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# Endostatin and Cystatin C as Potential Biomarkers for Early Prediction of Preeclampsia

Fatima Alshannag, Radwa M. M. Zaki, Eman Hemida, Mustafa M. M. ElBakry, and Amani F. H. Noureldeen*

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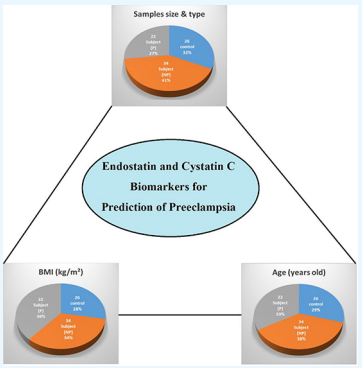
**ABSTRACT:** Preeclampsia (PE) is characterized by new onset hypertension and proteinuria. Undoubtedly, some individuals do not fit precisely into this description, and it could be challenging to spot newly developed PE in females who already have hypertension or renal illness. Monitoring the disease's progression enables the optimization of delivery time while minimizing premature births. The current study explores the diagnostic benefits of serum endostatin and cystatin C in addition to serum and urinary magnesium (Mg) and fractional excretion magnesium (FEMg) for early prediction of PE. The population sample included 82 pregnant women divided into 3 groups: normal pregnancy group served as a control ($n$ = 26), nonpreeclampsia (NPE, $n$ = 34) group included pregnant women with one or more risk factors but did not progress to PE, and pregnant women who developed preeclampsia (PE, $n$ = 22) group. Blood samples were withdrawn at two sampling times: at 12th to 16th and 24th to 26th weeks of gestation. Compared to normal pregnancy, results ($\bar{X} \pm$ SD) indicated a significant increase in serum endostatin in NPE at the first sample (10.78 ± 3.63 ng/mL) and the second sample (28.03 ± 3.79 ng/mL), while cystatin C was at the first sample (0.68 ± 0.06 mg/dL) and the second sample (0.71 ± 0.07 mg/dL). In the PE group, the serum endostatin was 18.86 ± 4.37 ng/mL at the first sampling time and 53.56 ± 9.76 ng/mL for the second sample. Serum cystatin C was also elevated in PE with $\bar{X} \pm$ SD equivalent to 0.73 ± 0.08 and 0.89 ± 0.08 mg/dL at the first and second samples, respectively. On the other hand, serum and urinary Mg in addition to FEMg levels did not significantly differ across the groups under study. Receiver operating characteristic (ROC) curve analysis proved that both endostatin and cystatin C could be good indicators for PE. The findings imply that measuring endostatin and cystatin C at early pregnancy and before progression to PE may be effective in detecting the likelihood of PE. Endostatin could be more precise and sensitive in assessing the probability of PE than cystatin C; however, coupling of the two parameters may be promising.




## 1. INTRODUCTION

Preeclampsia (PE) is a systemic syndrome characterized by new onset hypertension (systolic and diastolic blood pressure of ≥140 and 90 mmHg, respectively, on two occasions, at least 6 h apart) and proteinuria (protein excretion of ≥300 mg in a 24 h urine collection or a dipstick of ≥2+) that develop after 20 weeks of gestation in previously normotensive women.[1] Undoubtedly, some individuals do not fit precisely into this description, and it could be challenging to spot newly developed PE in females who already have hypertension and/or renal illness.[2] In addition, PE is one of the leading causes of maternal-fetal death and morbidity globally. It affects 3−5% of all pregnant women and disrupts about 10% of pregnancies in underdeveloped nations where there is nonexistent or insufficient emergency treatment.[3] In Egypt, it disrupts about 6−8% of all pregnancies, and in referral facilities like university hospitals, it might reach 15% of all pregnancies.[4] Placental dysfunction has been connected to the progression of PE, which manifests itself in two stages: aberrant placentation during the first trimester, followed by maternal syndrome in the latter second and third trimesters marked by an overabundance of antiangiogenic elements.[5,6] Accurate PE prediction is critical since it allows for early detection and management of those at risk.

Endostatin, a 20 kDa C-terminal fragment of collagen XVIII, is a powerful endothelial cell-specific antagonist for angiogenesis that selectively impacts endothelial cell apoptosis, growth, and *in vitro* migration.[7,8] Endothelial cell dysfunction is reflected in high plasma endostatin amounts in a wide range of illnesses.[9] The ischemia of the placenta is thought to contribute to the dysfunction of endothelial cells by changing the equilibrium of circulating amounts of antiangiogenic and angiogenic growth factors.[10] Endostatin is also involved in the

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pathophysiology of heart disease and chronic kidney disease.[11] On the other hand, cystatin C is an endogenous low molecular weight protein that is created at a steady rate by all nucleated cells. It is readily filtered in the kidneys and digested by the proximal tubular cells.[12,13] The serum level of cystatin C is thought to have higher diagnostic accuracy for PE than serum urate and creatinine.[14] The level of cystatin C in serum may play an important role as a marker of PE, especially when combined with the uric acid level.[12] Serum cystatin C is recommended as a prediction model for the diagnosis of PE in the third trimester of pregnancy.[15] Moreover, high blood pressure may be related to the low Mg levels in serum, which may lead to endothelial dysfunction.[16,17] The normal drop in Mg level during pregnancy is linked to endothelial dysfunction, which might be a risk factor for PE.[18] Thus, an expectant woman having an ionized Mg fraction of less than 24.67% has a higher risk of PE during the second trimester.[19] In nondiabetic chronic kidney disease, increased fractional excretion of Mg (FEMg) is a noninvasive indicator of renal function. The strong relationship between kidney performance indicators and FEMg reflects the importance of these markers in clinical practice.[20]

Monitoring PE markers in pregnant women can enable the optimization of delivery time while minimizing premature births.[21] Our interest in investigating cases of complicated pregnancy[22−25] has prompted us to explore some potential bioactive markers to predict the preeclampsia disease before its development during pregnancy. The current study intended to assess the diagnostic effectiveness of serum endostatin, cystatin C, and magnesium fractions as indicators for the early diagnosis of preeclampsia in expectant women.

