



CYSTATIN C IN PREECLAMPSIA: A CASE CONTROL STUDY

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ABSTRACT

**Background:** Preeclampsia is one of the common complications in pregnancy and contributes significantly to maternal and perinatal morbidity and mortality. It is a multisystem disorder with renal failure as one of its major complication. Assessment of renal function is important in the evaluation of these patients. Cystatin C, a chain of 120 amino acids is a new marker of renal function. Present study conducted to evaluate diagnostic value of Cystatin C serum levels as alternative marker of renal function in Pre-eclampsia (PE) and compare it with the traditional markers of renal function, Creatinine and BUN. Present study might give us an early marker of renal dysfunction in preeclampsia.

**Material and Methods:** In this case-control study markers of kidney function were investigated in two groups: one with newly diagnosed cases of preeclampsia PE ( $n = 50$ ) and the other of healthy primigravida as controls ( $n = 50$ ) of same gestational age. Serum Cystatin C levels were measured as well as levels of traditional renal markers like Creatinine and BUN. Data analyzed by Students Unpaired t test.

**Results** demonstrated that difference for BUN & Creatinine between cases (mean BUN=12.46 mg/dl, mean Creatinine=0.7 mg/dl) & controls (mean BUN=8.62 mg/dl, mean Creatinine=0.62 mg/dl) were not statistically significant. But the results showed that difference for Cystatin C (mean for cases 1.86 mg/L and for controls 0.73) was highly significant ( $P=0.0001$ ).

**Conclusion-** we found that Serum Cystatin C is early and superior marker of renal function in Preeclampsia.

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INTRODUCTION

Pregnancy can be the most wonderful experience life has to offer. But it can also be dangerous. Pregnancy is a physiological state associated with substantial and, on occasion, profound alterations in metabolic and biochemical processes. If there are no complications these biochemical changes are reversible following delivery (Frund *et al.*, 1990). Hypertension and proteinuria have long been recognized to be important complications of pregnancy (Dennis *et al.*, 1988). Hypertension in the pregnancy is the major cause of fetal growth retardation and perinatal mortality (Ferrazzani *et al.*, 2008). Preeclampsia affects 5–7% of all pregnancies and is a leading cause of maternal and perinatal morbidity and mortality (Nora Franceschini *et al.*, 2008; Roberts *et al.*, 2005; Zhang *et al.*, 2003). Preeclampsia is associated with increased risk of ischemic stroke (Nora Franceschini *et al.*, 2008; Wilson *et al.*, 2003), and cardiovascular disease (Nora Franceschini *et al.*,

2008; Smith *et al.*, 2001 and Arnadottir *et al.*, 2005). Preeclampsia manifests by proteinuria, hypertension, and impaired renal function (Nora Franceschini *et al.*, 2008 and Lafayette *et al.*, 1998). Preeclampsia was defined as sustained pregnancy-induced hypertension with proteinuria. Hypertension was defined as sustained blood pressure readings of  $\geq 140/90$  mmHg (with readings taking place  $\geq 6$  hours apart) and/or a sustained 15 mm Hg diastolic rise or a 30 mm Hg systolic blood pressure above first-trimester values. Proteinuria was defined as urine protein concentration  $\geq 30$  mg/dL (or 1+ on a urine dipstick) on  $\geq 2$  random specimens collected  $\geq 4$  hours apart. Regardless of precipitating aetiology, the cascade of events that leads to preeclampsia syndrome is characterized by a host of abnormalities that result in vascular endothelial damage with vasospasm, transudation of plasma and ischemic and thrombotic sequelae (Maybury and Waugh, 2004; Dutta, 2005). Extensive changes occur in the renal system in preeclampsia. As a part of the "end organ pathology" preeclamptic glomeruli undergo structural changes with pronounced endothelial vacuolization and hypertrophy of the cytoplasmic organelles first defined as glomerular

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endotheliosis (Maybury and Waugh, 2004; Dutta, 2005). The net effects are reduced renal blood flow and GFR, impaired tubular reabsorption or secretory function (Maybury and Waugh, 2004; Dutta, 2005). Renal damage in PE is one of the key components of the pathophysiological process, and among the most significant events which lead to this is endothelial dysfunction (Aleksandra *et al.*, 2012; Nora Franceschini *et al.*, 2008; Wang *et al.*, 2004).

