Cystatin C as Alternative Markers of Renal Functions and High Sensitive C-Reactive Protein in Early Diagnosis and Prediction of pre-eclampsia

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**Background:** Preeclampsia (PE) is a disorder peculiar to pregnancy and a major cause of maternal death. It is associated with fetal growth restriction, low birth weight, preterm delivery and respiratory distress syndrome. The exact pathophysiological mechanisms of PE are still out of reach. Studies to date have included multiple processes, including invasive abnormal trophoblastic invasion, vascular spasm, platelet activation, changes in the relative quantities of prostaglandins, and change in renal GFR. Early diagnosis and predicting of PE remains challenging due to the lack of a specific marker or one test sensitive and specific enough to identify high-risk pregnancies for PE. The aim of this study was to estimate the levels of cystatin C (Cys C) as renal dysfunction marker and high sensitive C-reaction protein (hs-CRP) as systematic inflammatory marker in early diagnostic and predicting and correlated with traditional markers at the second trimester in women with preeclampsia and its severity.

**Methods:** Eighty PE women aged (20-40) years, who proven at the second trimester (19-23 weeks of gestation) were divided to MPE and SPE, and another 40 of normotensive pregnant women (NP) as control were enrolled in this study. Cys C was measured using immunoturbidimetric assay, RX RANDOX, England, and hs-CRP was measured using enzyme-linked immunosorbent assay (ELISA) kits, Demeditec, Germany. Tukey HSD Post hoc ANONA test and One-way ANOVA were used to compare differences of mean between groups. Pearson Correlation was calculated to assess the relation between the biomarkers of this study. Receiver Operating Characteristics (ROC) curve was calculated to estimate and compare the sensitivity and specificity for the use of Cys C and hs-CRP as biomarker in the diagnosis and discriminatory ability of PE.

**Results:** Cys C and hs-CRP were significantly higher in PE women (MPE & SPE) compared to NP as controls (1.2±0.27 and 1.3±0.2 vs. 0.74±0.11) (p<0.01) for Cys C and (7.3±5.4 and 8.1±4.5 vs. 5.0±2.6) for hs-CRP. There were a significant differences in mean of each Cys C and hs-CRP values between two categories of preeclampsia were seen (p<0.01) and (p=0.04) respectively. The correlation of Cys C was a positive correlation with uric acid in MPE and SPE women (r =-0.37, p=0.01 and r =-0.85, p<0.01) and with creatinine (r =-0.51, p<0.01), urea (r =-0.77, p<0.01) in SPE women. Receiver operator characteristics (ROC) curve was calculated to define the optimal cut-off values for the use of each Cys C and hs-CRP as biomarkers in early diagnostic and distinguish of patients with MPE and SPE. ROC curve revealed to the optimal cutoff value for Cys C in MPE was 0.89 mg/L and SPE was 0.94 mg/L with sensitivity of 97.5% and 95% and specificity of 90% and 92.5% with area under curve AUC of 97.2% and 99%. The optimal cutoff value for hs-CRP in MPE was 4.5 ng/ml and SPE was 5.3 ng/ml with sensitivity of 57.5% and 77.5% and specificity of 55% and 70% with AUC of 57.5% and 71.5%.

**Conclusions:** Its conclude that Cys C together with hs-CRP assay may be used as promising biochemical markers for the early diagnosis and predicting of PE, but only Cys C considered as good discriminator between MPE and SPE women.
Keywords: Preeclampsia, Cystatin C, High sensitive C-reaction protein, Traditional renal markers

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Introduction

Preeclampsia (PE) is a multisystem, pregnancy-specific disorder that occurs in 3-5% of pregnant women \[1\], but can quickly progress to eclampsia and lead to multi-organ failure, seizure, and maternal-fetal death \[2\]. It is one of the leading causes of maternal and fetal morbidity and mortality worldwide \[3\]. PE is responsible over 35% of premature births in China and for 20% in the United States \[4\]. Preeclampsia is a disease manifested in the second half of pregnancy with a classic triad of symptoms including high blood pressure, proteinuria, edema \[5\], with mild symptoms as systolic blood pressure (SBP) and diastolic blood pressure (DBP) readings of ≥140 and ≥90 mmHg, respectively, on 2 occasions at least 4 hrs apart after 20weeks gestation in women with a previously normal blood pressure, or severe symptoms that SBP readings of ≥ 160 mmHg or ≥ 110 mmHg DBP, proteinuria ≥ 300 mg/ 24hrs or a Protein / creatinine ratio ≥ 0.3 mg/dl or a dipstick reading of ≥ 1+ \[6\], and peripheral oedema \[7\].

