Assessment of Renal Function in Women with Preeclampsia by Measuring of Serum Cystatin C Level

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Abstract

Background: Preeclampsia (PE) is a major health problem that increases the risk of renal impairment during pregnancy. Studies have described the utility of Cystatin C as a better indicator of kidney function during pregnancy compared to urea, creatinine, or uric acid. **Objective:** The objective of the study was to evaluate cystatin C as a diagnostic tool for renal impairment in women with severe PE and compare it to standard renal function tests. **Patients and Methods:** A case–control study was conducted at Al Yarmouk Teaching Hospital, Bagdad. From March 1, 2022, to September 1, 2022, 94 3rd-trimester pregnant women with newly diagnosed or a history of PE were included in the study and interviewed and subsequently divided into three groups. The GraphPad Prism Software was used for data analysis and graphical presentations in addition to SPSS version 28; 0.05 was selected as a cutoff point for statistical significance. **Results:** The mean of cystatin C for control 0.39 ± 0.15 was significantly lower than for severe PE 1.12 ± 0.53, $P < 0.001$. The mean cystatin C for mild PE 0.52 ± 0.13 was considerably lower than for severe PE 1.12 ± 0.53, $P < 0.001$. **Conclusions:** Patients with severe PE had higher serum cystatin C than patients with mild PE and healthy normotensive pregnant females. Serum cystatin C at a cutoff value of >0.61 had higher diagnostic accuracy than the other standard kidney tests (urea, creatinine, and uric acid), with a sensitivity and specificity of 90.32% and 93.75%, respectively.

Keywords: Cystatin, preeclampsia, pregnancy, renal impairment

Introduction

PE and renal dysfunction have a complex bidirectional interaction. Kidney disease, even if modest, can predispose women to preeclampsia (PE), which itself has subsequently been found to cause albuminuria, chronic renal disease, and even end-stage kidney disease.[1] In PE, the imbalance between soluble proangiogenic and antiangiogenic substances due to hypoxia is responsible for glomerular endotheliosis and podocyte damage causing kidney injury,[1-3] which if undiagnosed early on can lead to renal failure, PE in subsequent pregnancies, and other vascular disorders.[4] It is therefore imperative to try and diagnose renal impairment as early as possible to reduce future morbidity and mortality.

During pregnancy, there is a 50%–80% increase in plasma flow and a change in glomerular filtration rate (GFR), which complicates the use of serum creatinine as a marker of GFR.[5] Cystatin C is a basic protein found in almost all nucleated cells and is filtered by the kidneys. As it is not affected by body habitus and muscle mass, it is thought to give a more accurate estimate of GFR compared to serum creatinine. Assays for cystatin-c have been developed and validated for routine clinical use.[6] Some authors have also assessed cystatin C as an alternative marker of renal function in PE and compared it to creatinine and uric acid. They concluded that cystatin C may play an important role as a marker of PE, particularly when combined with uric acid levels.[7] Despite research indicating that cystatin C is a better predictor of renal function than creatinine, there are currently no widely accepted guidelines for its use, even during pregnancy.[8]

Our study aims to evaluate cystatin C as a diagnostic tool for renal impairment in women with severe PE and compare it to standard renal function tests, namely: Blood urea, creatinine, and serum uric acid.

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Patients and Methods

Setting and study design

This is a case–control study conducted through a collaboration between the Department of Obstetrics and Gynecology and the laboratory department of Al-Yarmouk Teaching Hospital between January 1, 2022 and the end of December 2022.

Sampling method

Convenience sampling was used in this study.

Study subjects

Pregnant women in their 3rd trimester presenting to the outpatient clinic and inpatient ward of Al-Yarmouk Teaching Hospital, between March 1, 2022, and September 1, 2022, were eligible for participation in this study. Inclusion criteria included pregnant women between 18 and 40 years of age with a gestational age of 20–40 weeks, singleton live fetus, and intact fetal membranes.

Exclusion criteria included women with systemic conditions such as chronic hypertension, thyroid, hepatic, renal, or adrenal disease, and certain inflammatory conditions (inflammatory bowel disorders, vasculitis, rheumatic disorders, and acute or chronic infections). Women with certain pregnancy-related conditions were also excluded including those with multifetal gestation, gestational diabetes, gross fetal anomalies, intrauterine fetal death, prelabor rupture of membrane

Study groups

Ninety-four participants included in the study were divided into three groups: the control group (32 women with normal blood pressure), the mild PE group (31 women with mild PE), and severe PE group (31 women with severe PE). Those with elevated blood pressure and albumin ≥+2 on dipstick or the presence of one of the following symptoms (headache, blurred vision, or epigastric pain) were included in the severe PE group. The rest with only raised blood pressure of <160/110 were considered to have mild PE.