Serum creatinine is an unreliable measure of kidney function in pregnancy (Nora Franceschini *et al.*, 2008; Strevens *et al.*, 2001). Cystatin C has been proposed as a measure of kidney function in population studies (Nora Franceschini *et al.*, 2008; Levin *et al.*, 2005) and in pregnancy (Nora Franceschini *et al.*, 2008; Strevens *et al.*, 2001). Several authors have proposed serum Cystatin C levels as a possible alternative marker of renal function (Aleksandra Novakov Mikic *et al.*, 2012; Roos *et al.*, 2007; Dharnidharka *et al.*, 2002; Strevens *et al.*, 2002), especially in detection of small changes in GFR (Aleksandra Novakov Mikic *et al.*, 2012; Laterza *et al.*, 2002; Coll *et al.*, 2000). Cystatin C, an endogenous low molecular weight protein (13 kDa), a member of the cysteine proteinase inhibitors family, which is produced at a constant rate in all nucleated cells, then freely filtered across the glomerular membrane, and finally reabsorbed and thoroughly metabolized in proximal tubules (Aleksandra Novakov Mikic *et al.*, 2012; Chew *et al.*, 2008).

Unlike creatinine, changes of cystatine C concentrations in serum are less susceptible to the influence of non-renal factors such as gender, age, muscular mass and inflammation (Aleksandra Novakov Mikic *et al.*, 2012; Hojs *et al.*, 2006; Stevens *et al.*, 2008). Cystatin C shows a high correlation with ioxeol clearance and <sup>51</sup>Cr-EDTA, which are considered to be gold standards for GFR evaluation (Aleksandra Novakov Mikic *et al.*, 2012; Larsson *et al.*, 2004; Garlipp *et al.*, 2008). Cystatin C level is stable until the third trimester when the level rises (Aleksandra Novakov Mikic *et al.*, 2012; Kristensen *et al.*, 2007). Apart from being marker of renal dysfunction, the hypothesis is that Cystatin C could have a direct role in etiology of PE, since placental expression of Cystatin C is increased in patients with PE (Aleksandra Novakov Mikic *et al.*, 2012; Thilaganathan *et al.*, 2009). Present study conducted to evaluate diagnostic value of Cystatin C serum levels as alternative marker of renal function in Preeclampsia (PE) and compare it with the traditional markers of renal function, Creatinine and BUN. Present study might give us an early marker of renal dysfunction in preeclampsia.

## MATERIAL AND METHODS

The study was conducted at the Department of Biochemistry, in a tertiary care centre hospital, after being approved by the Institutional Ethics committee. An informed consent was signed by all the participating women. Markers of kidney function were investigated in two groups of pregnant women: one with preeclampsia PE ( $n = 50$ ) and the other of healthy pregnant women ( $n = 50$ ). The second and third trimester primigravidae patients attending the obstetrics OPDs for routine follow up and patients from obstetrics ward were enrolled for the study. Subjects were grouped into two groups.

**The inclusion and exclusion criteria were-**

### Inclusion Criteria

The Study group included primigravida patients > 20weeks gestation with proteinuria with BP  $\geq 140/90$ mmHg and the Control group included primigravida women > 20weeks gestation normotensive and nonproteinuric. The common inclusion criteria for both groups were: singleton pregnancy, normal foetal morphology and the absence of concomitant disease and gestation between >20 and < 36 gestational weeks.

### Exclusion Criteria

(For both the groups) Multiple Pregnancy, Previous History Of Abortion, Hypertension, Diabetes Mellitus, Cardiac illness, Gestational trophoblastic diseases, High grade fever or Any Concomitant illness.