Although the aetiology and pathophysiology of PE remain unclear \[8\], preeclampsia development can occur in the following two-step process. The first step involves placental abnormality, which may be the result of an imbalance of the local maternal immune response against fetal tissue \[9\], associated with the abnormal differentiation of trophoblast cells during their invasion of the uterine spiral arteries \[10,11\]. These symptoms reduce the placenta’s size and restrict blood flow of the utero-placental, which does not meet the needs of the growing fetus, \[12\], this abnormal placentation occurs which leads to placental ischemia/hypoxia \[13\]. The ischemic placenta is thought to secrete soluble factors during the third trimester that in turn induces systemic endothelial dysfunction and the maternal syndrome of preeclampsia \[14,15\]. In general, these changes lead to the beginning of the second stage of PE that occurs after 20 weeks of pregnancy and are characterized by systemic endothelial dysfunction in many organs and systems including the kidneys, the liver, the cardiovascular system, the brain, and others \[16,17\].

Up to now, there are few effective indicators or methods for predicting, diagnosing, and treating PE \[18\]. PE seriously threatens the health of pregnant women as well as fetuses and has become a major public health problem that affects women's health \[19\].

Cystatin C (Cys C) is a non-glycosilated protein with low molecular weight of 13kDa consisting of 120 amino acids, belonging to cysteine-proteases family. It is produced at a constant rate by lysosomes of all nucleated cells in the organism and can be found in several biological fluids, such as serum, seminal liquid and cerebrospinal fluid \[20\]. Because of its small size and positive charge, Cys C is freely filtered by glomerulus, where it is taken up and degraded by proximal tubular cells. It also does not provide tubular secretion and can therefore be used as a biomarker for renal function \[21\]. Several studies have shown that Cys C is an effective marker for diagnosing kidney damage, such as acute kidney injury \[22\] and kidney failure, in patients with diabetes mellitus \[23\].

Kidney function and glomerular filtration rate (GFR) decline, the blood levels of cystatin C rise, previous studies have shown that serum levels of Cys C are more precise test of kidney function than serum creatinine levels \[24\]. Cys C levels are less dependent on age, gender, ethnicity and muscle mass compared to creatinine \[25,26\]. Increasing level of Cys C is associated with normal pregnancy during the third trimester, followed by a significant decrease in the postpartum period \[27\]. Previous studies have demonstrated that the expression Cys C is regulated by trophoblastic cells in placenta of preeclamptic women, indicating its possible role during placentation \[28\].
C-reactive protein (CRP) is a systemic inflammatory plasma protein. The CRP plasma concentration is deviated by at least 25% during inflammatory disorders. CRP is mainly classified as an acute marker of inflammation, but research has begun to indicate the important roles that CRP plays in inflammation \[^{[29]}\]. There are many factors that can change baseline CRP levels including age, weight, gender, fat levels, smoking status, and blood pressure \[^{[30]}\].

CRP levels are elevated in PE women than healthy pregnant and the later was higher extent than that seen in non-pregnant women \[^{[31]}\]. It has been shown that elevated levels of CRP during gestation have been linked to complicated pregnancy outcomes like preeclampsia \[^{[32]}\]. Many studies shown elevated level of CRP in PE women, but the predictive role of CRP in PE is still dialectical \[^{[33,34]}\]. Early diagnosis and predicting of PE remains challenging due to the lack of a specific marker or one test sensitive and specific enough to identify high-risk pregnancies for PE.

**Patients and Methods**

This case control study was executed during the term from March 2019 to September 2019, and approved by the Institutional Ethics Committee. 80 patients who were diagnosed with PE aged 20 to 40 years at second trimester (19-23 weeks of gestation), by their physician to physical examination, blood pressure measurement, and laboratory investigations including serum urea, uric acid, and creatinine. These patients were divided into two groups; One group consist of 40 patients with mild PE (MPE) (SBP ≥140 mmHg or DBP ≥90 mmHg) and one group consist of 40 patients with severe PE (SPE) (SBP ≥160 mmHg or DBP ≥110 mmHg), and another group of 40 normotensive pregnant (NP) women as control. All groups were collected from Al- Imamain Alkadhimain Teaching Hospita, al Elwea Maternity Hospital and Iraq Red Crescent hospital. Patients with a renal, liver, heart, diabetes mellitus, chronic inflammatory disorders, chronic hypertension, severe anemia, and malignant tumor were excluded from this study.

