



Research Article

## Evaluation of Serum Cystatin C as an Indicator of Glomerular Filtration Rate and its Correlations to other Biomarkers in Detection of Pre-eclampsia.

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**Keywords:**

Glomerular Filtration Rate, Pre-eclampsia, Serum Cystatin C, Serum Albumin, Serum Aspartate Aminotransferase

**ABSTRACT**

**Background:** The Glomerular Filtration Rate (GFR) is the best indicator of renal function. Serum cystatin C has been introduced as an endogenous marker of GFR. However, there are conflicting reports regarding its use in pregnancy and pre-eclampsia. We aimed to assess the GFR changes in pre-eclampsia, evaluate the use of serum cystatin C as an indicator of GFR and to identify other biomarkers for detection of pre-eclampsia. **Methods:** This was a case-control study, conducted between December 2008 and December 2010, in Omdurman Maternity Hospital. The study group was 72 pre-eclamptic cases compared to 96 normal pregnant and 63 non-pregnant Sudanese women. Clinical and laboratory parameters including blood samples and 24-hour urine output were recorded. Total blood count, serum liver enzymes, renal function tests and serum cystatin C. Creatinine clearance and GFR were calculated. **Results:** Serum cystatin C level in the pre-eclamptic cases was significantly higher than the normal pregnant ( $P=0.000$ ) and the non-pregnant participants ( $P=0.0001$ ). Mean GFR of the pre-eclamptic cases was significantly lower than that of normal pregnant ( $P=0.0001$ ) and the non-pregnant ( $P=0.0001$ ). GFR of pre-eclamptic cases did not correlate with serum cystatin C nor with serum uric acid, but was negatively correlated with serum creatinine ( $r=-0.31$ ,  $P=0.02$ ). Serum cystatin C correlated with serum albumin ( $r=-0.41$ ,  $P=0.0001$ ), serum aspartate aminotransferase ( $r=0.26$ ,  $P=0.03$ ) and serum uric acid ( $r=0.60$ ,  $P=0.0001$ ) in the pre-eclamptic group. The diagnostic accuracy tests showed that serum cystatin C was a good detector of pre-eclampsia but not a reliable marker of GFR changes in pre-eclampsia. **Conclusions:** In pre-eclampsia, serum cystatin C level was significantly higher than in normal pregnancy. Serum cystatin C was superior to serum uric acid and serum creatinine in detection of pre-eclampsia but not a reliable indicator of GFR. Serum albumin and serum aspartate transferase can be used as additional biomarkers in pre-eclampsia.

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### INTRODUCTION

The Glomerular Filtration Rate (GFR) decreases early, in kidney disease, before the onset of any symptoms of renal failure (Manjunath et al., 2001). The decrease corresponds with the extent of damage caused by the kidney disease (Schainuck et al., 1970; Striker et al., 1970).

Normal reproductive and renal functions are required during pregnancy (Phyllis, 1990). Physiological changes occur normally during pregnancy and marked renal and 'haemodynamic' changes are obvious by the end of the first trimester. GFR and effective renal plasma flow (ERPF) increase by 50% (Frederiksen, 2001; Baylis, 1987). In the final weeks of pregnancy, GFR usually declines to nearly non-pregnant values (Linheimer and Katz, 2000).

Pre-eclampsia affects about five percent of all pregnancies (Siddiquiet al., 2010), (which is a multi-system disorder, occurring in the second half of

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pregnancy and is accompanied with increased rates of fetal and maternal morbidity and mortality (Redman and Sargent, 2005; Fisher et.al., 1981).

The syndrome is characterized by vasospasm, haemo-concentration and ischaemic changes in the placenta, kidney, liver and brain (James et.al., 2003). Both renal plasma flow and GFR decrease in pre-eclampsia, although absolute values may remain above the non-pregnant range (Moran et. al. 2003). Altered renal function is a basic element of the pathophysiological process of pre-eclampsia (Hladunewich et.al., 2007).

Serum cystatin concentration is exclusively glomerular rate dependent and therefore its serum assay shows a more precise analysis of the renal function than serum creatinine. Cystatin C gives a better view for mild renal damage and is a more sensitive marker of changes in GFR than creatinine (Amgad et.al., 2004). It was diagnostically superior to serum creatinine (Villa et.al., 2005). Small increases of cystatin C was associated with increased mortality in intensive care unit patients independent of acute kidney injury by acute kidney injury network criteria (Kwon et.al., 2011). Serum cystatin C is also used as a new biomarker for early detection of renal injury associated with metabolic syndrome and cardiovascular risk (Sheen and Sheu, 2011).