The fasting blood samples were collected in plain evacuated tubes. Samples were transferred to the laboratory where serum was separated and biochemical tests were performed. Results of Preeclampsia (PE) group were compared with the results of control group of healthy pregnant women group, matched for age and gestation. Serum Cystatin C levels were measured by automated (LEIT) latex enhanced immunoturbidimetric assay using nephelometer. BUN and Creatinine tests were performed on Beckman AU400 Fully automated chemistry analyzer.

### Statistical Analysis

For each parameter studied, mean and standard deviation was calculated to estimate the significance. The difference between the groups was measured by Students Unpaired 't' test and chi square test. P Value less than 0.05 considered as statistically significant. The calculations were performed using the statistical program SPSS for Windows Version 13, with a  $p$  value of < 0.05 considered significant.

## RESULTS

The demographic characteristics of the study and control groups are as follows-

Majority of subjects belonged to age group between 22-26 years. The period of gestation of all subjects was between 26-36 weeks. All the subjects in both the groups were primigravida patients. Our results demonstrated that difference between demographic characteristics between cases and controls was not statistically significant.

Figure 1 shows Age distribution of Study group and Control group. Figure 2 shows Gestational Age of Study group and Control group. Table no. 1 shows Comparison of blood urea nitrogen (BUN), Serum Creatinine and Serum Cystatin C levels between cases and controls. Third figure shows Comparison of BUN between Study and Control group. BUN was found to be increased in 3 preeclampsia patients only and it was found to be slightly higher in study group compared to control group but values were within reference range. Maximum value of BUN was 34 mg% in study group and mean was 10.4mg%. Fourth

figure shows Comparison of Serum creatinine between Study and Control group.

group but it was non-significant. Maximum value was 1.3 mg%.

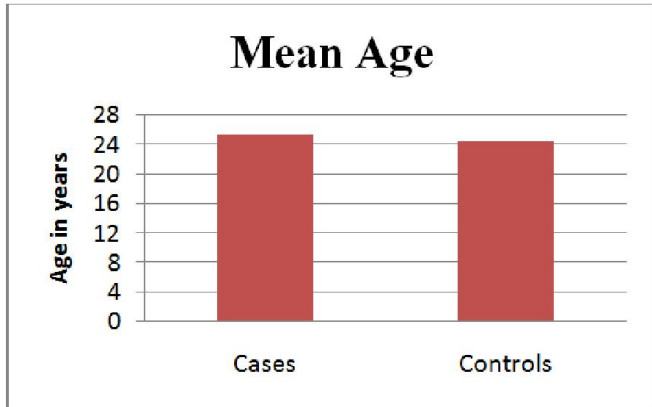


Fig.1. Age distribution of Study group and Control group

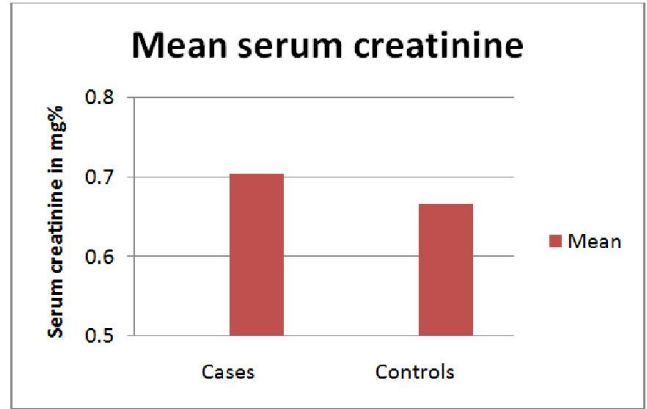


Fig.4. Comparison of Serum creatinine between Study and Control group

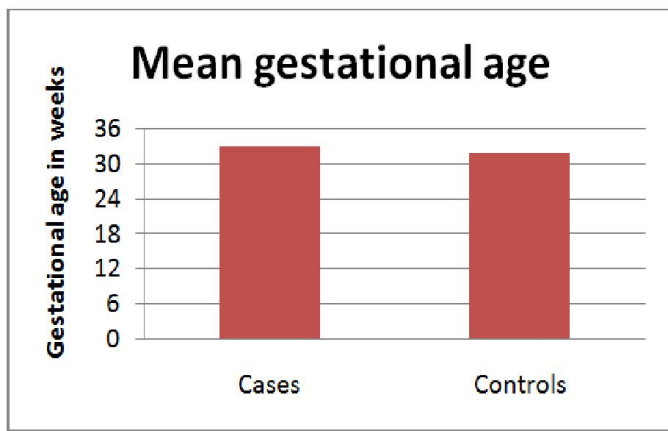


Fig.2. Gestational Age of Study group and Control group

Fifth figure Comparison of Serum Cystatin C between Study and Control group.