About seven milliliters of blood samples from all subjects were collected by venipuncture. Blood samples were left for 20 minutes at room temperature. After coagulation, sera were aspirated and divided into small aliquots for immediate measurements of serum urea, uric acid, and creatinine were done using were done using appropriate enzymatic colorimetric method, Abbott architect c4000, USA. The rest will be stored at -20 until assayed for serum cystatin C that measured using Immunoturbidimetric assay, RANDOX, England, and hs CRP that measured using enzyme-linked immunosorbent assay (ELISA) kits, Demeditec, Germany.

**Statistical Analysis**

Statistical analysis was carried out using Microsoft excel 2013 and SPSS version 20. The numerical data expressed as mean ±SD. For three groups, one-way ANOVA was performed. Furthermore, the Tukey HSD Post hoc ANONA test was used to compare between mean serum levels of Cystatin C and hs-CRP together with uric acid, for MPE, SPE and control were performed. A p ≤0.05 was considered statistically significant. Pearson correlation was calculated to assess the relation between each of Cystatin C and hs-CRP with urea, uric acid, and creatinine. Receiver Operating Characteristics (ROC) curve was calculated to estimate the sensitivity and specificity of the used Cystatin C and hs-CRP as biomarkers in the diagnosis and discriminatory ability of PE.

**Results**

Baseline and clinical characteristics of the patients and control groups are given in Table 1. One-way ANOVA was used to arrive at the p-value between NP, MPE and SPE for creatinine, urea, uric acid, Cys C and hs-CRP.

The total number of PE patients (MPE & SPE) and NP as control group were 40 for each group. There were no significant differences in age, creatinine and urea between all groups, whereas the uric acid, Cys C and hs-CRP were significantly elevated in MPE and SPE groups comparing with control (P<0.01), (P<0.01) and (p= 0.04).

Table (1): Baseline and clinical characteristics of the PE patients and control groups

Tukey HSD Post hoc ANOVA test results between each two groups of PE and control are given in Table 2. The results showed that the uric acid level has a significantly elevated in SPE group comparing with NP group (P<0.01) and SPE group comparing with MPE group (P<0.01), but there were no significant differences between NP and MPE. The Cys C level has a significantly elevated in MPE group comparing with NP group (P<0.01), SPE group comparing with NP group (P<0.01) and SPE group comparing with MPE group (p=0.01), whereas the hs-CRP level has a significantly elevated in SPE group comparing with NP group (P<0.01), but there were no significant differences between NP and MPE and between MPE and SPE.

Table (2): Comparison of the mean Uric acid, Cystatin C and hs-CRP values between each two groups of patients and control

The correlation of each Cys C and hs-CRP with creatinine, urea and uric acid in MPE and SPE patients are given in table 3. The results revealed that there was a positive correlation between cystatin C with uric acid in MPE and SPE women (r = -0.37, p=0.01 and r = -0.85, p<0.01) and with creatinine (r = -0.51, p<0.01), urea (r = -0.77, p<0.01) in SPE women, whereas there was no correlation between hs-CRP and conventional biomarkers in MPE and SPE patients.

Table (3): Correlation of each Cys C and hs-CRP with creatinine, urea and uric acid in PE patients (MPE & SPE)
The ROC curve of Cys C, hs-CRP and uric acid for diagnosis of MPE was shown in Figure 1 and table 4. The AUC for Cys C, hs-CRP and uric acid in MPE were 97.2%, 57.5% and 46% respectively, and the cut-off value was 0.89 mg/L with sensitivity of 97.5% and specificity of 90% for Cys C, 4.5 ng/ml with sensitivity of 57.5% and specificity of 55% for hs-CRP whereas the cut-off value was 4.7 mg/dl with sensitivity of 52.5% and specificity of 45% for uric acid.

The ROC curve of Cys C, hs-CRP and uric acid for diagnosis of SPE was shown in Figure 2 and table 5. The AUC for Cys C, hs-CRP and uric acid in SPE were 99%, 71.5% and 92.3% respectively, and the cut-off value was 0.94 mg/L with sensitivity of 95% and specificity of 92.5% for Cys C, 5.3 ng/ml with sensitivity of 77.5% and specificity of 70% for hs-CRP whereas the cut-off value was 6.6 mg/dl with sensitivity of 85% and specificity of 82.5% for uric acid.

