Role Of Soluble Endoglin In The Diagnosis Of Preeclampsia Severity In Iraqi Women

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ABSTRACT

Background: Preeclampsia is a multiorgan disorder characterised by an imbalance in angiogenic factors, including soluble endoglin (sEng). Till now, the relationship between serum levels of sEng with the severity of preeclampsia is not fully elucidated. The study aimed to clarify the precision of serum soluble endoglin (sEng) in the diagnosis of preeclampsia.

Materials and Methods: A case–control study was conducted in the Department of Obstetrics and Gynaecology and clinical biochemistry department, Al-Yarmouk Teaching Hospital, college of medicine/ Mustansiriyah university, Baghdad-Iraq for six months duration. A total of 180 subjects were enrolled. Among them, 40 subjects of non-severe preeclampsia patients (Group 1), 40 of severe preeclampsia (Group 2), and 50 healthy pregnant normotensive women served as controls. Levels were estimated by enzyme-linked immunosorbent assay technique (EUSA) in both cases and controls.

Results: There are no significant differences in age among the three groups with a mean age of controls (27.62 ± 4.76), non-severe PE (27.72 ± 5.99), and severe PE (27.90 ± 5.34) years with (p > 0.05). Similarly, no significant differences between non-severe PE and severe PE groups regarding gestational age when compared to the control group (p = 0.691). Mean level of sEng was the highest in severe preeclampsia group (4.04 ± 2.60 ng/ml) as compared to non-severe group (1.63 ± 0.41) and in controls (0.11 ± 0.28) with p-value (p < 0.001). The results showed that the area-under-the-curve (AUC) of serum soluble Endoglin in cases compared with control was (1.00) with a confidence interval (99% CI). The sensitivity and specificity for sEng in PE groups were (100%). On the other hand, compared with the control group, PE groups showed significantly higher creatinine, blood urea, uric acid, ALT, and AST (p < 0.001). Platelet, hemoglobin, PCV, PT, and PTT showed a significant decrease when compared to control at p-value (p < 0.001) for the first three, (p = 0.001) for PT and low significant for the last (p = 0.03). sEng levels were moderately correlated with platelets (r = 0.312), (p<0.05) in non-severe preeclampsia also, it correlated moderately but negatively with ALT (r = −0.324) and AST (r = −0.363), (p<0.05) in severe group. Soluble Endoglin levels were strongly correlated with systolic and diastolic blood pressure with a correlation coefficient in non-severe cases with systolic BP was (r = 0.471), diastolic BP (r = 0.313) while in severe cases of PE, for systolic BP was (r= 0.773) and diastolic BP (r= 0.632) and it was statistically significant (p < 0.001).

In conclusion, soluble endoglin (sEng) can be considered as a novel biomarker for the diagnosis and prediction of severity of preeclampsia.

KEYWORDS: Soluble Endoglin, diagnosis, prediction, pre eclampsia.

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INTRODUCTION

Preeclampsia (PE) is a pregnancy-specific disease. It is described by new-onset abnormal high blood pressure and proteinuria after 20 weeks of gestation which is associated with placental hypo-perfusion. Preeclampsia is one of the major causes of restricted fetal growth, and in severe cases, dysfunction can advance into multiorgan maternal affection or even mortality of both the mother and her fetus [1]. Approximately 5–10% of pregnancies worldwide are influenced by preeclampsia [2]. Globally the disease occurs in 2–8% of pregnancies which accounting for approximately 16% of maternal mortality in developing countries [3]. The accurate pathogenesis of preeclampsia remains "controversial", while any damage in coagulation-fibrinolysis systems [4] and abnormal stimulation for immune responses and inflammation are often mentioned [5]. There are different mechanisms which may participate in the dysfunction of endothelial cells and hypoxia with poor perfusion of the placenta. Consequently, this leads to an imbalance of angiogenic factors, a state of excessive oxidative stress, the release of antiangiogenic proteins, and other inflammatory mediators [6]. Measurements of pro-and anti-angiogenic factors in the serum of preeclampsia patients mainly [placental growth factor PIGF], [soluble vascular endothelial growth factor receptor-1, sFlt-1] and [soluble fms-like tyrosine kinase-1] is crucial because of the high prevalence of preeclampsia and seriousness. Additionally, these markers are tested as a diagnostic as well as study their ability for predicting the development of PE severity [7,8].

The regulation of placental vasculature is by the essential role of angiogenesis factors and their cellular receptors. In this process, successful placentation is achieved through the regulation between anti-angiogenic and pro-angiogenic influences. Therefore, an imbalance in this equilibrium leads to loss of functional organization between vasodilator and vasoactive substances with the possible subsequent resultant preeclampsia disease [9].