*Measurement of the three angiogenic molecules:* soluble endoglin, placental growth factor and soluble FMS-Like Tyrosine Kinase was more strongly predictive of pre-eclampsia than the individual biomarkers (Levine et.al., 2006). Renal biopsies were performed and showed that serum cystatin C reflected glomerular endotheliosis in normal, hypertensive and pre-eclamptic pregnancies. They reported a significant correlation of serum cystatin C with glomerular volume (Strevens et.al., 2001). On the other hand, trophoblast invasion, in pregnancy, is controlled by proteases and their inhibitors (e.g cystatin C), so serum level of cystatin C is increased in late pregnancy (Christensson et.al., 2003). In pre-eclampsia, placental expression and secretion of cystatin C could contribute to the elevated maternal plasma levels of cystatin C (Karl and Mansson, 2007).

The number of Sudanese women who develop pre-eclampsia and subsequently eclampsia is high and a genuine concern. Maternal deaths in Sudan were 957 out of 457491 live births (LB) in 2010. Direct maternal deaths were 567/957 (63.1%). The main causes were haemorrhage, eclampsia and sepsis in that order. Maternal mortality in association with pre-eclampsia in the years 2010 and 2011 were 14.9% and 11.3% respectively (Umbeliet al., 2012).

We aimed to assess GFR changes in pre-eclampsia, evaluate the use of serum cystatin C as an indicator of

GFR and identify other biomarkers for detection of pre-eclampsia.

## Methods

### *Study Setting*

This was a cross-sectional, case-control and hospital-based study performed during a two-year period from December 2008 to December 2010; in Omdurman Maternity Hospital, Sudan. Omdurman Maternity Hospital has a large catchment area and covers a large population of the National Capital. Two obstetricians were responsible for the recruitment of the study participants. The pre-eclamptic cases were pregnant women with blood pressure  $\geq 140/90$  mmHg and proteinuria and in their second half of pregnancy (20 weeks of pregnancy and onwards) (ACOG, 2002). The control pregnant women had normal blood pressure and no proteinuria and were in their second half of pregnancy. Normal pregnant and pre-eclamptic cases were selected randomly (1 in 3) in the antenatal care clinics and those who fulfilled the requirements were included. The pre-eclamptic participants included newly discovered and already diagnosed and followed-up cases in their recent pregnancies. The non-pregnant group was selected from the midwives and nurses of Omdurman Maternity Hospital, the female officers and trainees of Sudan Police Headquarters and the female students of Ribat University. All selected participants were informed about the study; and their written consent was taken in advance.

The inclusion criteria required that all the pregnant participants and non-pregnant control group to have no history of diabetes mellitus, essential hypertension, renal disease or any other chronic disease. The exclusion criteria included subjects with multiple pregnancies and/or have any obstetrical abnormality. The participants who showed abnormal thyroid function tests were also excluded (a post-test exclusion criterion) (Niet et al., 1998).

### *Data Collection*

All participants were interviewed according to a pre-constructed questionnaire. The assigned obstetricians (authors 3 and 4) performed complete medical and obstetrical examination. All pregnancies were dated by the obstetrical examination and followed by ultrasonography measurement. The selected pre-eclamptic and normal pregnant groups had follow-up cards to monitor their pregnancies. They were admitted in the morning into the antenatal wards after their consent was taken. The blood samples were collected and the blood was centrifuged and the sera collected. Each subject emptied her bladder completely after cleaning the vulva and the mid-stream urine was taken and tested for proteins. The time was recorded. The

subjects were watched closely by nurses trained by the first author. They accompanied the subjects to toilets every time from that time and onwards whenever was required. The urine passed out was collected in the 24-hours in a urine container in which 5 ml of hydrochloric acid was added in advance.

The subjects were given meals with free intake of fluids and told to lie on their lateral and not the supine position to avoid pressure over the ureters and bladder by the pregnant uterus. Another urine sample was taken from the subject before discharge and urine analysis for proteins was performed again. The 24-hour urine volumes were measured and the amounts recorded in the same laboratory. A urine sample for each subject was kept for urine analysis. All the urine and serum samples were kept at  $-80^{\circ}\text{C}$ . The liver enzymes, serum creatinine and urine creatinine, serum cystatin C, serum albumin, uric acid and urine proteins were measured. Creatinine clearance was used to estimate GFR. Serum creatinine, urine creatinine and the 24-hour urine output were used to calculate the creatinine clearance. GFR was calculated in ml/min/m<sup>2</sup> in accordance with John Hopkins' method and using creatinine clearance corrected with body surface area (ABX, 2010).