## 2. RESULTS AND DISCUSSION

**2.1. Characteristics of Pregnant Women in the Included Groups.** Preeclampsia (PE) is one of the main causes of worldwide maternal death and morbidity. It presents as a wide-ranging sickness, with detrimental effects on expecting women and their unborn children. PE can develop into eclampsia, a convulsive illness if it is not properly diagnosed.[1,26] Early diagnosis of PE is critical for reducing risk factors and improving accuracy in predicting pregnancy development. The current study examined several biochemical markers, including endostatin, cystatin C, and magnesium (Mg) for early prediction of PE. It included three groups of expecting women: (1) normotensive pregnant women, (2) pregnant women with one or more risk factors without progressing to preeclampsia (NPE), and (3) women with risk factors who progressed to preeclampsia later during pregnancy (PE). Two blood samples were withdrawn from each woman, one at the 12th to 16th weeks and the second sampling taken during the 24th to 28th weeks of gestation. The groups were matched for perinatal ages and parity. The expecting women with difficulties were found to have higher average scores for maternal age and BMI values relative to normotensive pregnant women. It was found that the mean values of maternal age and BMI showed significant differences between normal pregnancy, women with risk factors (NPE), and preeclampsia (PE) groups, with elevated BMI mean values for NPE and PE compared to the normal control group. No statistical differences were found between normal pregnancy, NPE, and PE groups regarding gestational age, at both sampling times, and parity (Table 1).

**Table 1. Differences between Normal, NPE, and PE Groups Regarding Initial Characteristics[a]**

| group parameter | normal pregnancy ($n$ = 26) | NPE ($n$ = 34) | PE ($n$ = 22) | $P$ value |
|---|---|---|---|---|
| maternal age (years) | 25.2 ± 3.86 | 32.2 ± 6.36 | 28.3 ± 5.88 | 0.001 |
| $P$* | | 0.001 | NS | |
| $P$** | | | 0.001 | |
| gestational age at 2nd trimester (weeks) | 14.0 ± 1.35 | 13.9 ± 1.50 | 13.6 ± 1.33 | NS |
| gestational age at 3rd trimester (weeks) | 25.5 ± 1.51 | 26.1 ± 1.59 | 26.0 ± 1.47 | NS |
| BMI (kg/m$^2$) | 23.7 ± 3.72 | 28.6 ± 5.82 | 32.7 ± 7.41 | 0.001 |
| $P$* | | 0.001 | 0.001 | |
| $P$** | | | 0.01 | |
| parity (median & IQR) | 1 (1−2) | 2 (1−3) | 2 (1−3) | NS |

[a]BMI: body mass index; $P$*: $P$ value compared to the normal pregnancy group; $P$**: $P$ value compared to the NPE group; NS: nonsignificant, $P$ value >0.05.

**2.2. Serum Glucose, Liver, and Kidney Functions in the Included Groups.** The typical clinical care of PE involves liver and renal performance tests. The current investigation involved measuring serum AST and ALT activities along with levels of serum albumin in the recruited subjects for the samples of the two periods, as indicators for liver function. At the second trimester, the study indicated significant differences between normal pregnancy, NPE, and PE groups for the mean values of serum insulin, ALT activity, and albumin level, with elevated serum insulin and decreased mean value of albumin in the PE group compared to other groups. Other tested biochemical parameters were comparable between the studied groups (Table 2). At the third trimester, all of the tested biochemical parameters were not significantly different among the three groups (Table 3). There was no prominent difference in serum AST activity between the investigated groups. On the other hand, compared to conventional pregnancy, considerable decreases in serum ALT activity and serum albumin concentration in the PE cluster were indicated at the first sampling phase. Notably, the average scores of both markers (AST and ALT) in the two groups were matched at 24th to 28th weeks. Although variances in the biomarkers of liver activity between the groups were observed, the values were within the normal ranges, suggesting acceptable hepatic performance in PE.[27−29] Furthermore, no significant variations were observed in the ALT activities or serum albumin levels in the normotensive and PE prenatal women during or beyond the 20 weeks of gestation.[30] Reduced serum albumin levels in the PE patients were also detected and were ascribed to nonspecific factors such as inflammations, which might be a result of the syndrome rather than a cause.[18] Earlier studies reported substantial relationships involving ALT, AST, and LDH activities, destructive consequences in PE, and increased levels of liver enzymes in both moderate and severe PE.[31−33]

Considering that hypoxia causes necrosis, which leads to hepatocyte degradation, it was previously hypothesized that higher transaminases in PE subjects may be caused by the PE's hypotoxic impact on the liver.[34] Various studies noted increased transaminase activity in PE that may be a result of placenta ischemia together with the periodic inflammatory


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Table 2. Mean Value ± SD of Serum Glucose and Related Markers and Liver and Kidney Function Parameters in Normal, NPE, and PE Pregnant Women at 12−16 Week Gestation[a]

| group parameter | normal pregnancy ($n$ = 26) | NPE ($n$ = 34) | PE ($n$ = 22) | $P$ value |
|---|---|---|---|---|
| serum glucose (mg/dL) | 74.62 ± 9.46 | 80.97 ± 28.5 | 75.24 ± 13.58 | NS |
| serum insulin ($\mu$LU/mL) | 15.02 ± 6.14 | 17.10 ± 12.67 | 25.32 ± 23.88 | 0.001 |
| $P$* | | 0.05 | NS | |
| $P$** | | | NS | |
| HOMA-IR | 2.83 ± 1.34 | 3.8 ± 3.8 | 4.97 ± 5.4 | NS |
| serum ALT (U/L) | 16.54 ± 5.38 | 15.27 ± 4.40 | 12.66 ± 4.40 | 0.05 |
| $P$* | | NS | 0.05 | |
| $P$** | | | NS | |
| serum AST (U/L) | 17.00 ± 7.76 | 17.73 ± 7.51 | 17.32 ± 5.63 | NS |
| serum albumin (g/dL) | 4.09 ± 0.90 | 3.55 ± 0.65 | 3.90 ± 0.87 | 0.001 |
| $P$* | | 0.01 | NS | |
| $P$** | | | NS | |
| serum urea (mg/dL) | 56.09 ± 20.21 | 51.73 ± 10.54 | 56.49 ± 21.20 | NS |
| serum creatinine (mg/dL) | 0.78 ± 0.14 | 0.72 ± 0.14 | 0.69 ± 0.25 | NS |
| urinary creatinine (mg/dL) | 128.06 ± 62.80 | 98.44 ± 68.10 | 107.43 ± 69.56 | NS |
| serum uric acid (mg/dL) | 4.69 ± 0.76 | 4.48 ± 0.72 | 4.94 ± 0.84 | NS |

