Identification of vascular endothelial growth factor in preeclampsia in Iraqi women

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ABSTRACT
Preeclampsia (PE) is a major obstetric syndrome and represents a pregnancy hypertensive disease affecting about 2–8% of pregnancies. Typically, it occurs after 20 weeks of pregnancy, being classified as early or late in accordance with the gestational age at diagnosis or delivery. An imbalance between angiogenic and antiangiogenic factors has an important role in the pathophysiology of PE. It was hypothesized that the dysfunctional endothelium contributes to the pathogenesis of PE. A change in the production of Vascular endothelial growth factor (VEGF), a biomarker of endothelial dysfunction, is associated with this disease, whether presenting an increase, decrease, or being at a normal level. This study examined the associations between VEGF and preeclampsia and the importance of this VEGF as a predictor of its severity. This case-control study included 50 patients with preeclampsia and 50 normotensive pregnant women in the control group. Venous blood was aspirated from each patient, and VEGF levels were measured from sera. The mean VEGF for patients with mild PE was 29.410±18.976 pg/ml, for those with severe PE it was 36.188±36.98 pg/ml, and for normotensive women it was 92.104±154.715 pg/ml. There were significant differences in VEGF levels between the studied groups (P=0.024). This study showed that serum VEGF levels were significantly reduced in patients with preeclampsia compared with normotensive pregnant women, suggesting marked endothelial dysfunction. This led to widespread vasoconstriction and, in turn, caused hypertension and proteinuria.

KEYWORDS: vascular endothelial growth factor, preeclampsia, growth factor in preeclampsia.

INTRODUCTION
Preeclampsia (PE) is a major obstetric syndrome [1] and is a pregnancy hypertensive disease affecting about 2–8% of pregnancies. Typically it occurs after 20 weeks of pregnancy. The diagnosis of preeclampsia requires a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg plus one or more of the following: thrombocytopenia, proteinuria, liver function impairment, renal insufficiency, cerebral or pulmonary edema, visual disturbances. Severe preeclampsia usually occurs before the 20th week of gestation and with systolic blood pressure ≥160 mmHg [2, 3]. The syndrome is classified as early or late in accordance with gestational age at diagnosis or delivery. Early PE is associated with more complications, such as multiple organ involvement and placental vascular lesion due to hypoperfusion [4]. It was proposed that an imbalance between angiogenic and antiangiogenic factors has an important role in the pathophysiology of PE [4]. Alterations on the concentrations of angiogenic factors, placental growth factor (PIGF), soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), known as fms-like tyrosine kinase -1 (sflt-1) in maternal circulation may precede preeclampsia [5, 6]. Despite extensive research, the exact cause of preeclampsia is still unknown [7, 8]. It was hypothesized that dysfunctional endothelium contributes to the pathogenesis of PE. Changes in the production of vascular endothelial growth factor (VEGF), a biomarker of endothelial dysfunction, are associated with this disease whether the production increases, decreases, or remains at a normal level. It was believed that PE is associated with changes in maternal VEGF plasma concentrations and other growth factors. Vascular endothelial growth factor was thought to be implicated in the pathogenesis of PE due to its role in the physiology of vasculogenesis and vascular permeability [10]. VEGF increased the production of nitric oxide, which was believed to be...
a strong vasodilator in normal gestation [11]. Sufficient knowledge regarding the role of VEGF in PE is important in order to design a paradigm for a screening strategy targeting the early detection and treatment of this disease. Many studies try to investigate the possible role of these angiogenic factors in the pathogenesis of PE. However, results remain elusive and conflicting [6, 12–19]. This study investigated the associations between VEGF and preeclampsia and its importance as a predictor of disease severity.

### MATERIAL AND METHODS

This case-control study was performed in the Al-Zahraa teaching hospital for maternity and pediatrics and included 50 patients with preeclampsia and 50 normotensive pregnant women as the control group. Data was collected on the following variables: maternal age, parity, blood pressure, urinary protein, complete blood count, renal function test and liver function test. Preeclampsia was diagnosed as follows: systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg with proteinuria (2-4++) on dipstick test for patients at more than 20 weeks of gestation, provided that the patient was previously normotensive. Diabetic patients, patients with chronic renal diseases, and chronic hypertensive women were excluded from this study. Venous blood was aspirated from each patient, and VEGF levels were measured from sera using human VEGF (NBPI-91272) by ELISA (enzyme-linked immunoassay method). Statistical analysis was performed using the analysis of variance (ANOVA) test at ≤0.05 significance level. All statistical procedures were done by SPSS version 23 and Microsoft Excel 2013.
RESULTS

The research included 50 pregnant women with preeclampsia with an age range of 19–30 years for all groups, as well as 50 normotensive pregnant women as a control group. Laboratory results of each group are shown in Table 1.