#### *Techniques of Biochemical Analysis*

Atomic absorption spectrophotometer (Siemens Advia 2400 Chemistry System) was used to analyze Aspartate Aminotransferase, Alanine Aminotransferase, serum cystatin C, serum and urine creatinine, blood urea and uric acid. DAKO Cystatin C PET kit for quantitative determination of Cystatin C concentration in human serum was used. Siemens Advia Centaur XP Immunoassay System was used to analyze Thyroid Stimulating Hormone and Free Thyroxine.

The urine was analyzed for proteins using the dipstick, Traces of proteins was equivalent to 5-20 mg/dl; 1+ equivalent to 30 mg/dl; 2+ equivalent to 100 mg/dl; 3+ equivalent to 300 mg/dl and 4+ equivalent to >300 mg/dl.

#### *Ethical Consideration*

Ethical approval was given by the Research Committee (Faculty of Medicine, U of K). Permission was given from Omdurman Maternity Hospital to conduct the study. A written consent of all the study subjects was given prior to entry in the study. There is no conflict of interest to any of the authors.

#### *Statistical Analysis*

The sample size calculation was done from the statistical records of Omdurman Maternity Hospital. The samples selection was done by a systematic random selection.

The data were presented as Means  $\pm$  S.E. of the means and confidence intervals. Correlation between quantitative data was determined using Spearman's test.  $P < 0.05$  was considered statistically significant. The diagnostic value of serum cystatin C, serum creatinine and serum uric acid for identifying renal dysfunction was evaluated using sensitivity, specificity, positive and negative likelihood ratios and/or receiving operating characteristic curve analysis (ROC) (Bewick et. al., 2004). The SPSS R 9.0 (SPSS, Inv., Chicago, IL, USA) program Version 20 was used in data analysis. Mann Whitney and Kruskal Wallis tests were used for comparison of means of continuous variables between groups.

#### **Results**

The pre-eclamptic cases were 72, the normal pregnant subjects 93 and the non-pregnant subjects were 63. Mean Body Mass Index (BMI – kg/m<sup>2</sup>) of the pre-eclamptic ( $29.5 \pm 1.0$  range 27.75-31.21) was significantly higher than the non-pregnant ( $23.9 \pm 0.6$  range - 22.59-24.98) ( $P=0.0001$ ) and the normal pregnant ( $25.6 \pm 0.5$  range - 24.69-26.63) ( $P=0.0001$ ).

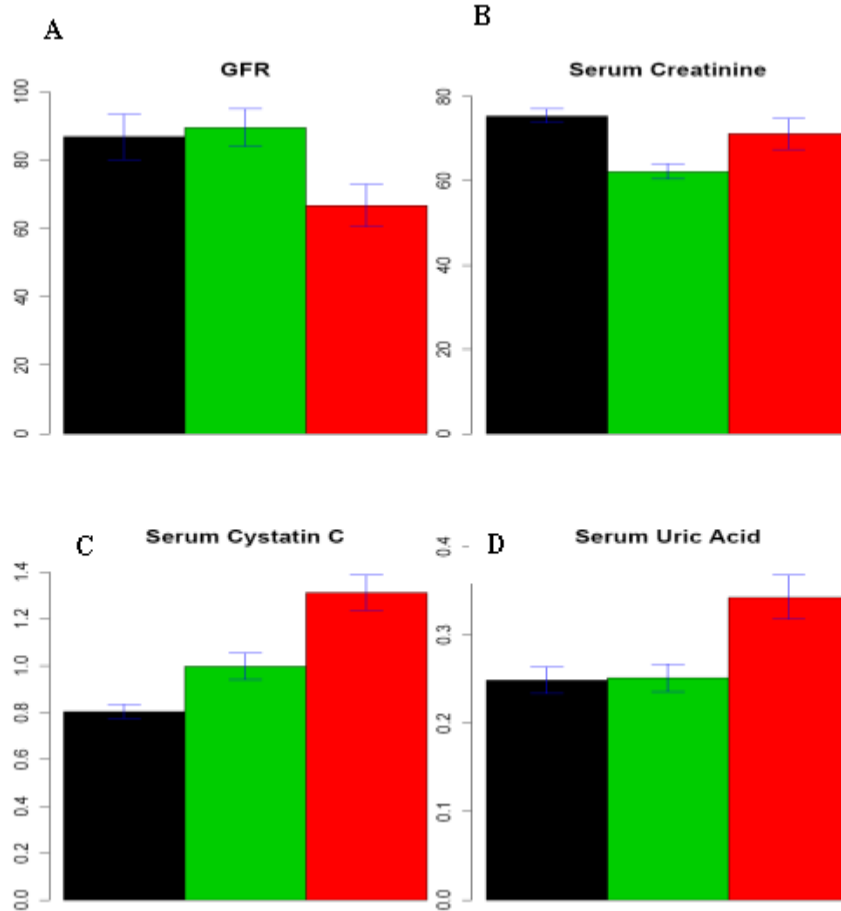
Mean systemic blood pressure of the pre-eclamptic ( $142.7$  mmHg  $\pm 2.0$  range- 138.31-145.91) was significantly higher from the non-pregnant ( $115.00$  mmHg  $\pm 1.3$  range- 112.30-117.32) ( $P=0.0001$ ) and the normal pregnant ( $114.00 \pm 1.2$  mmHg range- 111.76-116.51) ( $P=0.0001$ ).