[a]HOMA-IR: homeostasis model assessment of insulin resistance; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Table 3. Mean Value ± SD of Serum Glucose and Related Parameters and Liver and Kidney Function Parameters in Normal, NPE, and PE Pregnant Women at 24th to 28th Week Gestation

| group parameter | normal pregnancy ($n$ = 26) | NPE ($n$ = 34) | PE ($n$ = 22) | $P$ value |
|---|---|---|---|---|
| serum glucose (mg/dL) | 68.25 ± 10.45 | 76.15 ± 19.85 | 75.2 ± 13.27 | NS |
| serum insulin ($\mu$LU/mL) | 35.31 ± 22.19 | 46.26 ± 36.33 | 72.64 ± 92.68 | NS |
| HOMA-IR | 6.29 ± 5.33 | 9.45 ± 8.66 | 14.13 ± 20.04 | NS |
| serum ALT (U/L) | 16.23 ± 5.25 | 14.56 ± 5.59 | 17.14 ± 9.02 | NS |
| serum AST (U/L) | 16.38 ± 5.37 | 15.47 ± 6.90 | 16.00 ± 8.59 | NS |
| serum albumin (g/dL) | 4.01 ± 1.00 | 3.49 ± 0.92 | 3.60 ± 0.63 | NS |
| serum urea (mg/dL) | 57.55 ± 7.30 | 57.63 ± 9.52 | 58.23 ± 10.03 | NS |
| serum creatinine (mg/dL) | 0.81 ± 0.15 | 0.85 ± 0.19 | 0.81 ± 0.22 | NS |
| urinary creatinine (mg/dL) | 97.35 ± 72.38 | 92.71 ± 52.89 | 120.15 ± 90.43 | NS |
| serum uric acid (mg/dL) | 4.61 ± 0.63 | 5.02 ± 1.23 | 4.83 ± 1.13 | NS |

responses.[35,36] As a result, there was endothelial dysfunction, which caused vasoconstriction and, ultimately, liver and kidney dysfunction. HELLP (Hemolysis, Elevated Liver enzymes and Low Platelets) is a hepatic dysfunction syndrome in PE. It is a perilous condition accompanied by indications of organ comorbidities, particularly kidney and brain hemolysis.[37,38] In the current study, serum urea, creatinine, and uric acid concentrations were evaluated in normal and complicated pregnancy. Predicted urine creatinine levels were also estimated to determine renal function. There were no apparent differences detected in these variables among the investigated groups. Consequently, the findings suggested that both PE and expectant women in danger of PE had normal renal functions.[29] Higher levels of serum uric acid were previously detected in PE relative to a control group.[36,39,40] In addition, elevated serum urea, creatinine, and uric acid levels in severe PE were reported.[41−43] The present finding is consistent with these previous observations as the included PE individuals did not have severe PE at either sampling period. It is worth mentioning that only 5 women in the risk category had advanced to PE at the second sampling, while the majority in the PE cohort had acquired PE eventually after 34 weeks of pregnancy. On the other hand, women with aberrant uric acid concentrations were found to be four times more chance prone to develop severe PE than women with standard uric acid levels, demonstrating a substantial relationship between the enormity of PE and amounts of serum uric acid.[44] Therefore, the levels of uric acid are claimed to be good indicators of the intensity of PE disease.[45] Uric acid, as a powerful inflammatory mediator, increases endothelial dysfunction, which stimulates hypertension, vascular illness, and renal disease. Before proceeding to PE, the liver and renal performance (at the 12−16 weeks of pregnancy) was assessed. PE was not established in all instances in the second sample. However, it is important to indicate that with the increase in gestational age, there was a subsequent increase in serum transaminases and serum uric acid levels.[36]

**2.3. Serum Endostatin-, Cystatin C-, and Mg-Related Parameters in the Included Groups.** Significant variations between normal, NPE, and PE groups for serum endostatin and cystatin C were detected at second and third trimester stages. However, urinary and serum Mg in addition to fractional excretion magnesium (FEMg) showed no significant differences between the groups. Notably, the mean values of both endostatin and cystatin C were significantly higher in complicated NPE and PE groups compared with the normotensive pregnancy, with the highest estimated values observed for the PE group (Tables 4 and 5). It is worth mentioning that in the complicated pregnancy, the mean values of both endostatin and cystatin C were elevated at the first sampling time and continued to raise further at the second sampling.



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**Table 4. Mean Value ± SD of Serum Endostatin-, Cystatin C-, and Mg-Related Parameters in Included Groups at 12−16 Week Gestation**

| group parameter | normal pregnancy ($n$ = 26) | NPE ($n$ = 34) | PE ($n$ = 22) | $P$ value |
|---|---|---|---|---|
| serum endostatin (ng/mL) | 5.34 ± 3.51 | 10.78 ± 3.63 | 18.86 ± 4.37 | 0.001 |
| $P$* | | 0.001 | 0.001 | |
| $P$** | | | 0.001 | |
| serum cystatin C (mg/dL) | 0.55 ± 0.06 | 0.68 ± 0.06 | 0.73 ± 0.08 | 0.001 |
| $P$* | | 0.001 | 0.001 | |
| $P$** | | | 0.01 | |
| serum Mg (mg/dL) | 2.09 ± 0.18 | 2.13 ± 0.22 | 2.12 ± 0.22 | NS |
| urinary Mg (mg/dL) | 2.93 ± 0.48 | 2.78 ± 0.40 | 3.16 ± 2.14 | NS |
| FEMg (%) | 1.47 ± 0.74 | 1.98 ± 0.94 | 1.59 ± 0.62 | NS |