Fourteen patients with a mean age of 25.78±2.90 years had mild PE, while 36 patients with a mean age of 23.94±2.86 years had severe PE. There were 50 pregnant normotensive women with a mean age of 23.94±2.86 years in the control group (Table 1). The mean VEGF for patients with mild PE was 29.41±18.97 pg/ml; for those with severe PE, it was 36.19±36.89 pg/ml, and for normotensive women, it was 92.10±154.71 pg/ml. There were significant differences in the VEGF level between the studied groups (P=0.024), as presented in Table 2 and Figure 1.

The serum level of VEGF was significantly higher (92.10±154.71 pg/ml) in normotensive pregnant women (control group) than in patients with PE (33.76±35.29 pg/ml) (P=0.011) (Table 3 and Figure 2).

There were no significant differences in VEGF serum levels between patients with severe PE and those with mild PE (P-value=0.519) (Table 4).

DISCUSSION

The possible contribution of VEGF in the pathophysiology of preeclampsia has been extensively studied; however, the results of previous studies are conflicting [9, 10, 20]. This study showed that the serum level of VEGF was significantly reduced in patients with preeclampsia compared with normotensive pregnant women (p<0.05). The results of our study were supported by the results of other studies [21–25]. The reduction of serum VEGF in preeclampsia could be caused by high levels of sflt-1 (soluble fms-like tyrosine kinase-1). Sflt-1 binds and inhibits several proangiogenic proteins such as placental growth factor (PIGF) and VEGF. This inhibition occurs by preventing the communication of these angiogenic factors with their receptors [26]. The physiologic action of VEGF is mediated by binding into two high-affinity tyrosine kinase receptors. Vascular endothelial cells selectively expressed these receptors [9, 20–23]. Soluble flt-1, a splice variant of flt-1, represents a potent antagonist. This antagonistic effect is mediated by binding to VEGF and inhibiting its biological activity [26]. A high level of soluble flt-1 was secreted in severe preeclampsia, which neutralized the effect of PIGF and VEGF [27]. A marked reduction of VEGF in preeclampsia denotes severe endothelial dysfunction and dysregulation, manifested as severe hypertension and proteinuria. A high level of flt-1 was associated with the level of VEGF and severe endothelial dysfunctions [28], leading to severe clinical features. In our study, we did not estimate serum flt-1 simultaneously with VEGF.

However, there was good evidence from other studies that demonstrated an increased level of flt-1 in preeclampsia, which led to decreased circulating free VEGF [28]. High levels of flt-1 are associated with glomerular endotheliosis, proteinuria, and hypertension in non-pregnant and pregnant rats, which are important features of preeclampsia [29]. This evidence suggests that low levels of VEGF lead to endothelial dysfunction, which

Table 3. Serum VEGF in patients with PE and normotensive pregnant women.

| Groups              | Number of patients | Mean VEGF (pg/ml)±SD |
|---------------------|--------------------|----------------------|
| Preeclampsia        | 50                 | 33.76±35.298         |
| Control normotensive| 50                 | 92.10±154.715        |

* = P-value=0.011.

Table 4. Serum VEGF in patients with severe and mild PE.

| Groups               | Number of patients | Mean VEGF (pg/ml)±SD |
|----------------------|--------------------|----------------------|
| Mild preeclampsia    | 14                 | 29.41±18.976         |
| Severe preeclampsia  | 36                 | 36.19±36.890         |

* = P-value=0.519.

Figure 2. VEGF in patients with PE and normotensive women.
Circulating angiogenic factors

Plasma soluble fms-like tyrosine kinase-1 (sFlt-1) may contribute to endothelial dysfunction, and in normotensive pregnant women could be attributed to the detection method and the date of VEGF measurement with respect to gestational age. Restoration of endothelial function may be achieved by administering exogenous VEGF [10]. The use of pro-angiogenic analogue might be beneficial in the management of preeclampsia by improving the function of the endothelium, while the use of nicotine may be beneficial in the management of preeclampsia as it improves angiogenesis, yet it is not advisable as it is associated with intrauterine growth retardation [32]. Meanwhile, a lower incidence of preeclampsia was shown in smoker women due to decreased serum levels of sFlt-1 [33].

CONCLUSION

This study showed that VEGF levels were significantly reduced in patients with preeclampsia compared with normotensive pregnant women, suggesting marked endothelial dysfunction. This led to widespread vasoconstriction, causing hypertension and proteinuria. However, serum VEGF was reduced in both mild and severe preeclampsia, suggesting the importance of VEGF in the pathogenesis of preeclampsia as a cause of endothelial dysfunction. Nonetheless, clinical evidence of severity may not reflect the true extent of endothelial damage and dysfunction.

ACKNOWLEDGMENTS

Conflicts of interest

The authors declare no conflict of interest.

Ethics approval

This study was approved by the local Ethics Committee of the University of Kufa, Faculty of Medicine (EC:169 in 15.10.2019).

Consent to participate

Written informed consent was obtained from the participants.

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Authorship

A.Sh, HAS-H, and BA-G contributed to the conception and design of the study. HAS-H and BA-G offered administrative support. SH, HAS-H and MK provided study materials and included patients in the study. BA-G, HAS-H and NRH contributed to data analysis and interpretation. All authors contributed to writing the manuscript and approving the final version.

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