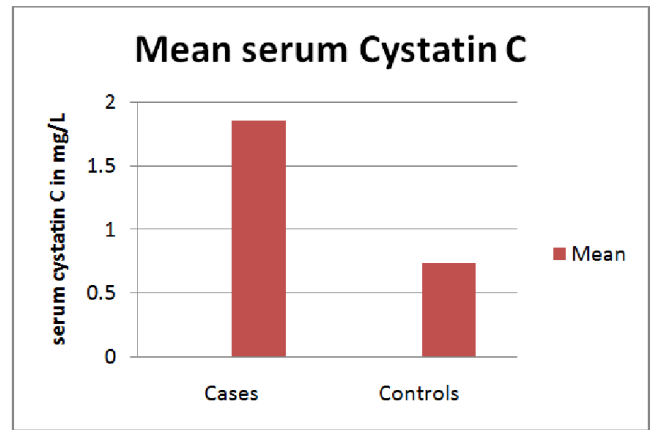


Fig.5. Comparison of Serum Cystatin C between Study and Control group

Table 1. Comparison of blood urea nitrogen (BUN), Serum Creatinine and Serum Cystatin C levels between cases and controls

Parameter	Cases (n= 50) (Mean ± SD)	Controls (n= 50) (Mean ± SD)	P value
BUN (mg%)	10.44 ± 4.8	9.64 ± 1.54	0.26, NS
Serum Creatinine (mg%)	0.70 ± 0.157	0.66 ± 0.10	0.13, NS
Serum Cystatin C (mg/L)	1.861± 0.673	0.735± 0.084	0.0001, S

Cystatin C showed highly significant increase in cases of preeclampsia as compared to controls. It was found to be increased in 96% of cases, i.e. increased in 48 patients out of 50 (n=50).

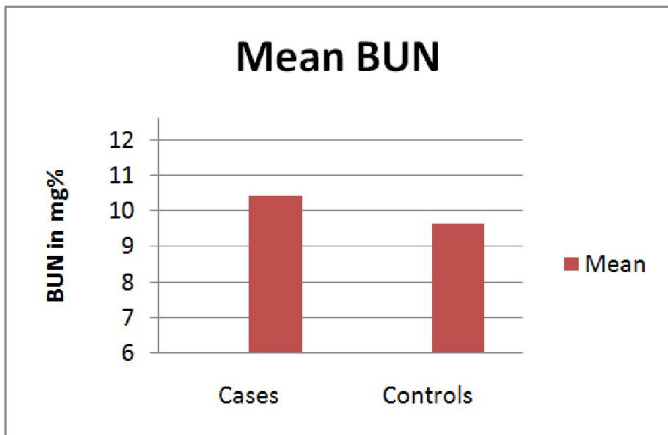


Fig.3. Comparison of BUN between Study and Control group

Serum creatinine was found increased in only 2 cases of preeclampsia and mean was found to be slightly higher in study

## DISCUSSION

Preeclampsia can cause changes virtually in all organ systems, most notably the cardiovascular, renal, hematologic and immunologic systems. Some of these changes are present before the clinical diagnosis of preeclampsia, as been clearly demonstrated by several investigators (Rodriguez *et al.*, 1998). It is responsible for majority of maternal and foetal complications. The predominant pathology, which is endothelial dysfunction, sets in as early as 8-18 week, however, the signs and symptoms appear in the late mid-trimester. In order to arrest the disease process in the initial stages or to prevent complications especially in women predisposed to preeclampsia, various predictors have been proposed time and again (Madira *et al.*, 2008). One of the most common complications of preeclampsia is renal failure. BUN and S. Creatinine are commonly elevated parameters in any renal failure. In our study, the mean blood urea nitrogen (BUN)