Mean diastolic blood pressure of the pre-eclamptic ( $96.9$  mmHg  $\pm 1.2$  range 94.94-99.09) was significantly higher than the non-pregnant ( $78.6$  mmHg  $\pm 0.7$  range 77.13-79.79) ( $P=0.0001$ ) and the normal pregnant ( $77.2$  mmHg  $\pm 1.01$  range 75.28-79.29)

Mean serum albumin of the pre-eclamptic ( $34.60$ g/L  $\pm 0.87$  range 32.85-36.34) was significantly lower from the non-pregnant ( $45.83$ g/L  $\pm 0.29$  range 45.25-46.41) ( $P=0.0001$ ) and the normal pregnant ( $35.63$ g/L  $\pm 0.34$  range 34.95-36.31) ( $P=0.0001$ ).

Mean serum alanine transferase (ALT) of the pre-eclamptic ( $12.1$ U/L  $\pm 2.27$  range 7.49-16.53) was significantly higher from the non-pregnant ( $10.89$ U/L  $\pm$

## Serum Cystatin C, GFR and Preeclampsia



**Fig.1:** Means and Confidence Intervals for GFR (ml/min/1.73m<sup>2</sup>), Serum Creatinine (μmol/L), Serum Cystatin C (mg/L) and Serum Uric Acid (mmol/L) in Non-pregnant, Pregnant and Pre-eclamptic Subjects

.6 range 9.66-12.10) (P=0.002) and the normal pregnant (8.61U/L ± 0.29 range 8.03-9.19) (P=0.001). Mean serum aspartate transferase (AST) of the pre-eclamptic (22.94U/L ± 3.71 range 15.55-30.33) was not significantly different from the non-pregnant (20.52U/L ± 1.18 range 18.16-22.88) but significantly different from the normal pregnant (16.00U/L ± 0.52 range 14.96-17.03) (P=0.02).

Mean serum cystatin C of the pre-eclamptic (1.3mg/L±0.0 range 1.23-1.38) was significantly higher than the non-pregnant (0.8mg/L±0.0 range 0.77-0.83) (P=0.0001) and the normal pregnant (1.0mg/L±0.0 range 0.94-1.05) (P=0.0001). (Fig.1C)

Mean serum uric acid of the pre-eclamptic (0.342mmol/L±0.01 range 0.317-0.367) was significantly higher from the non-pregnant (0.247mmol/L±0.01 range 0.232-0.262) (P=0.0001) and the normal pregnant (0.250mmol/L ± 0.01 range 0.234-0.266) (P=0.0001). (Fig.1D)