Data collection procedure

A structured questionnaire was used to record patient information including detailed patient history and general examination. Blood pressure was measured in a sitting position on two occasions by a mercury sphygmomanometer. Obstetrical ultrasound was also performed for all participants.

Biological sample collection and analysis

For standard blood tests, maternal blood samples were collected at the time of admission (before any intervention or medications were given) and sent to the laboratories for blood group and crossmatch, random blood sugar, complete blood count, liver function tests, blood urea, and serum creatinine, and serum uric acid. Maternal midstream urine samples were also collected for albumin measurement.

For measurement of cystatin C, 3 ml of blood was collected from a visible vein under an aseptic technique and centrifuged at 10,000 rpm for 10 min; the separated serum was transferred into another tube and stored at −20°C. Enzyme-linked immunosorbent assay kit was used for biological sample analysis; during which, a Biotin double antibody sandwich technique was used for quantitative measurement of cystatin c in biological fluids such as plasma, serum, plasma, and tissue homogenates.

Statistical analysis

GraphPad Prism Software and SPSS Version 26 (IBM, Chicago, United States) were used for data entry, analysis, and graphical presentation. Descriptive statistics included mean and standard deviation for continuous variables, whereas qualitative variables were expressed as a frequency of cases (n) and percent. The Kolmogorov–Smirnov test for normality was used before data analysis. Associations for quantitative variables were tested using one-way ANOVA and Student *t*-test. The Chi-square test was used to test for association between categorical variables. Receiver operating curve (ROC) analysis was conducted to establish the best value of the studied biochemical indicator to differentiate between the 3 study groups. $P < 0.05$ was used as a cutoff point for statistical significance.

Results

Sample demographics

This study included 94 pregnant women in their 3rd trimester; 32 (34%) of them had normal blood pressure and were included in the control group, 31 (33%) had mild PE, and 31 (33%) had severe PE.

Sample demographics are summarized in Table 1. Accordingly, the difference in the mean age of individuals was not statistically significant ($P = 0.08$) to reduce selection bias. Gestational age, however, was statistically different across all three groups ($P < 0.0001$) and during pairwise analysis using *t*-test. There was also no statistically significant difference in terms of gravidity and parity.

Maternal blood pressure

As expected, both systolic blood pressure and diastolic blood pressure were significantly higher in severe and mild PE with

Table 1: Sample demographics according to study group

Variables	Groups	*n*	Mean±SD	*P*
Maternal age (years)	Control	32	27.41±5.26	0.08
	Mild PE	31	30.64±5.64	
	Severe PE	31	29.98±7.07	
GA (weeks)	Control	32	38.89±1.22	<0.0001
	Mild PE	31	37.53±1.75	
	Severe PE	31	34.50±2.38	
Gravida	Control	32	3.62±1.72	0.17
	Mild PE	31	3.65±1.23	
	Severe PE	31	4.51±2.98	
Para	Control	32	2.06±1.52	0.22
	Mild PE	31	1.99±1.33	
	Severe PE	31	2.63±1.87	

Results for difference were calculated using one-way ANOVA.
SD: Standard deviation, PE: Preeclampsia, GA: Gestational age, ANOVA: Analysis of variance

$P < 0.0001$ and higher in severe PE than mild as shown in Table 2.

Renal function tests

In terms of standard renal function tests, no statistically significant difference was found in the levels of urea and creatinine across the three groups, with a $P = 0.11$ and >0.05, respectively, as shown in Table 2. For uric acid, although there was no statistically significant difference between controls and participants with mild PE ($P = 0.96$), the difference was statistically significant between participants with severe PE and the two other study groups.

Liver function tests

Mean alanine transaminase levels were significantly higher in severe PE compared to both controls and those with mild PE. Similar results are shown for aspartate transferase (AST) with severe PE having statistically ($P < 0.001$) higher AST levels compared to both controls and mild PE.

Urine albumin

All normotensive women in the control group tested negative for albumin in their urine, whereas patients in the mild PE group had albumin levels that were 1+. Patients in the severe PE group had albumin ≥2+; results are summarized in Figure 1.

