



## SERUM CYSTATIN C – AN EARLY DIAGNOSTIC MARKER OF DIABETIC NEPHROPATHY.

### Biochemistry

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### KEYWORDS

#### INTRODUCTION:

Diabetes Mellitus is a group of metabolic diseases characterized by hyperglycemia resulting due to insulin resistance. Long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels can be associated with chronic hyperglycemic state of Diabetes mellitus.<sup>1</sup>

Diabetic nephropathy can lead to end-stage renal disease (ESRD) and a leading cause of Diabetes Mellitus related morbidity and mortality.<sup>2</sup> With global epidemic of type II Diabetes Mellitus and ageing of the world population, the prevalence of Chronic Kidney disease (CKD) and End stage renal disease (ESRD) is increasing worldwide.<sup>3</sup>

Therefore it is important to detect renal dysfunction in early stages as timely intervention can reduce its progression to end stage renal disease and also decrease the risk of cardio vascular complications.<sup>4</sup>

Glomerular filtration rate using endogenous marker serum Creatinine is most commonly used method for assessment of renal function in clinical practice. But this marker is found to be influenced by muscle mass, sex, age, diet, race, nutritional status and analytical interference. Though simple and easily available but the general view is that up to 50% of GFR can be lost before there is significant elevation in levels of serum Creatinine occur.<sup>5</sup> Mild renal dysfunction may go unrecognized due to poor sensitivity of serum Creatinine.<sup>6</sup>

Cystatin C is a 13-kDa protein, of the cysteine proteinase inhibitor superfamily, having constantly produced throughout the ages of 1 to 60 years. It is not affected by gender, muscle mass, malignancy and its plasma concentration is dependent only on GFR. It is freely filtered at the glomerulus and then reabsorbed and fully catabolised by proximal renal tubules. Due to catabolism direct measurement of its clearance is not possible, but its concentration in serum can be a good marker of GFR. Serum levels of Cystatin C can have more advantages over traditional established markers like serum Creatinine.<sup>7</sup>

The ability to assess renal function in diabetic patients rapidly and early is of major importance for the possibility of preventing the development of nephropathy. Therefore, it is worth to discover a more sensitive or specific indicator for detecting early renal impairment in diabetic patients. So, corrective measures could be adopted to prevent the progression of kidney function impairment towards frank nephropathy.

This study was undertaken to compare levels serum Creatinine and serum Cystatin C in newly diagnosed type II Diabetes Mellitus and already diagnosed type II Diabetes Mellitus patients with healthy controls, to evaluate the value of this newer molecule in assessment of renal dysfunction at a phase when timely interventions can be instituted and the progression of nephropathy can be delayed.

#### MATERIAL AND METHOD

The present study was carried out in the Department of Biochemistry and Central Investigation Laboratory in collaboration with the Department of Medicine in MGM's Medical College, Aurangabad. The study was approved by Institutional Ethical and Research Committee to use human subjects in the research study.

40 Cases of Type II Diabetes Mellitus of any sex of varied socioeconomic status between ages 30-60 years without any complications are selected from Outpatient Department and Inpatient Department patients in General Medicine Department. Cases were

categorized in two groups as newly diagnosed 20 cases and already diagnosed 20 cases taking treatment regularly. 20 healthy persons were chosen as controls. Informed consent was taken from patients and controls.

Patient suffering from urinary tract infection, Type I Diabetes mellitus, thyroid disorder, chronic inflammatory disease, pregnant females were excluded from study. History and personal physical data was obtained from both cases and controls.

5 ml of venous blood sample was collected from both cases and controls, centrifuged and separated for the estimations. Estimations of serum Creatinine was done by Jaffe's method and serum Cystatin C was done by immunoturbidimetric method.

#### Statistical Analysis

The statistical software SPSS 17.0 was used for the analysis of the data. Descriptive statistical analysis was carried out in the present study. Results on continuous measurements were presented as Mean  $\pm$  SD. P value  $<0.05$  (95% confidence interval) was considered significant. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups.

#### RESULT

Total of 40 cases categorized in two groups and 20 controls were studied. The variables studied in cases and controls are shown in Table 1. There was significant difference in Body mass index (BMI) between cases and controls.