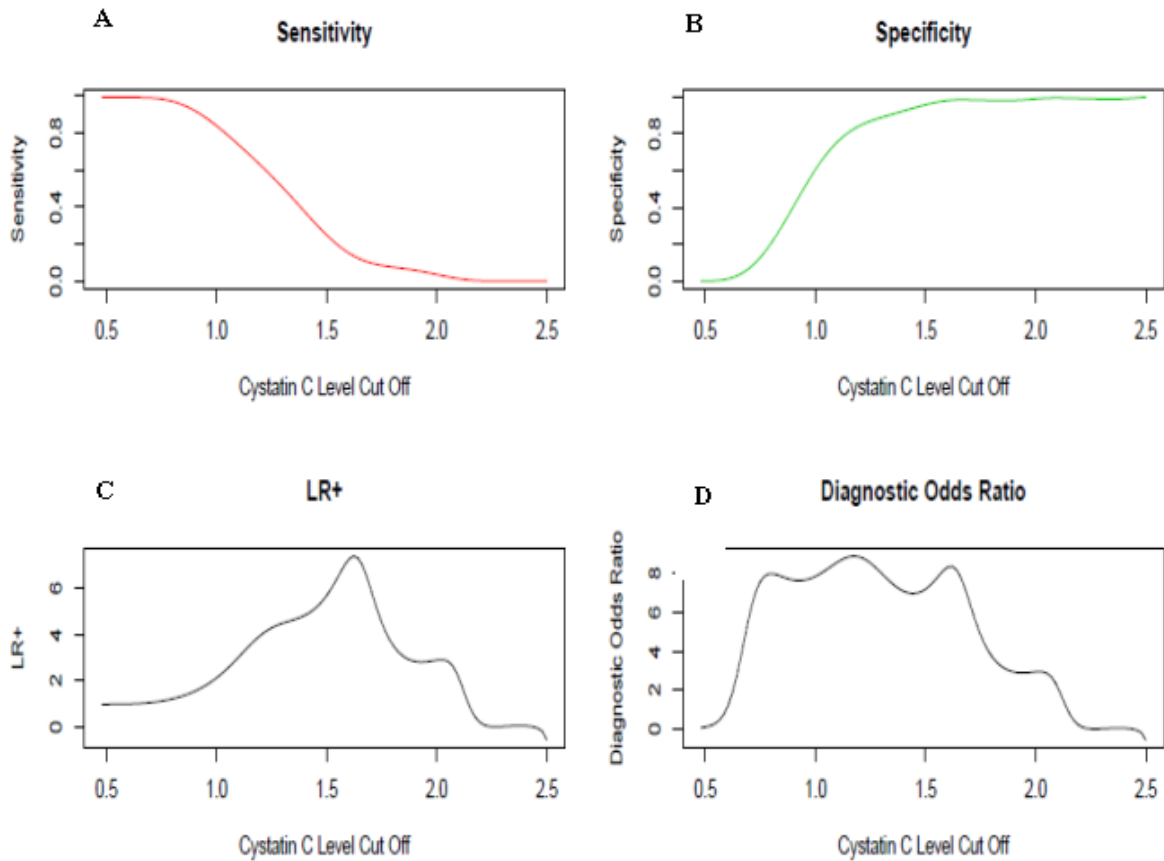
Mean serum creatinine of the pre-eclamptic (68.6μmol/L ± 1.8 range 67.39-74.82) was significantly different from the non-pregnant (75.5μmol/L ± 0.8

range 73.73-77.03) (P=0.0001) and the normal pregnant (62.21 μmol/L ± 0.91 range 60.39-63.95) (P=0.0001). (Fig.1B)

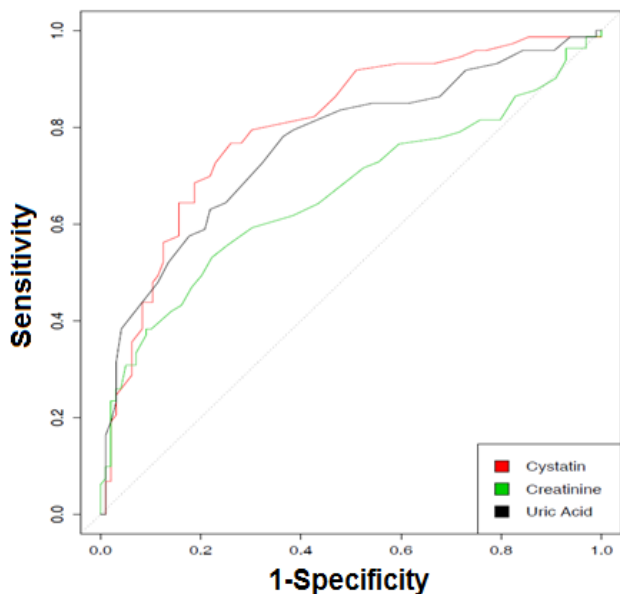
Mean urine creatinine of the pre-eclamptic (5.6mmol/L ± 0.6 range 4.47-6.78) was significantly lower from the non-pregnant (11.9mmol/L ± 1.0 range 9.90-13.70) (P=0.0001) and the normal pregnant (6.8mmol/L ± 0.3 range 6.08-7.44) (P=0.0001)

