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Assessment of High Serum Cystatin C as an Early Marker of Renal Impairment in Pre Eclampsia

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Abstract

Introduction: *Preeclampsia is the most common medical complication during pregnancy and one of the leading causes of maternal and perinatal morbidity and mortality in Bangladesh. Kidney has role in both adaptive physiology of normal pregnancy and in pathophysiology of preeclampsia. Among the new biomarkers, serum Cystatin C can reliably reflect the GFR in both healthy and hypertensive pregnant women. It is important to evaluate the diagnostic efficiency of Cystatin C as a marker of renal function in preeclampsia.* ***Aim:*** *To assess high serum Cystatin C level as an early marker of renal impairment in pre-eclamptic patients.* ***Materials and Methods:*** *From March,2021 to February,2022 (A total of 12 months) a cohort study was conducted among 66 pregnant women, aged 18 to 40 years with pre-eclampsia and normal serum creatinine (0.5-0.8mg/dl) at their 20-28 weeks of gestation attending the antenatal clinic and admitted in the Department of Feto maternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, selected by non random purposive and convenient sampling.* ***Results:*** *Out of 66 respondents, final analysis was done with 62 patients. Among 62 study samples, 76% patients had "High Cystatin C" level that was greater than 0.84 and renal impairment developed in 15% pregnant women. Majority of the participants were in 25-30 age group (46.8%). The mean serum creatinine level of the pregnant women increased throughout follow up and it was statistically significant ($p<0.05$) in both cases. No significant difference was found between serum Cystatin C in renal impaired and normal renal function pre eclampsia patients at a cut off value of 0.84. ROC analysis of serum Cystatin C level for detection of renal impairment among Pre eclamptic patients found a cut-off value of ≥1.49 showed the highest Youden index of 0.721. The sensitivity, specificity, PPV, NPV and accuracy of serum Cystatin C were 77.78%, 94.34%, 70.00%, 96.15% and 91.94%.* ***Conclusion:*** *Higher Cystatin C level in pre-eclampsia reflected renal impairment at an early stage even before conventional marker like serum creatinine raise. The diagnostic efficiency of Cystatin C as a marker of renal function in pre eclampsia can be used to reduce maternal morbidity and mortality of Bangladesh.*

Keywords: *Cystatin C level, Preeclampsia, Early marker of renal impairment.*

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Introduction:

Pre-eclampsia is a pregnancy specific hypertensive disorder and one of the common medical complications during pregnancy. It is a significant cause of maternal and perinatal morbidity and mortality.