**Table 1: Comparison (Mean  $\pm$  SD) of study variables in cases and controls.**

Basic parameters	Cases (40)	Controls (20)	P value
Age (years)	50.15 $\pm$ 7.31	53.1 $\pm$ 5.13	0.112
Weight (kg)	70.0 $\pm$ 5.81	64.45 $\pm$ 6.6	0.4353
Height (cm)	156.23 $\pm$ 4.98	154.05 $\pm$ 3.17	0.08
BMI (kg/m <sup>2</sup> )	28.7 $\pm$ 2.26	27.2 $\pm$ 3.06	0.0359*

Statistical analysis done by paired student t-test

\*p value  $<0.05$  was considered as statistically significant

As shown in table 2, There was not significant increase in levels of serum Creatinine in cases compared to controls but there was significant increase in levels of serum Cystatin C when cases compared to controls

**Table 2: Comparison (Mean  $\pm$  SD) of serum creatinine and serum Cystatin C in cases and Controls.**

Parameters	Cases (40)	Controls (20)	P value
Serum Creatinine (mg/dl)	0.96 $\pm$ 0.16	0.91 $\pm$ 0.09	0.2003
Serum Cystatin C (mg/L)	1.23 $\pm$ 0.15	0.81 $\pm$ 0.14	0.0001*

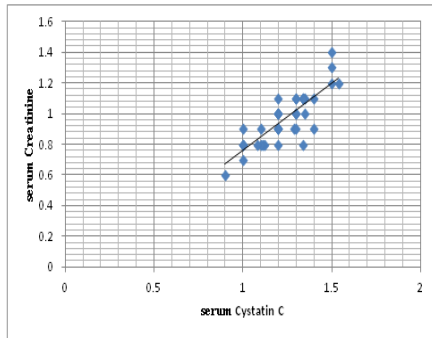
Statistical analysis done by paired student t-test

\*p value  $<0.05$  was considered as statistically significant

In cases, There is a strongly positive correlation of 80% (r value is 0.80) between serum Cystatin C and serum Creatinine, as shown in scatter plot (Figure:-1)

As, we compared patients according to groups, there was no significant difference in levels of Serum Creatinine and serum Cystatin C in Newly diagnosed patients and controls (table 3), but levels of serum

Cystatin C found to be significantly increased in already diagnosed cases taking regular treatment as compared with controls (Table 4).



**Figure 1: Scatter plot showing correlation between serum Cystatin C and serum Creatinine among cases.**

**Table 3:** Comparison (Mean ± SD) of serum Creatinine and serum Cystatin C in Newly diagnosed type II Diabetes Mellitus cases and controls.

Parameters	Cases- Newly diagnosed patients (20)	Controls (20)	P value
Serum Creatinine (mg/dl)	0.96 ± 0.19	0.91 ± 0.09	0.219
Serum Cystatin C (mg/L)	0.90 ± 0.23	0.81 ± 0.14	0.1432

Statistical analysis done by paired student t-test  
\*p-value <0.05 was considered as statistically significant

**Table 4:** Comparison (Mean ± SD) of serum Creatinine and serum Cystatin C in already diagnosed type II Diabetes Mellitus taking treatment regularly and controls

Parameters	Cases- already diagnosed cases taking treatment regularly (20)	Controls (20)	P value
Serum Creatinine (mg/dl)	0.95 ± 0.25	0.91 ± 0.09	0.5049
Serum Cystatin C (mg/L)	0.99 ± 0.29	0.81 ± 0.14	0.0169*

Statistical analysis done by paired student t test  
\*p value <0.05 was considered as statistically significant

**DISCUSSION**

Diabetes Mellitus being one of the most common chronic diseases in the young and adult and is a substantial cause of morbidity and mortality at all ages. Diabetes mellitus patients in long term have a greater risk of developing cardiac diseases, visual problems, nerve disease as well as renal disease which indirectly increases risk of premature deaths.

Ideally Diabetic nephropathy should be prevented in order to prevent end stage renal disease which is unachievable in current situation therefore it is prudent to identify and manage it in early stages. It will halt complications and renal function can revert back to normal<sup>7,8</sup>

Currently serum Creatinine is the most widely used endogenous marker of assessment renal function in routine laboratory and clinical practice. It is popular due to its convenience and cost effectiveness,<sup>9</sup> but its levels remain within the normal range even when though renal function is significantly impaired<sup>10</sup> that is normal levels of serum Creatinine does not imply normal GFR or stable disease<sup>9,10</sup>. National kidney foundation does not recommend serum Creatinine as the solo means to assess renal functions. Serum Cystatin C, a low molecular weight protein produced at a constant rate by all nucleated cells. It was proposed as a potential marker to assess Renal functions.<sup>11</sup>

Superiority of serum cystatin C is being studied over serum Creatinine in patients with early and moderately decreased renal function. Despite of the reports that its levels are modulated by several non-renal factors like steroids, thyroid status, smoking, C-reactive protein and malignancy<sup>11</sup>. Measurement of serum Cystatin C is simple but accurate method for detecting early renal impairment and to follow changes in kidney function in subjects with diabetes<sup>12,13</sup>.

