

A Comparison Of Diagnostic Accuracy Of Cystatin C With Creatinine In The Sample Of Patient Of T2 DM With Diabetic Nephropathy

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Abstract:

Objective: To compare the diagnostic accuracy of cystatin C with creatinine in the sample of patient of T2 DM with diabetic nephropathy.

Methods: The target population of this study comprised the adult males and females who have T2DM for different period of time and aged between 30-60 years from outpatient diabetic clinics. Another group of apparently healthy individuals represented the control group. The sample size was 70 patients which were previously diagnosed as having T2DM as cases and 70 healthy individuals.

Results: The cystatin was found to be significantly ($p=0.0001$) higher in cases (3.05 ± 1.29) compared to controls (0.92 ± 0.12). The creatinine was found to be significantly ($p=0.0001$) higher in cases (6.08 ± 4.22) compared to controls (0.90 ± 0.15). The sensitivity and specificity of cystatin (≥ 1.17) was 95.7% and 97.1% respectively. The sensitivity and specificity of creatinine (≥ 1.07) was 95.7% and 77.1%.

Conclusion: The study found high sensitivity and specificity of cystatin C and creatinine for T2 DM with diabetic nephropathy.

Keywords: Diagnostic accuracy, Diabetic nephropathy, Cystatin C, Creatinine

I. Introduction

Diabetes mellitus is a metabolic disease characterized by defects in insulin secretion, insulin action or both. The number of people with diabetes is increasing due to population growth, aging, urbanization and the increasing prevalence of obesity and physical inactivity. Approximately 40% of patients with type I diabetes and 5-15% of patients with type II diabetes eventually develop End Stage Renal Disease (ESRD) (Evans and Cappel, 2000). Even a diabetic patient is under treatment, there is a risk of development of nephropathy. The risk is related to the length of time the person has diabetes. There is good evidence that early treatment delays or prevents the onset of diabetic nephropathy. Therefore, prevention of diabetic renal disease or at least the postponement of or slowing down the disease process, has emerged as a key issue (Mussap et al, 2002). However, the ability to assess renal function is poor in early diabetic nephropathy, when active management is important (Tan et al, 2002). Glomerular filtration rate (GFR) is the best overall index of renal function in health and disease.

Serum creatinine and creatinine clearance are the most widely used indices for the routine noninvasive estimation of GFR. Creatinine is usually measured by the Jaffe reaction, based on a complex formation between alkaline picrate and creatinine (Hojs et al, 2006). The serum creatinine concentration may be significantly influenced by several extra renal factors. Serum creatinine is considered relatively specific but not very sensitive because serum creatinine remains in the normal range until 50% of renal function is lost.

The aim of this study was to compare the diagnostic accuracy of cystatin C with creatinine in the sample of patient of T2 DM with diabetic nephropathy.

II. Material And Methods

Study design

This was a case-control study.

Study site

The study was conducted in the Department of Pathology, Hind Institute of Medical Sciences, Barabanki.

Study subjects

The target population of this study comprised the adult males and females who have T2DM for different period of time and aged between 30-60 years from outpatient diabetic clinics. Another group of apparently healthy individuals represented the control group.

Sample size

The sample size was 70 patients which were previously diagnosed as having T2DM as cases and 70 healthy individuals

Exclusion criteria

A. Cases

Diabetics with urinary tract infections (UTI), Diabetics suffering from renal or liver disease, Diabetics who had high blood pressure: more than 130/80 mmHg, Females who were pregnant.

Controls

Subjects with fasting blood glucose ≥ 125 mg/dl, Subjects with UTI, Subjects suffering from renal or liver disease, Subjects who had high blood pressure: more than 130/80 mmHg, Females who were be pregnant.

Ethical considerations

The ethical clearance was taken from the Ethical Committee of the institute

Informed consent

The written informed consent was taken from each patient before enrolling in the study. The subjects were explained about the objectives of the study.

Data collection

A meeting interview was used for filling the questionnaire from both cases and controls. All the interviews was conducted face to face by the researcher. Personal data will be collected. The questionnaire included issues about personal information like name, sex, age, weight, height, blood pressure, time of diagnosis (for patients only), smoking and family history of diabetes in first-degree relatives, (father, mother, sister and brother). In addition, it included other information like type of medication and complication of diabetes (retinopathy, CVD and neuropathy).

Specimen collection

Convenient sampling method was used for selection of the study population, in order that every individual had to meet the criteria of being included in the sample. For both members of the case and control group, blood and urine samples were collected under quality control and safety procedure.

Blood sampling and processing

Fasting overnight venous blood sample (about 5 ml) will be drawn from each control and diabetic individual. The blood was collected in plain vacutainer tubes and left for a while to allow blood to clot. Then clear serum sample was obtained by centrifugation at room temperature at 3500 rpm for 10 minutes and collected into two plastic tubes, then will be stored at -20°C for no more than one month until the time of analysis.