<table>
<thead>
<tr>
<th>variables</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Area under curve</th>
<th>Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C</td>
<td>97.5%</td>
<td>90%</td>
<td>97.2%</td>
<td>0.89 mg/L</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>57.5%</td>
<td>55%</td>
<td>57.5%</td>
<td>4.5 ng/ml</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>52.5%</td>
<td>45%</td>
<td>46%</td>
<td>4.7 mg/dl</td>
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Figure (1): ROC curve of Cys C, hs-CRP and uric acid assay for comparing performance in MPE diagnosis.

Table (4): Cutoff, Sensitivity and Specificity of Cys C, hs-CRP and uric acid in patients with MPE.
Figure (2): ROC curve of Cys C, hs-CRP and uric acid assay for comparing performance in SPE diagnosis.

Table (5): Cutoff, Sensitivity and Specificity of Cys C, hs-CRP and uric acid in patients with SPE

<table>
<thead>
<tr>
<th>variables</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Area under curve</th>
<th>Cutoff</th>
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</thead>
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<tr>
<td>hs-CRP</td>
<td>77.5%</td>
<td>70%</td>
<td>71.5%</td>
<td>5.3 ng/ml</td>
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<tr>
<td>Uric Acid</td>
<td>85%</td>
<td>82.5%</td>
<td>92.3%</td>
<td>6.6 mg/dl</td>
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Discussion

The present study showed in table 1, the mean and standard deviation (Mean ± SD) of maternal age for MPE, SPE and NP were 29.8±6.1, 30.7±6.25 and 29.37±6.7 respectively, and it has no significant difference between ACS groups and control group (P=0.027). This result is agreed with the conclusion obtained from Shahanaj et al [35]

This study showed, the mean and standard deviation (Mean ± SD) of MPE, SPE and NP for creatinine were 29.8±6.1, 0.64±0.1, and 29.37±6.7 (mg/dl) respectively, and for urea were 22.38±3.2, 22.9±2.6, and 21.7±2.3 (mg/dl) respectively, with no significance differences. This result is agreed with the conclusion obtained from John and Abraham [36], that creatinine considered as marker for kidney function, but it's not suitable for detect the early stage of kidney dysfunction which associated with PE, and disagreement with the study presented by Aleksandra et al [37], which may be contributed to the differences of gestational age used.

The results of uric acids that shown in table 1 for MPE, SPE and NP were 4.7±0.67, 6.6±0.98, and 4.8±0.74 (mg/dl) respectively, with significantly elevated in PE groups comparing with the control group (P<0.01). These values showed that high levels of uric acid was associated with increased risk of renal dysfunction which reported by Aleksandra et al [37]. Hyperuricemia in PE is mainly as a result of increasing tubular reabsorption with decreasing GFR, but it may occur by increasing of placental for production of uric acid as a result of increasing purine degradation by increasing the activity of xanthine oxidase or xanthine dehydrogenase making uric acid plying an important role in PE [38,39]. As
well as these results showed that the level of Cys C was significantly higher in PE groups as compared to the control group (P<0.01). The high level of Cys C may be associated with different explanation. From one hand

PE may increase the level of CysC, which causes defects in the endovascular trophoblast invasion into uterine spiral arteries during pregnancy results in vascular modifying and breakdown of these maternal vessels [40], and leads to the implantation and growth of the placenta, which is associated with the production of the cysteine protease (cathsepine), that helps to invade of trophoblast into the desidua [41,42]. The desidua works to produce the Cys C, which is a cysteine protease inhibitor act to limit of the trophoplast invasion [43,44]. The control of balanced between expression of cysteine proteases and Cys C supports strongly the development of normal placentation [43,45]. According to the explanations above it is suggested the imbalance between cysteine proteases and its inhibitor may be explain the inability of trophoblast invasion in PE [46,47].