Our study shows statistically significant increase in serum Cystatin C levels in diagnosed diabetic patients taking treatment as compared to

controls. This coincides with a study conducted by Borges et al<sup>14</sup>.

According to some researchers a Cystatin C increase has an almost 100% specificity and sensitivity for decreased GFR<sup>15</sup>. Some cross-sectional studies have shown that Cystatin C presents a higher sensitivity than serum Creatinine to detect mild kidney disease. Cystatin C concentration has been reported to increase when GFR ranges between 70 and 90 ml/min which is Creatinine blind range zone<sup>16</sup>.

Thus our shows that serum Cystatin C can be used for assessment of renal function in Type II Diabetes Mellitus compared to serum Creatinine.

**CONCLUSION**

Serum Cystatin C levels can be routinely used as a better marker to assess early renal dysfunction in Type II Diabetic mellitus patients as compared to traditional serum Creatinine.

**Limitation**

The incidence of diabetes and its associated complications such as diabetic nephropathy are increasing, hence it is difficult to conclude from small sized studies. Further large sizes studies using other gold standard parameters are required to assess usefulness of serum Cystatin C for assessment of renal function and early diagnosis of diabetic nephropathy.

Also we have considered reference range of Cystatin C that are published in studies in western population, hence there is need to establish reference range of serum Cystatin C in Indian population. This will help to diagnose diabetic nephropathy in early stages and timely intervention can be done.

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**REFERENCES**

- American Diabetes Association Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2010 January; 33(1):562-569. care.diabetesjournals.org
- Powers AC. HARRISON'S PRINCIPLES OF Internal Medicine. 16th ed. chapter 323. Mc Graw-Hill publication. 2152-2180
- Chew JSC, Saleem M, Florowski CM, George PM. Cystatin C – A Paradigm of Evidence Based Laboratory Medicine Clin Biochem Rev. 2008 May; 29(2): 47–62.
- Unnikrishnan R, Rema M, Pradeepa R, Deepa M, Shanthirani CS, Deepa R, Mohan V. Prevalence and risk factors of diabetic nephropathy in an urban South Indian population: the Chennai Urban Rural Epidemiology Study (CURES 45). Diabetes Care. 2007 Aug;30(8):2019-24. Epub 2007 May 8.
- Roos JF, Doust J, Tett SE, Kirkpatrick CM. Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children: a meta-analysis. Clin Biochem. 2007;40:383-91.
- Yun Kyung Jeon, Mi Ra Kim, Jung Eun Huh, Ji Young Mok, Sang Heon Song, Sang Soo Kim, et al. Cystatin C as an early biomarker of nephropathy in patients with type 2 Diabetes. J Korean Med Sci. 2011;26:258-63.
- Jayapaul MK, Messersmith R, Bennett-Jones DN, Mead PA, Large DM. The joint diabetic-renal clinic in clinical practice: 10 years of data from a District General Hospital. Q J Med 2006;99:153-160
- Zubairi A M, Hussain A. The Glomerular Filtration Rate: Comparison of various predictive equations based on Serum Creatinine with Conventional Creatinine Clearance test in Pakistani Population. J Pak Med Assoc. 2008 Apr; 58(4): 182-185
- Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. Kidney int. 1985 Nov;28(5):830-8.
- Kannapiran M, Nisha D, Rao MA. Underestimation of Impaired Kidney Function with Serum Creatinine. Ind J Clin Biochem 2010; 25(4):380-384
- Hojs R, Bevc S, Ekart R, Gorenjak M, Puklavec L. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. Nephrol Dial Transplant. 2006;21:1855-62.
- Richard JM, Premaratne E, Jerums G. Estimating Glomerular Filtration Rate in Diabetes Using Serum Cystatin C. Clin Biochem 2011 May;32(2):61-67
- Christensson AG, Grubb AO, Nilsson JA, Norrgren K, Sterner G, Sundkvist G. Serum cystatin C advantageous compared with serum creatinine in the detection of mild but not severe diabetic nephropathy. J Intern Med. 2004;256:510-8.
- Borges RL, Hirota AH, Quinto BM, Ribeiro AB, Zanella MT, Batista MC. Is cystatin C a useful marker in the detection of diabetic kidney disease? Nephron Clin Pract. 2010;114(2):127-34.
- Temesgen Fiseha. Clinical Significance of Cystatin C-Based Estimates of Renal Function in Type 2 Diabetic Patients: Review. Annals of clinical and laboratory Research. 2015;3(2):11
- Mares J, Stejskal D, Vavrouskova J, Urbanek K, Herzig R, Hlustik P. Use Of Cystatin C Determination In Clinical Diagnostics. Biomed Papers 2003;147(2):177-180