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It complicates 5-10% of pregnancy worldwide[1]. The incidence of pre-eclampsia in nulliparous patient is 2-7% and in multiparous 5%[2]. Etiology of pre-eclampsia is explained by multiple inflammation theories like inadequate trophoblast invasion, endothelial cell activation, inflammation along with immunological, genetic, pro and antiangiogenic factors etc. Uterine natural killer cells (NK) produce a series of cytokines involved in angiogenesis and vascular stability of growing placenta, including vascular endothelial growth factor (VEGF), placental growth factors (PLGF) and angiopoietin 2. These cytokines play key role in regulating trophoblast invasion and the maternal placental bed vascular changes[3]. Pre-celampsia is characterized by poor trophoblastic invasion resulting oxidative stress, hypoxia and release of factors that promotes endothelial dysfunction, inflammation and end organ ischemia[4]. This endothelial dysfunction leads to imbalance between angiogenic and antiangiogenic factors[5]. Kidney is the most important organ affected by pre-eclampsia. It has role in both adaptive physiology of normal pregnancy and in pathophysiology of pre-eclampsia[6]. The diagnosis of renal impairment in pregnancy is based on raised serum creatinine level. However, serum creatinine level does not rise until moderate to severe decrease of glomerular filtration rate (GFR) (<40 ml/min/1.73m^2) in renal function impaired patients. This insensitivity for small to moderate decrease in GFR in creatinine blind GFR area (40-70 ml/min/1.73 m^2) gives a false sense of normal renal function and late detection of renal impairment[7]. There are different tests available for diagnosing renal function impairment. However, these have technical limitations and contraindicated during pregnancy[8]. Therefore, an appropriate marker which provides an accurate GFR based value for early detection of renal impairment is the keynote of this study. Among new biomarkers Cystatin C is found in virtually all body fluids and tissues. Due to its low molecular weight (13-kDa) and basic isoelectric point, Cystatin C is removed from the bloodstream by glomerular filtration, reabsorbed and catabolized by tubular epithelial cells[9]. After glomerular filtration, it is fully catabolized in the proximal convoluted tubule and is not returned in blood. Concentration of serum Cystatin C is not affected by gender, age, race, protein intake and muscle mass like serum creatinine. Cystatin-C has drawn the attention of a battery of research studies. In order to overcome the obstacles in estimating renal function in pregnant women, studies have demonstrated that serum Cystatin-C can reliably reflect the GFR in both healthy and hypertensive pregnant women[10]. It is more sensitive marker for kidney dysfunction, capable to detect small reductions in GFR, and a pre-clinical status of kidney dysfunction, which cannot be detected by serum creatinine or creatinine based GFR. Thus, mild renal dysfunction can be detected earlier with raised Cystatin C[11]. The measurement of serum Cystatin C is very simple, non-invasive and safe method for early detection of renal impairment in pre eclamptic patient. Studies have reflected that Cystatin C can reliably reflect the GFR in both healthy and hypertensive pregnant women[10]. This investigation is used for early detection of renal impairment in developed countries. KDIGO (2012) and NICE (2021) have both recommended Cystatin C as a superior biomarker of in assessing renal function with accuracy in CKD patients[12,13]. However, to the best of our knowledge, there is no such study regarding this topic in our country conducted beforehand and no established protocol in detecting renal insufficiency in this group of patients. Therefore, the objective of this study was to evaluate the diagnostic efficiency of Cystatin-C as a marker of renal function in pre-eclampsia.

Materials and Methods:

The cohort study was carried out in the Department of Feto maternal Medicine (indoor and outdoor) BSMMU, Dhaka from January, 2021 to December, 2021. Ethical clearance for this study was taken from the Institutional Review Board of BSMMU. Detailed sociodemographic information, obstetrics history, gestational age was recorded in a predesigned data sheet. Then physical examination was performed and recorded. Patient who developed pre-eclampsia in between 20 to 28 weeks and normal serum creatinine (0.5-0.8 mg/dL) were included in this study. Serum Cystatin C level measurement was done after first detection of pre-eclampsia. Then Patients were divided into two groups based on serum Cystatin C being <0.84 mg/L and ≥0.84 mg/L. These patients were followed up with serum creatinine 2-3 weekly upto delivery for research purpose. Patients were considered renal impaired when their S.creatinine was ≥1.1mg/dl during subsequent follow up. Other investigations were also done accordingly for management purpose. Data was analyzed using SPSS 24 software. With all aseptic precaution 5 ml blood from antecubital vein was collected from each subject and taken in a sterile vacuum container and send to the department of Microbiology of BSMMU. Serum Cystatin level was done by N Latex Cystatin C Kit14 and serum creatinine level was analyzed by a kinetic colorimetric assay on a Hitachi 912 Analyzer. Statistical analysis was carried out by using Windows based Statistical Package for Social Sciences (SPSS) version 24.0.

Result:

This cohort study was carried among 66 pregnant women with diagnosis of pre-eclampsia with normal renal function at their 20-28 weeks of gestation, attended at Feto maternal Medicine (indoor and outdoor) and among them four patients were lost during follow up. The age of majority of the participants were in 25-30 group (46.8%) with a mean of 28.4±4.5 years. About half (48.4%) of the participants were educated to HSC level and above. Half of the participants were housewives (50.0%) and monthly income of half of the participants (48.4%) were more than 25,000 Taka. Most of the patients in high Cystatin C were in the age group of 25 to 30 years. No significant difference was

found for age between high and normal Cystatin C patients (P=0.117) (Table I).