**Table 5. Mean Value ± SD of Serum Endostatin-, Cystatin C-, and Mg-Related Parameters in NPE and PE Pregnancy at 24−28 Week Gestation**

| group parameter | normal pregnancy ($n$ = 26) | NPE ($n$ = 34) | PE ($n$ = 22) | $P$ value |
|---|---|---|---|---|
| serum endostatin (ng/mL) | 19.42 ± 4.32 | 28.03 ± 3.79 | 53.56 ± 9.76 | 0.001 |
| $P$* | | 0.001 | 0.001 | |
| $P$** | | | 0.001 | |
| serum cystatin C (mg/dL) | 0.68 ± 0.06 | 0.71 ± 0.07 | 0.89 ± 0.08 | 0.001 |
| $P$* | | NS | 0.001 | |
| $P$** | | | 0.01 | |
| serum Mg (mg/dL) | 1.95 ± 0.07 | 1.99 ± 0.12 | 1.99 ± 0.10 | NS |
| urinary Mg ( mg/dL) | 2.61 ± 0.37 | 2.72 ± 0.58 | 2.74 ± 0.44 | NS |
| FEMg (%) | 2.53 ± 1.90 | 2.27 ± 1.15 | 2.14 ± 1.83 | NS |

Most previous investigations were concentrated on measuring the amounts of circulating endostatin in PE-complicated pregnancy during the late trimester (around 34th to 37th weeks). However, few studies assessed the endostatin level just before the disease's development. This study evaluated the serum endostatin values at two different gestational ages, 12th to 16th weeks and 24th to 28th weeks. In comparison to normotensive women, the groups of women with eventual PE and those with just a risk of developing PE had substantially higher serum endostatin levels at all sampling periods. Likewise, serum endostatin levels in the PE category were significantly higher than those in the risk category. Endostatin levels continued to raise from 12th to 16th weeks to 24th to 28th weeks in individuals with PE and those at risk of PE.[46] Moreover, higher circulating endostatin concentrations in acute and moderate PE patients around the 34th to 37th weeks of pregnancy were observed relative to those in normal pregnant women at a similar gestational period.[28,47] Additionally, as compared to a normal or healthy pregnancy, it was observed that endostatin serum levels were higher in both moderate and severe PE, with a greater rise in severe than moderate PE.[48] The higher endostatin concentrations found in the serum of preeclamptic women may indicate that the condition is affecting the expression of this antiangiogenic factor.[47] It was previously hypothesized that the increased endostatin levels in pregnancies complicated by PE might be a defensive mechanism to guard the host against tumor progression as the host responds to trophoblast invasion as malignant.[48] Preeclamptic plasma may include a significant amount of elevated endostatin, which inhibits endothelial cell proliferation and may be a factor in the development of endothelial plaques. It could also harm some placental developmental processes including villi branching, angiogenesis, or trophoblastic division.[47] Elevated serum endostatin in PE may originate from various maternal routes. First is the ability of blood vessels or platelets to create pro- and antiangiogenic substances given the occurrence of certain conditions. The placenta is also a source since PE-related inflammation or hypoxia may cause secretion of the antagonist.[49]

The changes in serum vascular endothelial growth factor (VEGF) with endostatin, which have antagonistic effects when interacting with the KDR/Flk-1 receptor, may be considered a major factor in the disease. Endostatin inhibited the multiplication and movement of endothelial cells generated by VEGF by blocking the tyrosine phosphorylation of KDR/Flk-1 and the subsequent signaling processes.[50] Endostatin also prevents the synthesis of nitric oxide in response to VEGF, which prevents endothelial cell angiogenesis and mobility.[51] Moreover, endostatin binds with integrin on the surface of endothelial cells to inhibit cell linkages, which has an antimigratory impact.[52] Thus, during the second trimester, there may be inadequate stimulation of vascular development and endothelial modulation that causes placental illness and PE. This is shown by the reduced levels of unbound nitric oxide and VEGF and elevated levels of total sFlt-1 and VEGF.[53] As a result, endostatin may exacerbate the negative consequences of an unfavorable VEGF/antagonist equilibrium. Additionally, it can adhere to Flt-1 and prevent VEGF and Flt-1 from interacting.[48] Through the suppression of cyclin D1 and Wnt signaling, respectively, endostatin causes apoptosis and decreases the growth and mobility of endothelial cells.[54]

Serum cystatin C levels (Tables 4 and 5) were determined at two gestational intervals in conventional pregnancy, in pregnancies with PE risk but not advanced to PE, and in pregnancies with PE later manifested in the third trimester. Our results showed that by comparison to healthy pregnancy, pregnancies with PE risk had higher serum cystatin C levels ($p \leq 0.001$). Additionally, at 12th to 16th and 24th to 28th weeks of gestation, cystatin C was higher in expectant women who eventually developed PE relative to normal pregnant women ($p \leq 0.001$). These findings may suggest that cystatin C could function as an early predictor of the development of PE. Decidua can inhibit trophoblastic incursion owing to cystatin C, which inhibits cysteine protease. The modulation of proper placenta development invasion depends on the equilibrium of protease inhibitors.[55] According to a previous study, some months earlier before clinical manifestations, the women with PE illustrated higher levels of cystatin C relative to women without PE.[56] Additionally, the preeclamptic placenta showed higher cystatin C mRNA and protein transcription, indicating enhanced protein production and its release. This could be a probable factor in the heightened maternal levels seen in PE patients.[57] The PE patients had elevated cystatin C levels as soon as the second trimester started. It was demonstrated that


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the greater levels of oxidative strain in PE may have an impact on serum cystatin C levels.[58]