value in study group is  $10.44 \pm 4.81$  mg/dl when compared to the controls which had mean value of  $9.64 \pm 1.54$  mg/dl. (Table no. 2) Although the study group had slightly higher values than control group, the difference was not significant. Mean Serum creatinine value in study group is  $0.704 \pm 0.157$  mg/dl as compared to controls which had  $0.67 \pm 0.11$  mg/dl. Here also the study group had slightly higher values than control but were statistically insignificant.

Our data agrees with the studies carried out by Strevens *et al.* 2001, and Richard Lafayette *et al.* (1998). In both studies mean serum creatinine levels were increased in preeclampsia but were not above the reference interval. The biochemical changes due to renal failure are reflected by changes in serum creatinine which is considered as superior marker of renal function than BUN (blood urea nitrogen) but both these are not accurate markers of renal function in preeclampsia Lafayette *et al.* (1998). Moodley *et al.* (2004). concluded that the combined use of Serum Cystatin C and serum creatinine provides the best possible information on renal function in preeclampsia. of all tests of renal function, Serum Cystatin C measurement is the latest one. Some work has been done on alterations of Serum Cystatin C in preeclampsia; however the investigation has not yet become popular. Hence the present study was undertaken to find out the use of Serum Cystatin C as early and better marker of renal function in preeclampsia. The main aim of this study was to determine whether Serum Cystatin C is superior to other renal function markers in preeclampsia and whether Serum Cystatin C can be used as predictor of preeclampsia. In this study, the mean value of Serum Cystatin C in study group was  $1.86 \pm 0.673$  mg/L compared to control group which had mean of  $0.73 \pm 0.084$  mg/L. The increase in Serum Serum Cystatin C levels in study group as compared to controls is statistically highly significant ( $p$ -value  $< 0.0001$ ). Our results are consistent with the results of Strevens *et al.* (2001, 2002) Babay *et al.* (2005). Nora Franceschini *et al.* (2008). Thilaganathan Basky *et al.* (2009) Sherif Saleh *et al.* (2010), Mona k farag *et al.* (2011), Hong-Xia Guo *et al.* (2012) and Aleksandra Novakov Mikic *et al.* (2012). In 2001, Strevens *et al.* (2001, 2002) concluded that the serum level of Serum Cystatin C had a superior diagnostic accuracy for preeclampsia compared to those of serum creatinine. Our results are strongly in agreement with this study.

The findings are similar to Babay *et al.* (2005), who concluded that Serum Cystatin C can be used for close supervision and early diagnosis of renal impairment in pregnant patients and is reliable marker of renal function. The findings also are in agreement with Nora Franceschini *et al.* (2008), who concluded that increased plasma levels of Serum Cystatin C were independently associated with preeclampsia & Basky Thilaganathan *et al.* (2009). Who demonstrated that maternal Serum Cystatin C concentrations in early pregnancy may be of value in identifying women at high risk of developing preeclampsia. Similar finding were observed by Sherif Saleh *et al.* (2010), Mona k farag *et al.* (2011), Hong-Xia Guo *et al.* (2012) and Aleksandra Novakov Mikic *et al.* (2012). It is also to be noted that the results did not match with the results of Akbari (2005) *et al.* who concluded that Cys-C was a poor marker of renal function. Also our results were in partial agreement with the results of Aleksandra Novakov Mikic *et al.*

who concluded that Serum Cystatin C and serum creatinine were significantly higher in preeclampsia group.

## Conclusion

- It is concluded that amongst BUN, S.Creatinine & Serum Cystatin C, Serum Cystatin C is the earliest & a better marker of renal dysfunction in preeclampsia.
- Serum Cystatin C being better marker needs to be routinely tested in all case of preeclampsia which will not only give early diagnosis of renal failure but will also help to start early treatment, for better prognosis of the mother as well as the foetus.

**Conflict of interest:** Authors report no conflict of interest.

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