Table I: Association of age of the participants with serum Cystatin C level

Parameters	High Cystatin C (n=47)	Normal Cystatin C (n=15)	p-value
	Frequency (%)	Frequency (%)	
Age (years)			
18-24	11 (23.4)	2 (13.3)	0.117
25-30	24 (51.1)	5 (33.3)	
31-40	12 (25.5)	8 (53.3)	
Mean ± SD	27.89 ± 4.33	30.00 ± 4.91	

Table II demonstrates that there was no significant difference in terms of gravidity, parity or previous history of pre-eclampsia between high and normal Cystatin C in pregnant women.

Table II: Obstetric history of the participants and serum Cystatin C level

	High Cystatin C (n=47)	Normal Cystatin C (n=15)	P value
Gravidity			
Primi	15 (31.9)	5 (33.31)	0.919
Multi	32 (68.1)	10 (66.7)	
Parity			
Nulliparous	7 (14.9)	0 (0)	0.180
Multiparous	40 (85.1)	15 (100)	
Previous history of pre-eclampsia			
Yes	11 (23.4)	6 (40)	0.210
No	36 (76.6)	9 (60)	

Among high Cystatin C group only 19% patient developed renal impairment, 81% had normal renal function. Among normal Cystatin C group 100% patient had normal renal function. P-value was not significant (0.067) (Table – III).

Table III: Association of renal impairment with level of Cystatin C

Parameter	High Cystatin C (n=47)	Normal Cystatin C (n=15)	P value
Renal impairment	9 (19%)	0	0.067
No Renal impairment	38 (81%)	15 (100%)	

ROC analysis

ROC analysis of Serum Cystatin C level for detection of renal impairment among pregnant women found an AUC value of 0.945 (95% CI 0.881-1.000) which was statistically significant (P <0.001) (Figure 1 and Table IV).

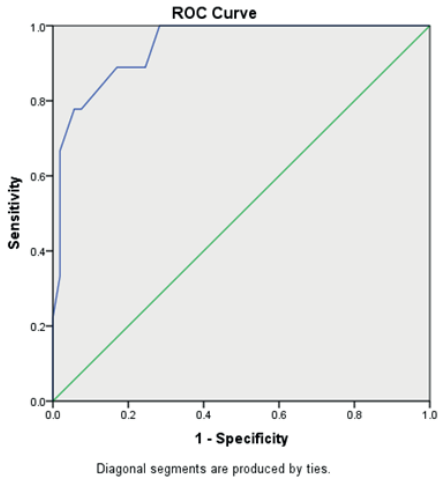

Figure 1: ROC analysis of Serum Cystatin C level

Table IV: Area Under the Curve

Test Result Variable (s): Serum Cystatin C				
Area	Std. Errora	P-value	95% Confidence Interval	
			Lower Bound	Upper Bound
0.945	0.033	<0.001	0.879	1.011

A cut-off value of ≥1.49 showed the highest Youden index (0.721) with 77.8% sensitivity and 94.34% specificity (Table V).

Table V: Determination of cut off value with Youden index (j=Sen+Spe-1)

Cut off point	Sensitivity	1-Specificity	Specificity	Youden index
1.385	0.889	0.17	0.83	0.719
1.44	0.778	0.075	0.925	0.703
1.49	**0.778**	**0.057**	**0.943**	**0.721**
1.525	0.667	0.019	0.981	0.648

In addition, the accuracy was 91.94%. Moreover, A cut-off value of ≥1.49 showed, PPV and NPV of 70% and 96.1% (Table VI).

Table VI: Diagnostic Accuracy of Serum Cystatin C Level for Early Diagnosis of Renal Impairment

Statistics	Value	95% CI
Sensitivity	77.78%	39.99% to 97.19%
Specificity	94.34%	84.34% to 98.82%
PPV	70.00%	42.41% to 88.09%
NPV	96.15%	88.03% to 98.84%
Accuracy	91.94%	82.17% to 97.33%

Discussion:

