was assessed by immunoturbidimetry. Evaluation Turbidimetric Immunoassay is based on the principle of agglutination reaction for the ultra sensitive determination of C-reactive protein in human plasma[10,11].

Gene expression by real time PCR (qRT-PCR): Total RNA was extracted from blood using the SV Total RNA Isolation System (Promega, Madison, WI, USA system). The extracted RNA was reverse transcribed into cDNA using a superscript III kit (Invitrogen Ltd., Paisley, UK) following manufacturer's instructions. According to the amplification procedure, relative expression of genes was calculated. The β-actin was amplified with the same run of the tested gene as a housekeeping gene. Primers are listed in Table 1. All RT-PCR were achieved using SYBR Green, low ROX, Stratagene (Thermofisher scientific).

Statistical analysis: Data were expressed as Mean±SE. One-way ANOVA was used and $p<0.05$ was accepted as statistically significant.

RESULTS

Clinical characteristic: The control and pre-eclamptic group's clinical characteristics are presented in Table 2. Pre-eclamptic patients showed significant elevations ($p<0.05$) in both the systolic and diastolic blood pressure (SBP), compared to the control group.

Table 1: Primers sequence used

Genes	Primers sequence	Size (bp)	Reference or accession No.
β-actin	F: 5'-TCA CCC TGA AGT ACC CCA TGGAG-3'	436	Lam *et al.*[12]
	R: 5'-TTG GCC TTG GGG TTC AGG GGG-3'		
VEGF	F: 5'-TCCATGTGGGAGGTGGTAGT-3'	154	CM000262
	R: 5'-AGCACA AGC CCCTCTTAGTCCA-3'		
eNOS	F:5'-CTGCCCCTTTGCACGCT-3'	510	KJ628492
	R: 5'- CTCTCGGCCGGGTCCT3'		
p53	F: 5'-ATGGCCCCTGTCATCTTTTGTC-3'	1429	AB021961
	R: 5'-CTTCTTCTGTACGGCGGTCT-3'		

bp: Base pair

Table 2: Clinical characteristic, cytokines concentration (TNFα and IL10) and C-reactive protein values in healthy non-pregnant, pregnant women and pre-eclamptic patients

Groups	Age (y)	Gestation age (w)	DBP (mm/Hg)	SBP (mm/Hg)	TNFα (pg mL^{-1})	IL10 (pg mL^{-1})	CRP (mg L^{-1})
Non-pregnant	29±1.06	--	115.5±1.14	71±0.69	0.30±0.02	286.67±16.07	0.48±0.09
Normal pregnant	29±0.89	31±0.81	110.5±1.35	76±1.52	0.48±0.02	299.84±12.51	2.90±0.42
Pre-eclamptic	28±1.04	30±1.08	157.67± 269[ab]	8933±249[ab]	169±11[ab]	23164±996[ab]	72.00±46[ab]

a: Significant at p<0.05 compared to control group, b: Significant at p<0.05 compared to pregnant group, Values are expressed as Mean±SE

Immunological results

Cytokine concentration (TNFα and IL10): In pre-eclamptic patients, the concentration of TNFα was significantly increased (p<0.05), compared to both control and normal pregnant groups as illustrated in Table 2. On the other hand, serum concentration of IL10 was significantly decreased (p<0. 05) in pre-eclamptic women as compared to control non-pregnant and pregnant groups.

Serum level of C-reactive protein (CRP): The level of serum C-reactive protein (CRP) is present in Table 2. The CRP was significantly increased in pre-eclamptic women, compared to control non-pregnant and pregnant groups.

Molecular results

Expression of VEGF gene: The expression of VEGF was down-regulated in pre-eclampsia when compared to normal non-pregnant and pregnant groups (p<0.05) as shown in Fig. 1.

Expression of eNOS genes: The expression of eNOS was down-regulated in pre-eclampsia, compared to normal non-pregnant and pregnant groups (p<0.05) as shown in Fig. 2.

Expression of P53 gene: The expression of P53 was down-regulated in pre-eclampsia, compared to normal non-pregnant and pregnant groups (p<0.05) as shown in Fig. 3.

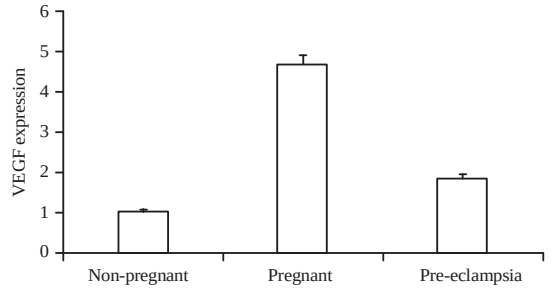

Fig. 1: VEGF gene expression in healthy non-pregnant, pregnant and pre-eclamptic patients