Table 2: Clinical and biochemical parameters according to study group

Variables	Groups	*n*	Mean±SD	*P*
SBP (mmHg)	Control	32	125.98±11.53	<0.0001
	Mild PE	31	144.6±8.15	
	Severe PE	31	178.97±11.44	
DBP (mmHg)	Control	32	74.73±5.74	<0.0001
	Mild PE	31	89.74±3.23	
	Severe PE	31	114.19±8.70	
Urea (mg/dL)	Control	32	16.11±6.17	0.11
	Mild PE	31	16.59±5.69	
	Severe PE	31	19.76±9.76	
Creatinine (mg/dL)	Control	32	0.58±0.24	>0.05
	Mild PE	31	0.84±0.35	
	Severe PE	31	1.11±0.22	
Uric acid (mg/dL)	Control	32	4.45±1.3	<0.0001
	Mild PE	31	4.54±1.2	
	Severe PE	31	6.3±1.7	
ALT (U/L)	Control	32	16.92±6.21	<0.0001
	Mild PE	31	14.97±5.29	
	Severe PE	31	38.19±30.83	
AST (U/L)	Control	32	18.75±7.59	<0.0001
	Mild PE	31	20.12±9.34	
	Severe PE	31	61.48±44.59	
ALP (U/L)	Control	32	139.2±33.94	0.93
	Mild PE	31	135.7±30.81	
	Severe PE	31	135.9±50.76	
Cystatin C (ng/mL)	Control	32	0.39±0.15	<0.0001
	Mild PE	31	0.52±0.13	
	Severe PE	31	1.12±0.53	

Results for difference were calculated using one-way ANOVA. SD: Standard deviation, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, ALT: Alanine transaminase, AST: Aspartate transferase, ALP: Alkaline phosphatase, ANOVA: Analysis of variance, PE: Preeclampsia

Cystatin C

Analysis of variance in cystatin C was significant ($F = 26.95$, $P < 0.001$) which means there were significant differences in levels between the three groups. The mean of cystatin C for control 0.39 ± 0.15 ng/ml was significantly lower than for severe PE 1.12 ± 0.53 ng/ml, $P < 0.001$ but not significantly different ($P = 0.23$) from mild PE (0.52 ± 0.13 ng/ml). The mean cystatin C for mild PE was significantly lower than for the severe PE ($P < 0.001$). The means and standard deviations are shown in Table 2 and Figure 2.

Receiver operating curve analysis of biochemical markers

Cystatin C showed more accurate diagnostic ability compared to urea and creatinine. From Figure 3, at a cutoff value of >0.61 ng/ml, cystatin c had a sensitivity of 90.32%, and a specificity of 93.75%. For creatinine, the cutoff value was 0.64 mg/dl and for urea 13.17 mg/dl.

The performance of uric acid at a value of >4.4 mg/dl was as follows: sensitivity: 87.10%, specificity: 71.87% indicating that uric acid exhibits a reasonably high diagnostic accuracy. The area under the curve of 0.834 suggests good discriminative ability, with the marker being able to effectively differentiate between the groups, as shown in Table 3.

Discussion

Serum cystatin C, in the present study, had higher levels in pregnant women with PE compared to healthy normotensive pregnant females. This is in agreement with other studies, which showed a significant difference ($P < 0.04$) in serum cystatin C between individuals with pregnancy-induced hypertension, PE, and healthy pregnant women.[9,10] Another study by Novakov Mikic *et al*. in 2012[7] also showed that the serum of pregnant women with PE had significantly higher levels of cystatin C, uric acid, and creatinine than the control group. In the current study, uric acid was also found to be higher in those with severe PE compared to mild PE, but no difference was observed in creatinine.

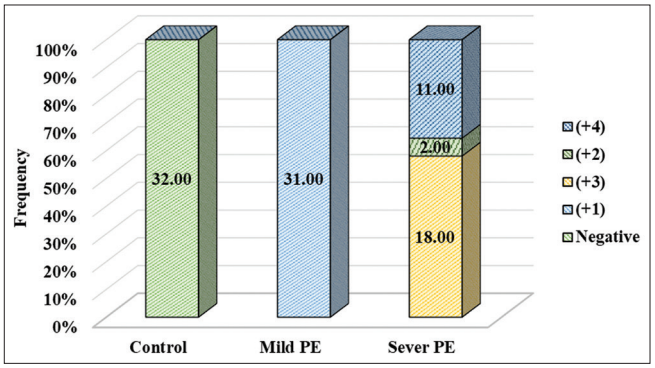

Figure 1: Frequency of different urine albumin levels by dipstick across study groups