This study reflected that Cystatin C has acceptable sensitivity and specificity in detecting renal impairment in pre-eclampsia at a cut off value of >1.49 mg/l which was significant. In this study the age of

majority of the participants were in 25-30 group (46.8%) with a mean of 28.4±4.5 years. No significant difference was found for age between high and normal Cystatin C patients. Dharnidharka et al. also found in their study that the risk of raised Cystatin C among pre-eclampsia patients was independent of maternal age and the finding supports our statement[15]. Niraula et al had also showed that the concentration of serum Cystatin-C was unchanged between various age and the difference was not significant[16]. In this study the level of serum Cystatin C >0.84 was considered as "High Cystatin C". About 76% patients of this study had high level of Cystatin C. Production of Cystatin-C might be increased during pregnancy due to an increased number of nucleated cells which is supported by a study showing serum Cystatin-C is increased during twin pregnancy[17]. In another study Niraula et al., speculated that there could be a shift towards a more cationic glomerular barrier in pregnant women, resulting in higher serum concentrations of Cystatin-C during pregnancy[16]. Babay et al. reported serum Cystatin C levels were 0.651 ± 0.14 mg/l during the second trimester, and increased again to 0.82 ± 0.191 mg/l in the third trimester[18]. Obrenovic et al. reported a significant increase in serum Cystatin C level with increase of the gestational age (0.78 ± 0.26 mg/l in second trimester vs. 1.21 ± 0.30 mg/l in third trimester)[19]. Another study showed that the mean gestational age of pregnant women with PE was 35.02 ± 4.59 weeks and mean serum Cystatin-C level was higher in PE compared to the control group (1.15 ± 0.37 versus 0.55 ± 0.12) and was statistically significant[16]. No significant difference in terms of parity or previous history of PE among two groups were observed in this study. In another study no significant differences were observed between pre eclamptic patients and control groups regarding age, gravidity and parity[20]. The mean serum creatinine level of the PE patients was (0.64±0.08) during the time of enrollment at 20-28wks of gestation and increased with advancement of their gestational age throughout the follow-up period in both groups in this study. All the enrolled patients had normal serum creatinine at first visit. But 9 patients developed raised serum creatinine above baseline creatinine level of impaired renal function at their subsequent follow up and they were termed as impaired renal function. The CRADLE 2 trial was a prospective observational study done in South Africa in a tertiary hospital, where 17.6% had raised creatinine (>1.02 mg/dl) featuring impaired renal function[21]. Incidence of renal dysfunction was similar to this current study. Another study showed that serum creatinine was higher in PE compared to control group but the distribution between the two groups was statistically insignificant[16]. The present study revealed that 47 (76%) PE patients had high Cystatin C level above kit reference range among them only 9 patient developed renal impairment. So no significant association was found between renal impairment and high Cystatin C value as per 0.84mg/l threshold value. But all 9 renal function impaired PE patients had a high Cystatin C value. In this study ROC analysis of Cystatin C for detection of renal impairment among pre-eclampsia patients a cut off value of ≥1.49 showed the highest Youden index (0.72) with 77.8% sensitivity and 94.3% specificity. In addition, the accuracy was 91.94%, PPV and NPV of 70% and 96.1% respectively. Jummaat et al found that the sensitivity and specificity of Cystatin C in pre-eclampsia population was 84.6% and 86.7 %, respectively and ROC curve identified the level of Cystatin C between 0.574 and 0.898 and area under curve was 0.736[22]. Niraula et al., had almost the same result with a diagnostic efficacy showing sensitivity 88.24% and specificity 98.04 with a cut of value of >0.9[16]. The cut off value was higher in our study probably due to the different characteristics of sample population and lower number of sample size, different kit. Therefore, it can be said that Cystatin C of PE patients with impaired renal function were significantly higher than normal renal function PE patients.

Conclusion:

In this study, mean Serum Cystatin C level is significantly higher in renal function impaired PE patients. Higher Cystatin C above a cutoff range (≥1.49mg/l) level in pre-eclampsia likely reflects renal impairment at an early stage even before conventional marker like serum creatinine raise. This serum Cystatin C is not affected by age, BMI, parity or previous history of pre-eclampsia. Therefore, it can be used as an early predictor of renal impairment in pre-eclampsia with more diagnostic accuracy.

Conflict of Interest: None.

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