On the other hand it is known that Cys C is used in kidney function tests and it is considered a good marker used to know the GFR [48]. The increase in GFR by almost 40% in pregnant women due to increased renal plasma flow during pregnancy so it makes sense to expect a decrease in the value of Cys C in the blood [49]. However, it was found that Cys C is not really decrease because its production increases due to the increase in the number of nucleated cells it creates [49,50]. In the case of PE, a decrease in GFR was found due to decreased renal plasma flow due to renal morphological changes leading to a decrease in glomerular filtration rate then lead to renal dysfunction [51,52]. It is likely that the increase in the levels of Cys-C in PE is due to a change in its filtration process versus an increase in the production rate [53]. The changes in the filtration barrier of the glomerular endotheliocsis in the case of PE will lead to decrease of the glomerular barrier size and the selectivity of the glomerular filtration, which strongly impedes the filtering of large particles or particles with positive charge and since the Cys C has a strong positive charge with a large volume it escapes from filtering and increases level [54].

It appears that preventive management to reduce the negative effects of PE and its complications are currently suboptimal may be due to the fact that PE and its severity are clinically unpredictable. Thus this study used to evaluate the role of hs-CRP as a systemic inflammatory marker synthesis in response to infection and tissue injury. The results of present study showed that serum hs-CRP level was significantly higher in PE groups as compared to the control group, thus in agreement with study of Reihane and Elham [55]. Increasing the level of hs-CRP in PE may be contributed to endothelial dysfunction that related to renal dysfunction as one of the main pathogenesis mechanisms associated to the severity of PE [56].

In the present study, uric acid, Cys C, and hs-CRP concentrations were compared between each two groups of PE in the 2nd trimester according to their severity and control group as shown in table 2. Although there were an increase in the mean and standard deviation (Mean±SD) of both uric acid and hs-CRP the results revealed, that there were no significance differences in the concentrations of uric acid in MPE as compared with the NP and hs-CRP in MPE as compared with the SPE. These results are in agreement with other study Kristensen et al [57], that serum uric acid levels increase with the progressing of pregnancy with high levels in the 3rd trimester. The results that obtained for hs-CRP are in agreement with other study Shahanaj et al [35], that hs-CRP as a systemic inflammatory marker may be used to predict the PE.

Table 2 also shown the higher significant of Cys C between each MPE and SE with NP (p<0.01) and MPE with SE (p=0.01). These results are in agreement with other study Vijayalakshmi and Usha [58]. Cystatin C has a wide extracellular distribution and may reflect the elevation in the second trimester in response to tissue damage. In PE there is a partial or complete failure to invade the trophoblastic of spiral arteries and may be lead to ischemic damage, which in turn will release cysteine protease [36]. Thus the results revealed that Cys C may be used as a good tool and batter than uric acid and hs-CRP in early diagnosis and predicting of PE and their severity [59].

In the present study, each Cys C, and hs-CRP levels were correlated with creatinine, urea and uric acid in MPE and SPE patients are given in table 3. The results found a correlation between Cys C
and uric acid in both MPE and SPE and Cys C with creatinine and urea in SPE. PE women were susceptible for increasing risk of renal dysfunction and this explain the positive correlation between Cys C and traditional markers of renal impairment [60]. There was no correlation between h-CRP and conventional markers of renal function [56,61].

Optimal cut-off values of receiver operator characteristics (ROC) curve were calculated for Cys C, hs-CRP, and uric acid in MPE (figure 1 & table 4) and SPE (figure 2 & table 5) as biomarkers in early diagnostic and distinguish of women with MPE and SPE. The area under curve for Cys C, hs-CRP, and uric acid in MPE were 97.2%, 57.5%, and 46% respectively, and in SPE were 99%, 71.5%, and 92.3% respectively. The sensitivity that reflect the positive detection rate of Cys C, hs-CRP, and uric acid in MPE were 97.5%, 57.5%, and 52.5% respectively, and in SPE were 95%, 77.5%, and 85.0% respectively. The optimal cutoff values for Cys C, hs-CRP, and uric acid in MPE were 0.89 mg/L, 4.5 ng/ml, and 4.7 mg/dl respectively, and in SPE were 0.94 mg/L, 5.3 ng/ml, and 6.6 mg/dl respectively. This results may be indicated that Cys C has a superior diagnostic ability and accuracy when compared with hs-CRP and uric acid in PE [62,63]. The increasing level of Cys C in PE may be reflect the increasing of its reabsorption by the kidney [64].

Conclusions:

Measurement of Cys C with hs-CRP and uric acid as a markers of PE can be a useful tools in the diagnosis and prediction of women with MPE and SPE. Its conclude that Cys C together with hs-CRP and uric acid assay may be used as promising biochemical markers for the early diagnosis and predicting of PE, but only Cys C considered as good discriminator between MPE and SPE women.

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