All pre-eclamptic cases had positive urine protein as follows: 31.6% had 1+ protein, 31.6% had 2+ proteins, 30.3% had 3+ proteins, 1.3% had 4+ proteins and 5.3% had traces protein. The non-pregnant subjects, 80% of them, had negative urine protein; while of the normal pregnant 86.9% had negative urine protein. Mean urine protein of the pre-eclamptic (2.0±0.1) was significantly

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**Fig.2:** The Diagnostic Value of Serum Cystatin C at Different Cut-off Levels for Detection of Pre-eclamptic Subjects



**Fig.3:** ROC to Compare Serum Cystatin C, Serum Creatinine and Serum Uric Acid in Pre-eclamptic Cases for Detection of Pre-eclampsia.

higher from the non-pregnant ( $0.3 \pm 0.1$ ) ( $P=0.0001$ ) and the normal pregnant ( $0.3 \pm 0.1$ ) ( $P=0.0001$ ).

Mean GFR of the pre-eclamptic ( $68.6 \text{ ml/min}/1.73 \text{ m}^2 \pm 3.3$  range 62.06-75.14) was statistically significantly less than the non-pregnant ( $87.0 \text{ ml/min}/1.73 \text{ m}^2 \pm 3.4$  range 80.41-93.59) ( $P=0.0001$ ) and the normal pregnant ( $89.0 \text{ ml/min}/1.73 \text{ m}^2 \pm 2.8$  range 83.57-94.43) ( $P = 0.0001$ ). (Fig.1A)

The sensitivity at a cut-off value of serum cystatin C of 1.0 mg/L was 0.8 (Fig.2A) and the specificity at a cut-off of 1.5 mg/L was 0.95 for pre-eclampsia. (Fig. 2B)  $LR^+$  for serum cystatin C was found to be reasonably high in the range shown (1.25-1.75 mg/L) (Fig. 2C). DOR was reasonably high in range shown for serum cystatin C (0.75-1.75) (Fig.2D).

The diagnostic accuracy of serum cystatin C using ROC-plot was found to be better than serum creatinine and serum uric acid for detection of pre-eclampsia. (Fig.3)

A value for GFR of  $40 \text{ ml/min}/1.73 \text{ m}^2$  or less was considered critically low which was observed in 6 cases. (Fig. 4).

## Serum Cystatin C, GFR and Preeclampsia

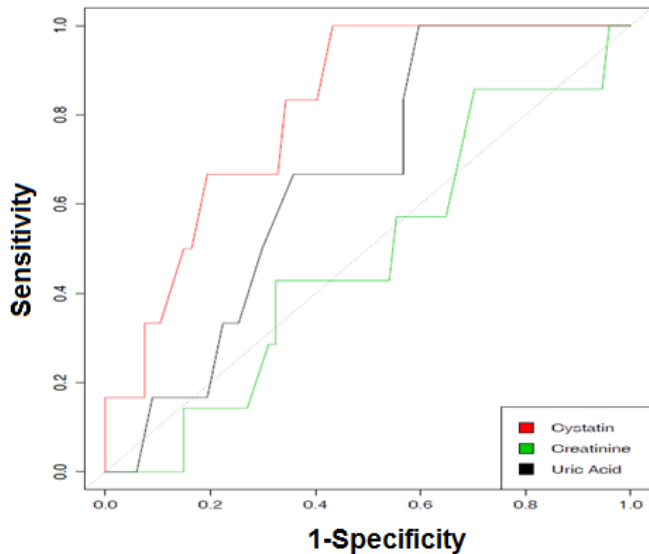


Fig. 4: ROC-40 Plot the Diagnostic Value of Serum Cystatin C, Serum Creatinine and Serum Uric Acid at Cut-off GFR 40 (ml/min/1.73m<sup>2</sup>) in pre-eclampsia