Urine sampling and processing

Urine sample from both patients and controls were collected in a plastic container. The urine samples were immediately centrifuged at 2000 rpm for 10 minutes. Routine urine test was performed to each sample in order to exclude cases that had UTI. 5 ml from each sample supernatant were distributed equally into two plastic tubes and then was stored at -20°C for no more than one month until the time of chemical assay.

Biochemical analysis

Serum cystatin C, glucose, urea, creatinine, cholesterol, triglycerides and urine albumin was analyzed using chemistry automated analyzer.

Statistical analysis

Data was analyzed using Statistical Packages for Social Science (SPSS 16.0 USA). The relationships between some qualitative categories was identified statistically by using Chi-square test. Independent t test was applied to compare means two cases and contros. The one-way analysis of variance (ANOVA) was used to determine whether there was any significant difference among the means. To assess the correlation between biochemical parameters, Pearson's correlation coefficient (r) was applied. ROC analysis was employed to calculate the AUC. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of cystatin and creatinine were calculated. All results will be considered significant if $P < 0.05$.

III. Results

About one third of cases (32.9%) and 10% of controls were between 41-50 years. However, 24.3% of cases and 45.7% of controls were between 31-40 years. There was no significant ($p > 0.05$) difference in the age between the groups showing comparability of the groups in terms of age. Majority of the cases (68.6%) and controls (71.4%) were males. There was no significant ($p > 0.05$) difference in the gender between the groups showing comparability of the groups in terms of gender (Table-1).

The cystatin was found to be significantly ($p = 0.0001$) higher in cases (3.05 ± 1.29) compared to controls (0.92 ± 0.12). The creatinine was found to be significantly ($p = 0.0001$) higher in cases (6.08 ± 4.22) compared to controls (0.90 ± 0.15) (Table-2).

The sensitivity and specificity of cystatin ($\square 1.17$) was 95.7% and 97.1% respectively (Table-3 & Fig.1).

The sensitivity and specificity of creatinine ($\square 1.07$) was 95.7% and 77.1% respectively (Table-4 & Fig.2).

IV. Discussion

Gold standard methods of assessing GFR are replaced by an estimated GFR derived from endogenous substances. Serum creatinine is the most widely used substance to estimate GFR. Creatinine concentration is influenced by sex, age, diet and muscle mass. It only increases once GFR reduction of about 50% is present. This leads to falsely high or low values, limiting its usefulness as an ideal marker of GFR (Richard et al, 2011). Cystatin C is a low molecular weight protein produced at a constant rate by all nucleated cells. It is freely filtered by glomerulus, completely reabsorbed and catabolized in the proximal tubule. Serum cystatin C is reported to be modulated by several non-renal factors like steroids, thyroid status, smoking, C-reactive protein and malignancy. Despite these limitations evidence continues to suggest superiority of serum cystatin C when compared with serum Creatinine in patients with early and moderately decreased renal function (Christensson et al, 2004; Hojs et al, 2006).

The study shows significant increase in serum cystatin C and creatinine levels in diabetic individuals compared to controls. In the present study, the cystatin was found to be significantly ($p=0.0001$) higher in cases (3.05 ± 1.29) compared to controls (0.92 ± 0.12). The creatinine was also found to be significantly ($p=0.0001$) higher in cases (6.08 ± 4.22) compared to controls (0.90 ± 0.15). These findings are similar to a study conducted by Borges et al (2010).

The inability of creatinine to detect early decline in GFR is due to the fact that SCr levels only begin to rise above the normal range when approximately 50% of renal function is already lost, suggesting that GFR can change before SCr becomes abnormal (Shemesh et al, 1985). Evidences suggest that serum cystatin c may rise faster than creatinine after a fall in GFR and is a reliable endogenous marker for assessing renal function in type 2 diabetic patients with renal impairment. In one study, including 52 type 2 diabetic patients, an early and more significantly increased levels of serum cystatin C than SCr was observed as GFR decreases, which indicated that serum cystatin C might be a useful marker for detecting early renal impairment in diabetic patients (Hamed et al, 2011).