Estimated sensitivity and specificity values of 85 and 84%, respectively, of serum cystatin C were suggested to consider it as a potential marker for PE diagnosis in the third trimester.[15] We have found that at 10−14 weeks of gestation, serum cystatin C in PE was shown to be greater than its corresponding value in healthy pregnancy.[59] In pregnancy, cystatin C plays a specialized role. The synthesis of cysteine protease is necessary for angiogenesis toward the decidua and trophoblastic incursion during placental development.[55] The family of cysteine lysosomal proteases includes cathepsin B. Like catabolizing intracellular proteins and digesting prohormones, it destroys extracellular matrix components. Available information suggests that cathepsin B has a role in the control of tissue regeneration and angiogenesis inside the endometrium following implantation and trophoblast invasions.[60] Cystatin C inhibits cathepsin B, and both participate in the trophoblast invasion regulation. Earlier studies showed that transverse cathepsin B levels were consistent during gestation and the PE is related to the higher plasma cathepsin B levels.[61]

Serum magnesium (Mg) and other relevant factors were examined to explain how these factors affected the likelihood of developing PE (Tables 4 and 5). Hypertension is one of the variables, which are thought to increase the risk of PE. Additionally, hypertension disorders are a contributing factor in the higher morbidity and death rates during pregnancy.[62] The relationship between pregnancy-related hypertension diseases (such as PE) and plasma electrolytes is indicated.[63−65] Therefore, it was necessary to evaluate the serum magnesium levels and associated metrics (urinary Mg and fractional Mg excretion) in pregnancy linked with susceptibility for PE relative to the normal control group. Furthermore, these factors were examined to explore if these indicators alter with progressing stages of pregnancy in both problematic and normotensive pregnancies.

At both sampling periods in this study, no significant difference in serum Mg and its related parameters between the included groups was found.[18,66,67] However, relative to healthy pregnancy, several investigations showed that PE patients had considerable low serum Mg levels.[65,68] Further, expectant women having PE had serum Mg levels that were within the healthy range.[69] The mean amounts of Mg in gestation were found to be 1.7 mg/dL relative to 2.0 mg/dL in nonpregnant women.[70] In the current study, the average values of Mg for normal pregnancies in the second and third trimesters were 2.09 ± 0.18 and 1.95 ± 0.07 mg/dL, respectively (Tables 4 and 5). On the other hand, in complicated gestation (without PE), the equivalent results were 2.13 ± 0.22 and 1.99 ± 0.12 mg/dL at the 12th to 16th and 24th to 28th weeks of pregnancy, respectively. Moreover, serum Mg levels in the expectant women who later had PE were 2.12 ± 0.22 and 1.99 ± 0.10 mg/dL at the first and second sampling times, respectively. It is widely recognized that reduced intestinal absorption or increased urine excretion might result in Mg deficiency.[71] Since Mg naturally exists in a variety of foods, reduced dietary intake cannot be a cause of Mg insufficiency.[72] Either alcoholics, patients with renal issues, individuals taking certain drugs, or people who have the celiac condition may have trouble in absorbing Mg.[73] We have found that there was no difference between PE and normal pregnancy in terms of urinary Mg levels or fractional excretion of Mg, demonstrating the lack of aberrant excretion. Additionally, our patients did not have variables that lead to gastrointestinal absorption defects. Therefore, in our opinion, PE does not significantly affect serum Mg, and variations in serum Mg might not be related to PE.

**2.4. Distribution of the Existing Risk Factors in NPE and PE Groups.** The current study accounted for a variety of disease progression risk variables (Figure 1). A total of 63.6%

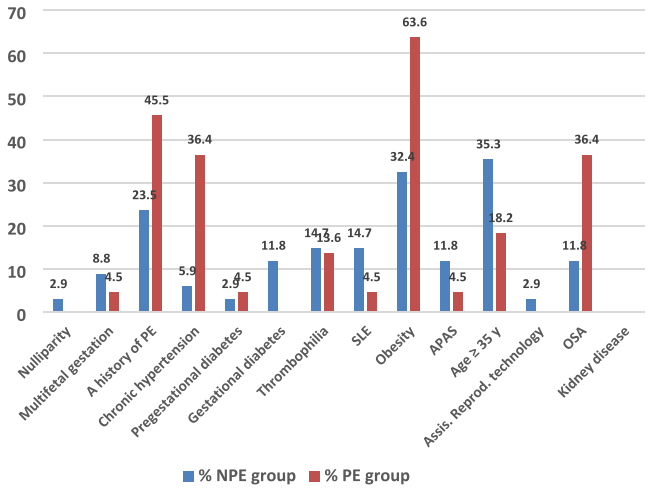


**Figure 1.** Representation of risk factors in the NPE and PE groups. SLE: systemic lupus erythematous; APAS: antiphospholipid antibody syndrome; OSA: obstructive sleep apnea.

of the examined women with PE were obese (BMI ≥ 30 kg/m$^2$), 45.5% had a preexisting PE, 36.4% had chronic hypertension, 36.4% had sleeping apnea, and 18.2% had a maternal age beyond 35 years. Additional risk indicators were also investigated such as systemic lupus erythematosus (4.5%), multiple pregnancies (4.5%), and thrombophilia (13.6%). Previous diverse percentage values for risk variables related to the occurrence of PE were reported. For example, 18.3 and 26% had chronic hypertension, 10.2% had preexisting PE, and 20.6% were obese.[74,75]

**2.5. Diagnostic Accuracy of Endostatin and Cystatin C.** Both serum endostatin and cystatin C mean values showed promising variations between pregnant women who progressed to PE and normal pregnant women and women with risk factors (NPE). Therefore, it was of interest to analyze the diagnostic accuracy of the two markers to predict the PE early and before it developed. Receiver Operating Characteristic (ROC) curves were constructed, and the area under each curve was calculated to evaluate the sensitivity and specificity. The curves were plotted for either endostatin or cystatin C to examine their sensitivity to differentiate between normal pregnancy and NPE (Figures 2−4and Tables 6−8).