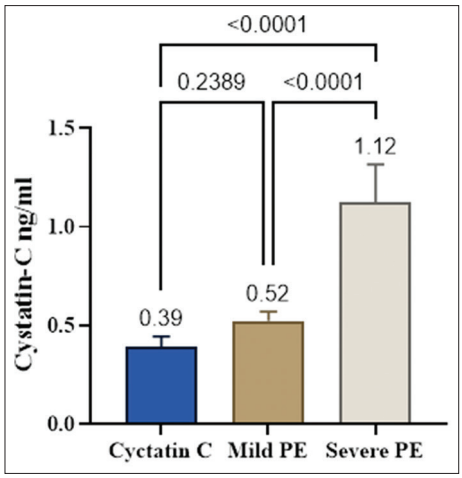

Figure 2: Comparisons in cystatin c levels across the study groups

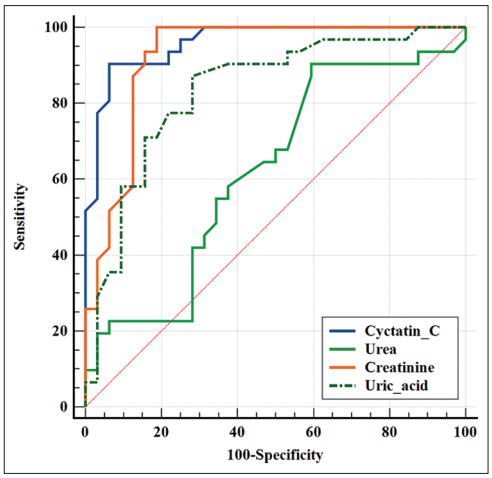

Figure 3: Receiver operating curve for cystatin c, creatinine, urea, and uric acid

Table 3: Receiver operating curve analysis for biochemical markers (cystatin-C, creatinine, urea, and uric acid) in differentiating between severe preeclampsia and controls

Analysis criteria	Cystatin-C	Urea	Creatinine	Uric acid
AUC	0.960	0.622	0.924	0.832
SE	0.0212	0.0722	0.0358	0.0527
95% CI	0.878–0.993	0.491–0.741	0.829–0.976	0.719–0.916
Cuff-off	>0.61	>13.17	>0.64	>4.4
Sensitivity	90.32	90.32	100.0	87.10
Specificity	93.75	40.63	81.25	71.87
Positive LR	14.45	1.52	5.33	3.10
Negative LR	0.10	0.24	0.00	0.18
PPV	93.3	59.6	83.8	75.0
NPV	90.9	81.2	100.0	85.2

AUC: Area under the curve, SE: Standard error, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value, LR: Likelihood ratio

Creatinine is considered the most extensively used biomarker of renal function, yet it is ineffective in the early stages of renal failure.[11] It also has limitations as a GFR marker because serum levels can be altered by a variety of nonrenal variables such as muscle mass, age, gender, and diet.[9] Our results show no difference in creatinine levels between groups, as well as results from other studies such as Strevens *et al*. 2001, Chen *et al*. in 2005, and Kristensen *et al*. in 2007, highlight the need for more biomarkers to assess renal impairment in PE patients.[9,10,12]

Using receiver operating characteristic analysis, serum cystatin C showed higher diagnostic accuracy when compared to other standard kidney markers, urea, creatinine, and uric acid, with a sensitivity and specificity for cystatin C detection calculated to be 90.32% and 93.75%, respectively, at a cutoff value of >0.61 ng/ml. These results are consistent with those found by Singh *et al*. in 2017, who found that cystatin C was more sensitive (94%) and specific (88%) than urea and creatinine.[13]

According to research done by Bramham *et al*. in 2010, pregnant women had higher levels of cystatin C in their blood.[14] In our study, the mean blood cystatin C levels of preeclamptic patients were substantially higher than those of healthy pregnant women and because cystatin C levels are also unaffected by age, gender, race, ethnicity, muscle mass, or diet, it is regarded as a superior marker for assessing renal function and GFR than standard renal markers such as creatinine and urea.[15,16]

Limitations of the study

Our study uses a convenient sampling method, not randomized which can cause an inability to generalize the result. In addition, our study focuses mainly on the usage of cystatin c during the 3rd trimester rather than as a predictor for future PE when measured earlier during the 1st and 2nd trimester. Future studies should be done to address these limitations.

Conclusions and Recommendations

Patients with severe PE had higher serum cystatin C than patients with mild PE and healthy normotensive pregnant females. Serum cystatin C at a cutoff value of >0.61 had higher diagnostic accuracy than urea and creatinine with a sensitivity and specificity of 90.32% and 93.75%, respectively. We, therefore, recommend performing a serum cystatin C assessment during the work-up of renal impairment in severe PE.

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Conflicts of interest

There are no conflicts of interest.

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