GFR of the normal pregnant group correlated negatively and significantly with serum cystatin C ( $r=-0.26$ ,  $P=0.02$ ) and with serum uric acid ( $r=-0.35$ ,  $P=0.001$ ). GFR of the pre-eclamptic group did not correlate neither with serum uric acid ( $r=-0.19$ ,  $P=0.171$ ) nor with serum cystatin C ( $r=-0.18$ ,  $P=0.192$ ). In the pre-eclamptic group, serum cystatin C correlated positively and significantly with serum uric acid ( $r=0.60$ ,  $P=0.000$ ) but not with serum creatinine ( $r=0.04$ ). Serum cystatin C correlated positively and significantly with serum aspartate aminotransferase ( $r=0.25$ ,  $P=0.01$ ) in the normal pregnant group. Serum cystatin C correlated negatively and significantly with serum albumin ( $r=-0.41$ ,  $P=0.0001$ ), and positively and significantly with serum aspartate aminotransferase ( $r=0.26$ ,  $P=0.03$ ) and serum uric acid ( $r=0.60$ ,  $P=0.0001$ ) in the pre-eclamptic cases.

### Discussion

In this study, mean GFR of pre-eclamptic cases was statistically significantly lower than that of non-pregnant and of normal pregnant women. This is due to the marked renal impairment and glomerular endotheliosis which accompanies pre-eclampsia (Strevenet.al., 2001). Unchecked steep decline in GFR in pre-eclamptic patients may result in renal failure. A similar significant decline in GFR of pre-eclamptic cases was reported compared to the normal pregnant control group (Lafayette et. al., 1998). In this study, serum cystatin C was measured in the three study groups. The results showed that it increased significantly in the normal pregnant women compared to the control group. A similar result was reported by

Streven et. al. who measured serum cystatin C in normal pregnant and non-pregnant subjects. They found an increased level of cystatin C in the normal pregnant subjects (Streven et.al., 2002).

In further research based on renal biopsies in pregnancy, they reported that there was a degree of glomerular endotheliosis in normal pregnancy. This may lead to a decrease in the filtration of serum cystatin C and thus increase its serum level though the GFR was high (Strevenet.al., 2001).

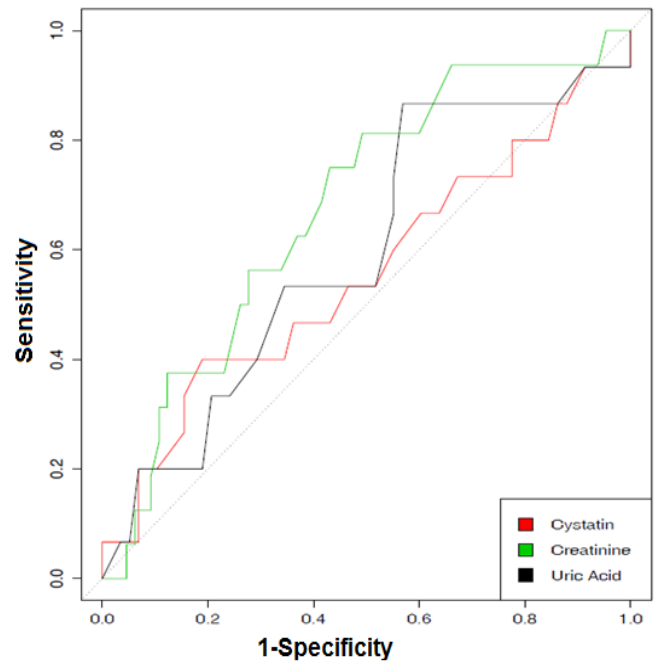


Fig. 5: ROC-50 Plot the Diagnostic Value of Serum Cystatin C, Serum Creatinine and Serum Uric Acid at Cut-off GFR 50 (ml/min/1.73m<sup>2</sup>) in Pre-eclamptic Cases.

This finding is further supported by Malyszkoet. al.. They performed a study to assess the use of Netrophilgelatinase associated lipocalin and cystatin C to reflect kidney function in pregnancy. NGAL correlated with cystatin C. They explained the steady rise of cystatin C in pregnancy by the fact that cysteine proteases are important in pregnancy for embedding and trophoblast invasion of the placenta. Cystatin C is synthesized by the embedded placenta and secreted by trophoblast cells into the maternal and fetal circulation (Malyszko, 2010). An increase of serum cystatin C in pregnancy was reported (Bramham et.al.,2010). In the current study, the pre-eclamptic cases mean serum cystatin C increased significantly when compared to non-pregnant and normal pregnant subjects. The mean serum cystatin C values were different in the three groups with a persistent rise and peak in pre-eclampsia.