The ability of serum endostatin to differentiate between expectant women who later developed PE and those at risk of PE but did not acquire PE indicated sensitivity and specificity values of 86.36 and 91.18%, respectively, and the AUC value was 0.878 (CI: 0.763−0.950) at the first sampling time. The AUC at the second sampling was 0.979 (CI: 0.900−0.999), with sensitivity and specificity values of 90.91 and 97.06%, respectively (Figure 4 and Table 8). Additionally, the ROC curve constructed to distinguish the groups of women who experienced PE and those who experienced normal pregnancy (Figure 3 and Table 7) showed an AUC value of 0.960 (CI: 0.860−0.995) at 12th to 16th weeks of gestation, with


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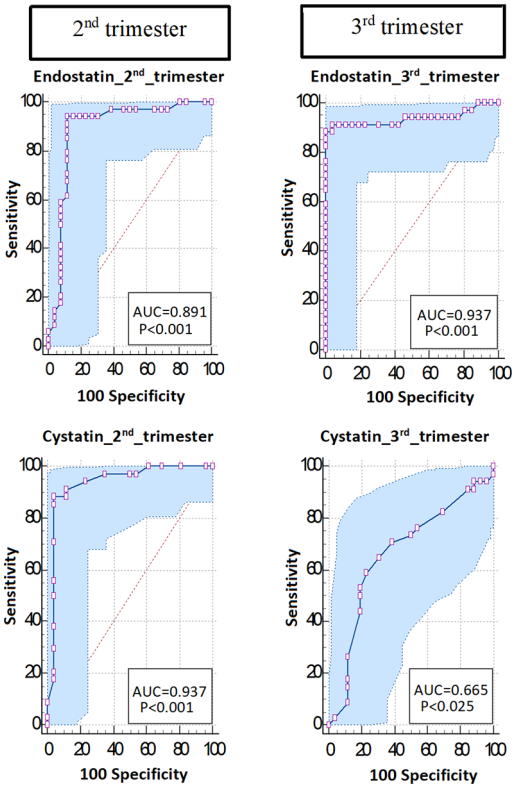


**Figure 2.** Receiver Operating Characteristic curve analysis using endostatin and cystatin C for discriminating the NPE group from normal pregnancy at 2nd and 3rd trimesters.

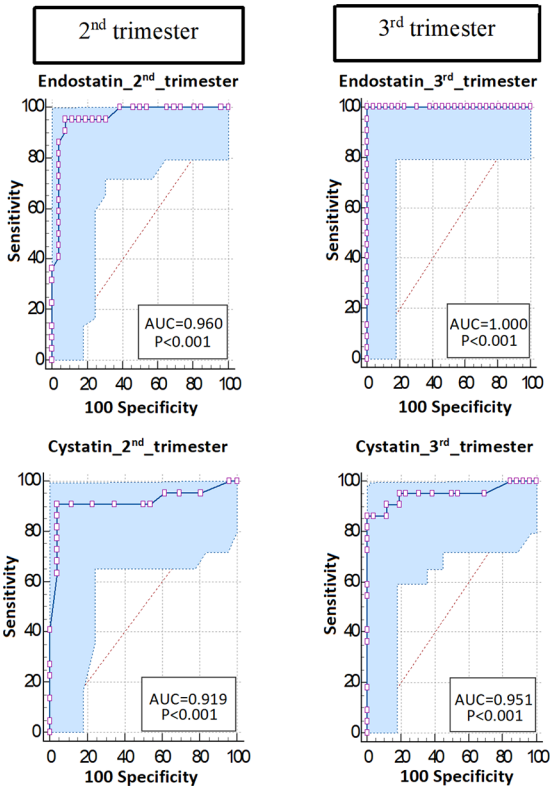


**Figure 3.** Receiver Operating Characteristic curve analysis using endostatin and cystatin C for discriminating the PE group from normal pregnancy at 2nd and 3rd trimesters.

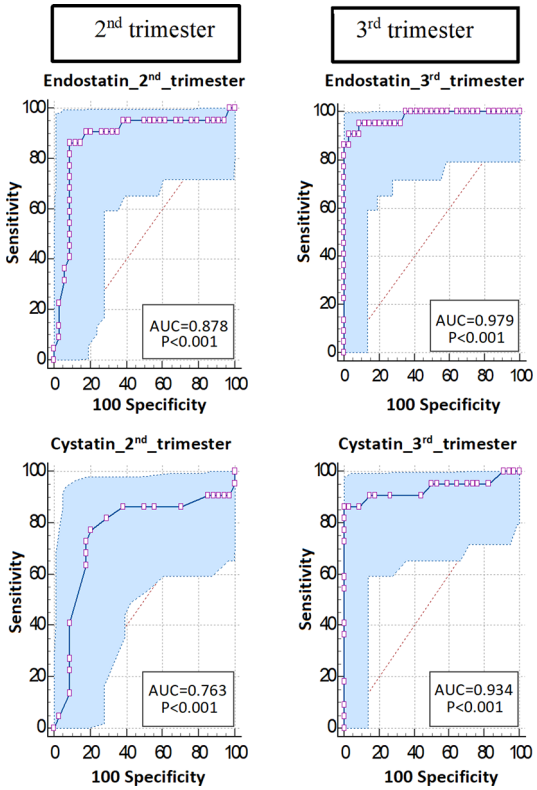


**Figure 4.** Receiver Operating Characteristic curve analysis using endostatin and cystatin C for discriminating the PE group from the NPE group at 2nd and 3rd trimesters.

sensitivity and specificity values of 95.45 and 92.31%, respectively. In the second sampling of that curve, the AUC value was 1.000% (CI: 0.926−1.000), and both sensitivity and specificity were 100%. Therefore, according to the ROC representation, high endostatin levels may accurately distinguish between pregnant women at risk for PE without developing the condition and pregnant women who are normotensive and have no history of PE.