Soluble endoglin (sEng) or (CD105) is an antiangiogenic factor. It is a homodimeric transmembrane glycoprotein with a molecular weight of 180 KD located on the "vascular endothelial" cell surface. sEng is a co-receptor for transforming growth factor (TGF-β1 and β3) and is highly expressed on cell membranes of vascular endothelium [10]. Soluble Endoglin has an antiangiogenic effect in preeclampsia by binding to TGF-β1 in maternal circulation, then preventing the signaling of TGF-β1 in endothelial cells,
in other words, the proangiogenic and vasodilators affect the normal endothelium [11]. There are lots of researches on the role of sFlt-1 and PlGF in preeclampsia; but, there is a lack of data on the relationship between serum level of sEng and PE cases. In our study, we evaluated serum sEng levels in preeclampsia patients to investigate its role in identifying those patients who are likely to develop a more severe condition so that to interfere before developing complications and to optimize the disease outcome.

MATERIAL AND METHODS

Study design
This is a prospective case-control study. The study protocol was approved by the scientific and ethical committees of the college of medicine/Mustansiriyah University. It was carried out on (130) pregnant ladies, who had been divided to (80) preeclampsia groups, (40) severe PE versus (40) non-severe PE and (50) normotensive women, from November 2019 to May 2020. Participants were collected from AL-Yarmouk Teaching Hospital and Baghdad Teaching Hospital, and all participating subjects signed informed consents. We studied singleton pregnancies diagnosed with preeclampsia who were admitted to the hospital or attended the outpatient clinic. They were at age of ≤ 40 years and gestational age of ≥ 32 weeks and full filled the diagnostic criteria for severe and non-severe PE with a matched control group concerning the age and gestational age.

Diagnostic criteria
A diagnostic criterion for preeclampsia was as follows:
(a)Types of preeclampsia defined as non-severe by systolic or diastolic blood pressure ≥140 or ≥90 mm Hg, respectively, measured twice at least 6 hours apart or severe preeclampsia as systolic or diastolic blood pressure ≥160 or ≥110 mm Hg respectively in one measurement, (b) with protein in urine dipstick ≥ +2 in both groups; additional feature for the severe type: persistent headache or other brain or visual problems; fetal growth restriction or death, and maternal end organ affection [3].

Sample collection
Serum specimens: All patients were given 5mL of venous blood on an empty stomach in the morning before the treatment, the blood was required to coagulate at 37 x C for 10 to 15 minutes, and the serum was aspirated after 4000 rpm/min in a centrifuge for 10 to 15 minutes and stored the serum in a refrigerator at −80°C.

METHODS

BP was checked by mercury sphygmomanometer for pregnant women in sitting position for the right arm, protein in urine analysis was by Sulfosalicylic acid test, and Quantitative Measurement Serum sEng concentrations were calculated in duplicate by enzyme-linked immunosorbent assay (ELISA).

Statistically analysis
The statistical analysis was performed using the SPSS version 26.0 software, whereas the continuous variable is the mean ± standard deviation. The ANOVA test was used to compare groups and group variations between study groups. The receiver operator characteristic (ROC), was done to analyze the optimum cut-off value for the assessment of diagnostic accuracy.

RESULTS

The mean age of the controls was (27.62±4.76 years), (27.72±5.99 years) in non-severe preeclampsia, and (27.90±5.34 years) in severe preeclampsia [Table 1]. No statistically significant difference was observed in the mean age of patients among different groups at p-value (p=0.9697) as well as no significant differences between the non-severe PE and severe PE groups regarding gestational age when compared to the control group (p = 0.691).

Soluble Endoglin (sEng) was shown to be increased with the progression of the disease. The mean value of sEng in severe preeclampsia patients (4.04 ± 2.60 ng/ml) which was significantly higher (P<0.001) than the mean value for non-severe preeclampsia patients and control group (1.63 ± 0.41 ng/ml) and (0.11 ± 0.28 ng/ml) respectively with p-values greater than (0.001) for both groups [Table 2]. ALT, AST, blood urea, creatinine, and uric acid had showed significant differences (p<0.001) between control and PE cases. Highly significant differences were apparent for platelet, HB, PCV (p<0.001), PT (0.001), and PTT (0.03) among the control and PE groups.

Soluble Endoglin levels were correlated with systolic and diastolic blood pressure. In non-severe cases, the correlation was moderate with a correlation coefficient for the systolic BP (r = 0.471), DBP (r = 0.313) while in severe cases of PE for the systolic BP was (r = 0.773) and DBP (r = 0.632) and it was statistically significant (P < 0.001). It is a well-known fact that the severity of preeclampsia is correlated to the increase in BP [Table 3].