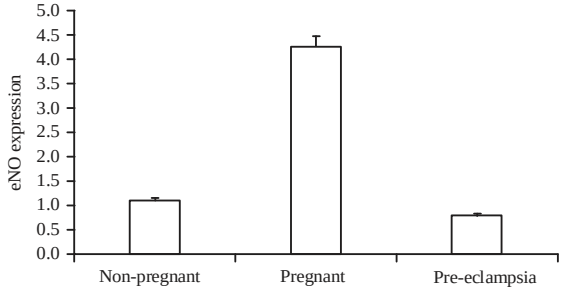

Fig. 2: eNOS gene expression in healthy non-pregnant, pregnant and pre-eclamptic patients

DISCUSSION

Pre-eclampsia (PE) is a specific pregnancy disorder that is characterized by hypertension and proteinuria after 20 weeks of gestation[13]. In pregnancy the activity of eNOS as vasodilator increases in the maternal systemic vasculature in general (as mentioned by the up-regulation of eNOS in the current study), this is even more pronounced in the uterine

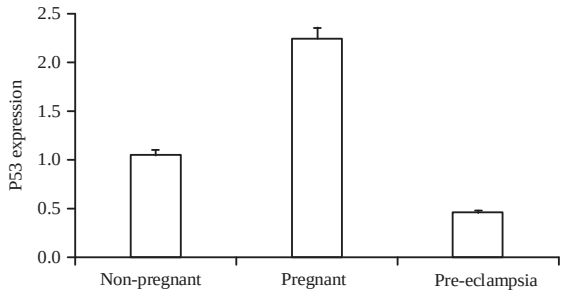

Fig. 3: P53 gene expression in healthy non-pregnant, pregnant and pre-eclamptic patients.

vasculature[14,15]. Vasodilation in the uterine vasculature during pregnancy is necessary to provide the growing fetus with both the nutrition and oxygen needed for proper development. However, the vascular endothelium cannot produce necessary levels of eNOS, which explains the maternal hypertension in PE as observed in the present study through the down-regulation of eNOS that results in decreased eNOS and insufficient vasodilation[14,16]. This impaired endothelial function leads to vasoconstriction and end-organ ischemia[17]. This ischemic placenta results in systemic oxidative stress and also releases cytotoxic, inflammatory and anti-angiogenic markers in the circulation resulting in systemic endothelial dysfunction and peripheral organ damage[18]. Inflammation has been shown to be an important contributor to the pathogenesis of PE[19]. Clinical and biochemical data suggested that endothelial dysfunction may be the primary cause of this condition and that this dysfunction is accompanied by an elevation in inflammatory markers, which have been investigated as possible predictors of preeclampsia, especially C-reactive protein (CRP)[20-22]. The study results showed significant elevation in the serum level of CRP in PE as a consequence of elevated TNF-α which is the primary stimuli for hepatic CRP production[23]. These results agree with many studies that have shown higher CRP concentrations in PE compared with normotensive pregnant women[24,25]. Rebelo *et al.*[26] revealed a positive association between CRP levels and development of PE. The p53 tumor suppressor gene is important in determining the fine balance between growth, differentiation and cell death. The p53 encodes a multi-functional transcription factor that is activated by DNA damage and hypoxia stimuli. So, the trophoblast hypoxia due to abnormal spiral arteries triggers apoptosis leading to the onset of PE[27]. Current results revealed a significant decreased in p53 in preeclamptic women, compared to non-pregnant and pregnant controls. Sharp *et al.*[9] showed that p53 mRNA levels were not significantly increased in pregnancies complicated by PE. However, p53 protein level was significantly raised in placentas from pre-eclamptic pregnancies. Moreover, p53 was localized to the trophoblast nuclei and the increased levels of p53 were associated with increased expression of the apoptotic factors. Consequently, apoptosis is particularly associated with trophoblast damage. Beside its role as a tumor suppressor gene, p53 plays a critical role in regulating angiogenesis regulations[28,29]. Alterations in the p53 gene product have been shown to be a potent inducer of angiogenesis via the vascular endothelial growth factor (VEGF) pathway[30].

The vascular endothelial growth factor (VEGF) family is important for establishing normal pregnancy. During pregnancy, maintenance of adequate blood circulation is required for placental growth, in addition to blood and oxygen supply for the normal foetal[31,32]. After pregnancy VEGF mRNA expression level in placental tissue and in peripheral blood increased[33,34]. Up-regulation VEGF level helps maintain the normal permeability of maternal blood vessels and regulating maternal cardiovascular adaptation to pregnancy[35]. In the present study, the levels of VEGF mRNA in peripheral blood of the PE group were significantly decrease than normal pregnancy group. However, the level of VEGF mRNA of the PE was significantly higher as compared to non-pregnant group. These results are in line with those of Ren *et al.*[36], Tandon *et al.*[37] and Zhou *et al.*[38] revealed that in the preeclampsia patients the VEGF mRNA expression in the placenta tissue more than serum levels. However, there is a substantial, critical and serious discrepancy in the literature concerning not only the level of circulating VEGF in pre-eclamptic plasma but also the level of VEGF mRNA[33,39-42].

CONCLUSION AND RECOMMENDATION

It could be concluded that elevation of the serum level of TNF-α and the decrease in VEGF mRNA expression in peripheral blood may consider as predictors and markers for PE. The TNF-α and VEGF gene were recommended used as markers for PE to be added to routine testes of pregnant women.

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