Utilizing cystatin C at the two investigated sample times, the ROC curve was constructed to distinguish between pregnancies that progressed to PE and pregnancies that were at risk for PE but did not advance to PE in the first and second samples (Figure 4 and Table 8). In the first sample, the sensitivity and specificity were 77.27 and 79.41%, respectively, with an AUC of 0.763 (CI: 0.630−0.866). In contrast, the AUC at the second sample was 0.934 (CI: 0.834−0.983), with sensitivity and specificity of 86.36 and 100%, respectively. Moreover, analyzing the ROC curve led to distinct differences in categories of expectant women who experienced PE versus those who had a healthy pregnancy (Figure 3 and Table 7). The data showed an AUC value of 0.910 (CI: 0.803−0.978) at 12th to 16th weeks of gestation, with values for sensitivity and specificity of 90.91 and 96.15%, respectively. In the second sample, the AUC value was 0.951% (CI: 0.847−0.992), with sensitivity and specificity values of 86.36 and 100%, respectively. The ROC analysis results also showed that serum cystatin C had good sensitivity for differentiating women with future PE from normotensive pregnant individuals and pregnant women with the likelihood of developing PE without resolving the illness (Figure 2 and Table 6). It was previously mentioned that the sensitivity and specificity for



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Table 6. Diagnostic Values of Endostatin and Cystatin C at 2nd and 3rd Trimesters in Normal and NPE Groups[a]

| | normal vs NPE | | | |
|---|---|---|---|---|
| | endostatin | | cystatin C | |
| groups | 2nd trimester | 3rd trimester | 2nd trimester | 3rd trimester |
| AUC | 0.891 | 0.937 | 0.937 | 0.665 |
| *P* value | <0.001 | <0.001 | <0.001 | 0.025 |
| cut-off value | >4.99 | >25.06 | >0.63 | >0.7 |
| sensitivity % | 94.12 | 88.24 | 88.24 | 58.82 |
| specificity % | 88.46 | 100.00 | 96.15 | 76.92 |
| positive group *n* (%) | 34 (56.67) | 34 (56.67) | 34 (56.67) | 34 (56.67) |
| negative group *n* (%) | 26 (43.33) | 26 (43.33) | 26 (43.33) | 26 (43.33) |
| standard error | 0.0520 | 0.0363 | 0.0375 | 0.0735 |
| 95% confidence interval | 0.784−0.975 | 0.843−0.984 | 0.843−0.984 | 0.532−0.782 |
| PPV % | 91.4 | 100 | 96.8 | 76.9 |
| NPV % | 92 | 86.7 | 86.2 | 58.8 |

[a]AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value.

Table 7. Diagnostic Values of Endostatin and Cystatin C at the 2nd and 3rd Trimesters in Normal and PE Groups

| | normal vs PE group | | | |
|---|---|---|---|---|
| | endostatin | | cystatin C | |
| groups | 2nd trimester | 3rd trimester | 2nd trimester | 3rd trimester |
| AUC | 0.960 | 1.000 | 0.910 | 0.951 |
| *P* value | <0.001 | <0.001 | <0.001 | <0.001 |
| cut-off value | >9.94 | >25.06 | >0.63 | >0.8 |
| sensitivity % | 95.45 | 100 | 90.91 | 86.36 |
| specificity % | 92.31 | 100 | 96.15 | 100 |
| positive group *n* (%) | 22 (45.83) | 22 (45.83) | 22 (45.83) | 22 (45.83) |
| negative group *n* (%) | 26 (54.17) | 26 (54.17) | 26 (54.17) | 26 (54.17) |
| standard error | 0.0281 | 0 | 0.0491 | 0.0365 |
| 95% confidence interval | 0.860−0.995 | 0.926−1.000 | 0.803−0.978 | 0.847−0.992 |
| PPV % | 91.3 | 100 | 95 | 100 |
| NPV % | 96 | 100 | 89.3 | 89.7 |

Table 8. Diagnostic Values of Endostatin and Cystatin C at 2nd and 3rd Trimesters in NPE and PE Groups

| | NPE vs PE group | | | |
|---|---|---|---|---|
| | endostatin | | cystatin C | |
| groups | 2nd trimester | 3rd trimester | 2nd trimester | 3rd trimester |
| AUC | 0.878 | 0.979 | 0.763 | 0.934 |
| *P* value | <0.001 | <0.001 | <0.001 | <0.001 |
| cut-off value | >11.25 | >35.01 | >0.7 | >0.8 |
| sensitivity % | 86.36 | 90.91 | 77.27 | 86.36 |
| specificity % | 91.18 | 97.06 | 79.41 | 100 |
| positive group *n* (%) | 22 (39.29) | 22 (39.29) | 22 (39.29) | 22 (39.29) |
| negative group *n* (%) | 34 (60.71) | 34 (60.71) | 34 (60.71) | 34 (60.71) |
| standard error | 0.0554 | 0.0167 | 0.0744 | 0.0444 |
| 95% confidence interval | 0.763−0.950 | 0.900−0.999 | 0.630−0.866 | 0.834−0.983 |
| PPV % | 86.4 | 95 | 70.8 | 100 |
| NPV % | 91.2 | 91.7 | 84.4 | 91.9 |

antepartum cystatin C levels were 80.0 and 50.0%, respectively, and the positive and negative prognostic values were 62.0 and 71.0%,[29,57] while in another investigations, the AUC value was found to be 0.993, with clinical sensitivity and specificity values of 88.24 and 98.04%, respectively.[13,59]

## 3. EXPERIMENTAL SECTION

**3.1. Subjects and Methods.** This study was approved by the Research Ethics Committee at the Faculty of Medicine, Ain Shams University, Cairo, Egypt. Each participant signed informed consent before participating in the study. Data were collected (using a standard interview-based questioner) from individual volunteers. Most of the risk factors associated with preeclampsia, such as nulliparity, multifetal gestation, a history of PE, chronic hypertension (CH), pregestational diabetes, gestational diabetes (GD), thrombophilia, systemic lupus erythematosus (SLE), obesity, antiphospholipid antibody syndrome (APAS), advanced maternal age (35 years or older), use of assisted reproductive technologies, and obstructive sleep apnea were registered.[76] PE has been



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diagnosed by the criteria of a systolic blood pressure ≥140 mmHg and a diastolic blood pressure ≥90 mmHg on two periods at least 4 h apart and visible dipstick proteinuria (≥"+").