Moderate correlation of sEng with platelets (p<0.05) with correlation coefficient (r = 0.312) in non-severe PE [Figure 1]. Negative correlation with ALT (r = - 0.324) and AST (r = - 0.363), (p<0.05) in severe group and a low negative correlation for uric acid in non-sever PE (r = - 0.23) while in severe PE (r = - 0.08) [Table 3]. The ROC-curves analysis for serum soluble Endoglin (sEng) levels in severe and non-severe preeclampsia with controls when used as a diagnosis biomarkers. The results showed that the area-under-the-curve in severe and non-severe PE patients compared with control was (AUC = 1) with a confidence interval (95% CI) and lower bound (1) and upper bound (1). The sensitivity and specificity for soluble Endoglin cutoff value in non-severe 0.81ng/ml and severe groups 1.06ng/ml were (100%). Positive predictive value (PPV) and negative predictive value (NPV) were (1.00) for both groups [Figure 2].

DISCUSSION

Preeclampsia is a complication of pregnancy that can lead to severe or even fatal complications for both the mother and her fetus. Although lots of researchers have described the role of several biochemical parameters for the diagnosis of preeclampsia, yet to date, no marker has been proved highly effective for the disease diagnosis and severity prediction. Consequently, this study was conducted to assess sEng diagnostic value as a biomarker of preeclampsia as well as to assess its correlation with the severity of the disease. In this study, no significant differences among the three groups were seen regarding age as they were matched groups to minimize the influence of the age difference on our results. These results were in agreement with other studies on preeclampsia patients that showed no significant differences between groups [12] while were not compatible with the study done by Zhongliang et al [13]. Similarly no significant differences in gestational age were found between preeclampsia cases and pregnant controls. Our observations were nearly identical to the study carried by Jural et al [14]. Significant elevation in the ALT and AST (p-value < 0.001) in both severe and non-severe preeclampsia patients compared with normal pregnant women. Other studies agreed with this study focused on the impact of liver function abnormalities mainly aminotransferase elevations in PE more than the normal [15]. On the contrary, a group of researchers proved a non-significant difference in alanine transaminase...
and aspartate transaminase in preeclampsia patients versus the control group [16]. In this study, we analyzed blood urea and creatinine and the results showed highly significant differences in PE groups as compared with the control group for urea. Our results were consistent with Hazari et al study [17]. No significant difference in the serum level of creatinine in all groups (p=0.05), these results are supported by another study [18]. One of the studies was in disagreement with our results when compare levels of creatinine (p<0.05) and blood urea (p<0.05) [19].

A highly significant difference has appeared in serum level of uric acid between preeclampsia groups and control group (p<0.001). Similarly, several studies are in agreement with our study done by a group of researchers [20, 21]. Several experiments have shown that oxidative stress triggered by hyperuricemia affects various systems in the body, including the kidneys. Pathologically, oxidative-stress-related to hyperuricemia leads to DNA damage, enzyme oxidation and inhibition, inflammatory generation of cytokines, as well as cell apoptosis. As well as, induces endothelial dysfunction through different mechanisms [22].

In this study, platelet showed significant differences in PE groups and the control group. Many studies are consistent with this study [23] while others have demonstrated no significant differences [24]. Another study suggested that the alterations in platelet count had an important role in inflammation [25]. Some studies point to the reasons behind the differences in results. One of these studies talked about the role of some vitamins for example folic acid and B12 during pregnancy that may improve the situation of platelet count [26]. Coagulation factor-like PT and PTT as well as PCV and HB were significantly different (p<0.05) that agreed with other studies [27, 28]. Some studies have contradicted the results of this study [29].

The current study has shown that the levels of serum Endoglin were strongly correlated with systolic and diastolic blood pressure in both preeclampsia cases with a p-value which is significantly high (p<0.001). Many studies were in agreement with our results [30, 31].

The present study included a large number of preeclamptic women demonstrating that serum sEng levels were significantly higher in patients with severe preeclampsia as compared with those in non-sever and normal pregnancy. Furthermore, we found that differences in serum levels of sEng were more pronounced as the severity of preeclampsia increased, suggesting that changes in levels of sEng can effectively reflect the extent and intensity of damage to the systemic vascular endothelium. In other words, the severity of the disease is strongly correlated with the high concentrations of sEng. Our results are consistent with previous reports which provided further support to the idea that alterations of this particular antiangiogenic factor not only related to preeclampsia but also with the abnormality of these biomarkers which become more pronounced as the severity of the disease increases [13].