## Serum Cystatin C, GFR and Preeclampsia

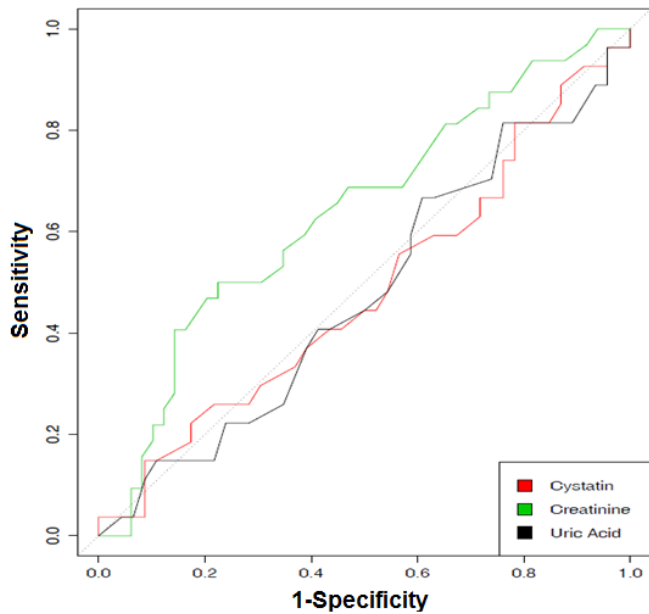


Fig. 6: ROC-60 Plot the Diagnostic Value of Serum Cystatin C, Serum Creatinine and Serum Uric Acid at Cut-off GFR 60 (ml/min/1.73m<sup>2</sup>) in Pre-eclamptic Cases

The pathological changes of marked glomerular endotheliosis, in pre-eclampsia, may have led to reduced GFR and higher serum cystatin level in the pre-eclamptic group (Strevens et al., 2001).

Malyszko et al. also stated that the imbalance of trophoblast cathepsin/cystatin C levels, which occurs in pre-eclampsia, may even increase more the serum cystatin C level. They explained that high serum levels of cystatin C, in pre-eclampsia, were not only due to impaired renal function but also to its increased secretion by the malformed placenta (Malyszko et al., 2010).

The diagnostic accuracy tests using ROC-plot for serum cystatin C at different cut-off values showed it to be a reliable indicator for detection of pre-eclampsia. It also showed the superiority of serum cystatin C on serum creatinine. Thus, serum cystatin C was found to be better than serum creatinine and serum uric acid for detection of pre-eclampsia.

In this study, in the pre-eclamptic cases, there was no correlation between the GFR and serum cystatin C.

Serum albumin level decreased significantly in pregnancy, due to the haemodilution of pregnancy. It decreased even more in pre-eclampsia.

Serum cystatin C correlated significantly with serum albumin, serum AST and serum uric acid in the pre-eclamptic cases. Serum cystatin C did not correlate significantly with the blood pressure and urine albumin in the pre-eclamptic cases.

The combination of multiple serum markers increased serum cystatin C, decreased serum albumin and

increased serum AST enables the detection ALT and AST are strong prognostic indicators of pre-eclampsia. Increase in their levels may indicate deterioration of the condition (Redman et al, 2005). In this study, both ALT and AST increased in the pre-eclamptic cases when compared to normal pregnant women.

### Conclusions

Serum cystatin C increased in normal pregnancy and more in pre-eclampsia. It was found to be diagnostic of pre-eclampsia using the diagnostic accuracy tests. Increased serum cystatin C, in the pre-eclamptic cases, correlated significantly with decreased serum albumin and increased serum aspartate aminotransferase. A combination of the three tests can be a better detection of pre-eclampsia. This finding also emphasizes compromised liver function in pre-eclampsia. Serum cystatin C had no correlation with GFR in pre-eclampsia. Thus, it cannot be used to reflect the GFR in pre-eclampsia. The limitations of the use of serum cystatin C, serum creatinine and serum uric acid for GFR changes in pre-eclampsia were confirmed. Serum cystatin C, in the pre-eclamptic cases did not correlate with the raised blood pressure and proteinuria.

**Abbreviations:** GFR, glomerular filtration rate; ERPF, effective renal plasma flow; ROC, receiving operating curve; BMI, body mass index; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DOR, diagnostic odd ratio; L<sup>+</sup>R, likelihood ratio; SPSS, statistical package for the social sciences.

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