**3.2. Study Population and Sample Collection.** A total number of 82 pregnant participants receiving antenatal care at the Obstetrics and Gynecology Hospital, Ain Shams University, Cairo, Egypt, were enrolled in the study. Participants were divided into the following groups: (a) the control group ($n$ = 26) included healthy women with normal pregnancy and without any risk or complications, (b) the nonpreeclampsia group (NPE, $n$ = 34) included pregnant women showing one or more risk factors for PE without progressing to PE later during pregnancy, and (c) the preeclampsia group (PE, $n$ = 22) included pregnant women who showed one or more risk factors and developed PE.

Fasting blood samples were collected from volunteers at two gestational periods. One sampling was taken during the second trimester (12th to 16th weeks), and the second sampling was at the third trimester (24th to 28th weeks) of pregnancy. Whole blood samples were obtained from all subjects' forearm antecubital regions. Serum was separated and stored in aliquots at −80 °C pending assay. Urine samples were collected from all participants at the same time of blood collection, placed into two different clean, leak-proof containers, and stored at −80 °C.

**3.3. Biochemical Analysis.** According to the manufacturer's protocol of each utilized kit for each parameter, the following were estimated:

I. Liver function parameters (LFTs): diagnostic kits provided by Biotechnology Company, Egypt, were used for determination of ALT (cat. no. 264001) and AST (cat. no. 260001) activities and the level of serum albumin (cat. no. 211011).

II. Kidney function parameters (KFTs): kits were supplied from Biotechnology Company, Egypt, to determine levels of serum urea (cat. no. 318001), serum and urinary creatinine (cat. no. 234001), and serum uric acid (cat. nos. 323001 and 323002).

III. Other biochemical indices:

a. The serum glucose concentration was assessed by using a spectrum diagnostic kit purchased from Biotechnology Company, Egypt (cat. no. 250001).

b. The serum insulin level was determined using an enzyme-linked immunosorbent assay kit obtained from Bioassay Technology Laboratory (BT LAB), Zhejiang, China (cat. no. E1712Hu). The detection range was 0−200 μLU/mL. The kit's sensitivity was 2.0 μLU/mL.

c. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by using the following formula:[77]

$$[\text{serum glucose level(mmol/L)} \times \text{serum insulin level(mU/L)}]/22.5$$

**3.4. Quantitative Determination of Serum Endostatin.** A commercial human ELISA kit obtained from the BT LAB, Zhejiang, China (cat. no. E1712Hu), was used to determine the levels of human endostatin in serum samples. The detection range of the kit was 0.3−90.0 ng/mL. The kit's sensitivity and coefficient of variation percentage were 0.15 ng/mL and <10%, respectively.

**3.5. Quantitative Determination of Serum Cystatin C.** A commercial human ELISA kit (cat. no. E1104Hu) from BT LAB (Zhejiang, China) was used to determine the level of human cystatin C in serum samples. The detection range for the kit was 0.05−4.00 mg/dL. The kit had a sensitivity and CV % of 0.02 mg/L and <10%, respectively.

**3.6. Quantitative Determination of Magnesium and Related Parameters in Serum and Urine.** A spectrum diagnostic kit purchased from the Biotechnology Company, Egypt (cat. no. 285001), was employed to determine serum and urinary Mg. The following formula was used to calculate the fractional excretion magnesium (FEMg) level:[78]

$$\text{FEMg} = [\text{SeCr} \times \text{UMg} \times 100]/[0.7 \times \text{SeMg} \times \text{UCr}]$$

where UMg indicates urinary Mg, SeCr indicates serum creatinine, UCr indicates urinary creatinine, and SeMg indicates serum Mg.

**3.7. Statistical Analysis.** Data analysis was performed with social science statistical software (SPSS V.22). To screen the data, normality tests, missing values, and outliers were checked. The Shapiro−Wilk and Kolmogorov−Smirnov tests were used to determine normality. Descriptive statistics, namely, median ± standard deviation, mean, and interquartile range, were performed to examine the distribution of explained serum levels. At the differences level, a one-way variance assessment was used. Multiple comparison Tukey test was used to identify which factors are associated with control, NPE, and PE groups. Factors associated with the PE group were obtained using regression analysis area under the curve. Sensitivity and specificity and/or Receiver Operating Characteristic (ROC) curve analysis were used to identify pregnant women with PE. For nonparametric analysis, Mann−Whitney's $U$-test, chi-squared test (with Yates' continuity correction when required), and Wilcoxon signed rank test were utilized. The $P$ value was set at 0.05 as low significant, 0.01 as medium significant, and 0.001 as highly significant.

## 4. CONCLUSIONS

The pathogenesis of preeclampsia is influenced by multiple of indicators. The findings illustrated that combining both serum endostatin and cystatin C at the beginning of pregnancy and before the emergence of PE might be reliable indicators for predicting the early development of preeclampsia. Both biomarkers are beneficial and appropriate for PE diagnosis between the 12th and 16th weeks of pregnancy. Furthermore, they are critical in differentiating between pregnancies, showing a likelihood of developing PE and those that might develop PE in the later stages from normotensive expectant women.

## AUTHOR INFORMATION


### Corresponding Author

**Amani F. H. Noureldeen** − *Biochemistry Department, Faculty of Science, Ain Shams University, Cairo 11566, Egypt;* orcid.org/0000-0002-6490-8443; Email: amaninoureldeen@sci.asu.edu.eg

### Authors

**Fatima Alshannag** − *Biochemistry Department, Faculty of Science, Ain Shams University, Cairo 11566, Egypt*




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**Radwa M. M. Zaki** − *Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University, Cairo 11566, Egypt*
**Eman Hemida** − *Ain Shams Specialized Hospital, Cairo 11568, Egypt*
**Mustafa M. M. ElBakry** − *Biochemistry Department, Faculty of Science, Ain Shams University, Cairo 11566, Egypt*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c05586

### Author Contributions

F.F.Z.A. and E.H.A.H. did the experimental work, collected the samples, and prepared figures. R.M.M.Z. diagnosed the cases and discussion. M.M.M.E. did the statistical part. A.F.H.N. wrote and revised the main manuscript text. All authors reviewed the manuscript.

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