In preeclampsia, dysfunction of maternal endothelial may cause a rise in the level of placenta-derived soluble VEGF receptor 1 (sVEGFR1 or sflt1) which is emerging as a substantial component in the pathogenesis of the disease. Soluble Endoglin (sEng), soluble TGF co-receptor, is a novel placenta-derived. It’s elevated in the sera of preeclampsia patients and strongly correlated with the severity of disease [32]. Jerome Groopman proposed that preeclampsia represents an inability of the fatal-maternal vascular interface to "remodel" and release some molecules from the placenta that injury the maternal endothelium. Therefore, excess in these molecules produced by the placenta like soluble FLT, a vascular endothelial growth factor (VEGF) receptor, causes maternal endothelial damage with low levels of VEGF. Soluble Endoglin, a placental derived TGF-co-receptor, is block endothelial repair if its concentration is elevated. Effects of FLT and sEng together may be responsible for the pathogenesis of preeclampsia [32]. Additionally to this, angiogenic and vasodilator molecule (TGF-β1), which is responsible for preserving the "lining of blood vessels" healthy as well as angiogenesis, is antagonized by the action of sEng. Because of this antagonistic effect cell lining of the blood vessels begin to be ill and die, which leads to a high level of proteinuria also elevated blood pressure [33].

In conclusion, soluble endoglin (sEng) can be regarded as a novel biomarker for the diagnosis of the severity of preeclampsia and more studies are needed with a large size of patients and multi-centers.

### Table 1: Comparison of Age and gestational age among the groups of the study.

<table>
<thead>
<tr>
<th>groups</th>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>Mean ± SD</th>
<th>P-value</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>controls</td>
<td>50</td>
<td>20</td>
<td>38</td>
<td>27</td>
<td>27.62 ± 4.76</td>
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<td>non sever PE</td>
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<td>16</td>
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<td>28</td>
<td>27.72 ± 5.99</td>
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<tr>
<td>severe PE</td>
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<td>17</td>
<td>39</td>
<td>28</td>
<td>27.90 ± 5.34</td>
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<tr>
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<td>All</td>
<td>130</td>
<td>32</td>
<td>39</td>
<td>37</td>
<td>36.515 ± 1.648</td>
<td>&lt;0.001*</td>
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<tr>
<td>controls</td>
<td>50</td>
<td>36</td>
<td>39</td>
<td>38</td>
<td>37.58 ± 0.785</td>
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<td>non sever PE</td>
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<td>39</td>
<td>37</td>
<td>36.45 ± 1.535</td>
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<tr>
<td>severe PE</td>
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<td>32</td>
<td>38</td>
<td>36</td>
<td>35.25 ± 1.66</td>
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### Table 2: Comparison of serum sEndoglin in the study groups.

<table>
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<th>Mean ± SD</th>
<th>Median ± SEM</th>
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</thead>
<tbody>
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<td>sEndoglin (ng/ml)</td>
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<td>0.28 ± 0.11</td>
<td>0.29 ± 0.02</td>
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<tr>
<td></td>
<td>non sever</td>
<td>40</td>
<td>1.63 ± 0.46</td>
<td>1.60 ± 0.07</td>
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<tr>
<td></td>
<td>severe</td>
<td>40</td>
<td>4.04 ± 2.60</td>
<td>2.91 ± 0.41</td>
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</table>

### Table 3: Correlations of soluble Endoglin with biological and biochemical parameters in preeclampsia patients

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<tr>
<th>Correlations</th>
<th>Systolic Bp.</th>
<th>Diastolic Bp.</th>
<th>Platelets</th>
<th>ALT</th>
<th>AST</th>
<th>Uric Acid</th>
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<table>
<thead>
<tr>
<th>Endoglin Concentration</th>
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</tr>
<tr>
<td></td>
<td>r</td>
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<td>.312*</td>
<td>.207</td>
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<tr>
<td></td>
<td>p</td>
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<td>&lt;.001</td>
<td>.050</td>
<td>.199</td>
<td>.680</td>
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<td>r</td>
<td>.773*</td>
<td>.632*</td>
<td>.187</td>
<td>-.324*</td>
<td>-.363*</td>
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</tr>
<tr>
<td></td>
<td>p</td>
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<td>0.001</td>
<td>.248</td>
<td>.042</td>
<td>.021</td>
<td>0.68</td>
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Figure 1: Scatter plot showing the correlation between platelet count and Endoglin concentration in PE patients. The regression line, correlation coefficient, and p-value were added to assess the interpretations.

Figure 2: Receiver operator characteristic curves of sEndoglin for non-sever and sever PE patients groups as discriminating them from control.

REFERENCES
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