In this study, the sensitivity and specificity of cystatin ($\square 1.17$) was 95.7% and 97.1% respectively. The sensitivity and specificity of creatinine ($\square 1.07$) was 95.7% and 77.1% respectively. Zati et al (2013) analysed blood and urine samples from 418 normal subjects and 37 Type 2 diabetes patients (T2DM) with chronic kidney disease (CKD). The calculated sensitivity, specificity and accuracy of eGFR-creatinine were 85%, 87.2% and 85% respectively. The eGFR- cystatin C showed higher sensitivity, specificity and accuracy than eGFR-creatinine in studied diabetic subjects. Zhang et al (2010) evaluated the usefulness of serum cystatin C (CysC) as a marker of renal function in 83 patients with diabetic nephropathy, considering multiple factors including tubular function and body mass index. Meta-analysis showed that the serum cystatin C had no heterogeneity ($P=0.418$, $I^2=2.2\%$, $DOR=25.03$), while creatinine heterogeneity was high ($P=0.109$, $I^2=37.5\%$, $DOR=9.11$). The values of SEN, SPE and SAUC were calculated as 0.86, 0.70 and 0.9015 for cystatin C and 0.78, 0.73 and 0.8285 for creatinine individually. This study utilized GFR detection and subgroups analysis by cutoff.

V. Conclusion

The study found high sensitivity and specificity of cystatin C and creatinine for T2 DM with diabetic nephropathy.

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Table-1: Distribution of age between cases and controls

| Age in years | Cases (n=70) | | Controls (n=70) | | p-value ¹ |
|--------------|--------------|------|-----------------|------|----------------------|
| | No. | % | No. | % | |
| 20-30 | 6 | 8.6 | 8 | 11.4 | 0.06 |
| 31-40 | 17 | 24.3 | 32 | 45.7 | |
| 41-50 | 23 | 32.9 | 7 | 10.0 | |
| 51-60 | 14 | 20.0 | 16 | 22.9 | |
| >60 | 10 | 14.3 | 7 | 10.0 | |
| Mean±SD | 46.51±13.94 | | 40.97±12.50 | | |
| Male | 48 | 68.6 | 50 | 71.4 | 0.71 |
| Female | 22 | 31.4 | 20 | 28.6 | |

¹Chi-square test

Table-2: Comparison of cystatin C between cases and controls

| Groups | Cystatin (mg/dl) (Mean±SD) | Creatinine (mg/l) (Mean±SD) |
|----------------------|----------------------------|-----------------------------|
| Cases | 3.05±1.29 | 6.08±4.22 |
| Controls | 0.92±0.12 | 0.90±0.15 |
| p-value ¹ | 0.0001* | 0.0001* |

¹Unpaired t-test, *Significant

Table-3: Diagnostic value of Cystatin for DN

| Cystatin cut off (≥1.17) | Cases | Control | Total |
|--------------------------|-------|---------|-------|
| ≥1.17 | 67 | 2 | 69 |
| <1.17 | 3 | 68 | 71 |
| Total | 70 | 70 | 140 |
| Sensitivity | 95.7 | | |
| Specificity | 97.1 | | |
| PPV | 97.1 | | |
| NPV | 95.8 | | |
| Accuracy | 96.4 | | |

AUC (95%CI)=0.98 (0.95-0.99), p=0.0001

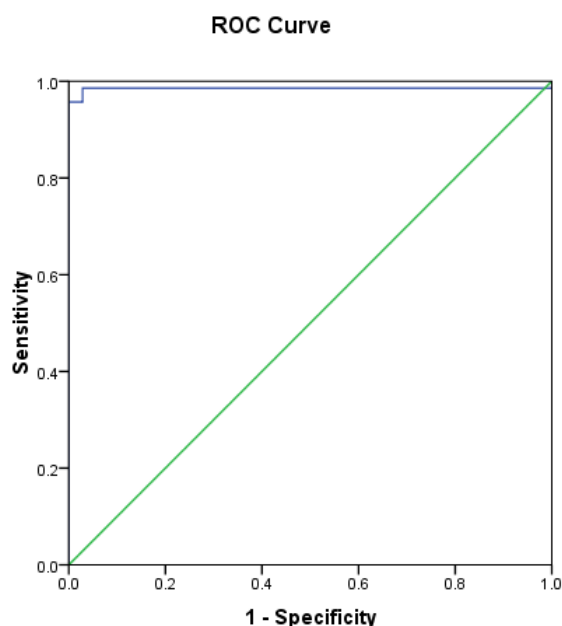


Fig. 1: ROC showing sensitivity and specificity of Cystatin for DN

Table-4: Diagnostic value of creatinine for DN

| Creatinine cut off (≥ 1.07) | Cases | Control | Total |
|------------------------------------|-------|---------|-------|
| ≥ 1.07 | 67 | 16 | 83 |
| < 1.07 | 3 | 54 | 57 |
| Total | 70 | 70 | 140 |
| Sensitivity | 95.7 | | |
| Specificity | 77.1 | | |
| PPV | 80.7 | | |
| NPV | 94.7 | | |
| Accuracy | 86.4 | | |

AUC (95%CI)=0.97 (0.94-0.99), p=0.0001

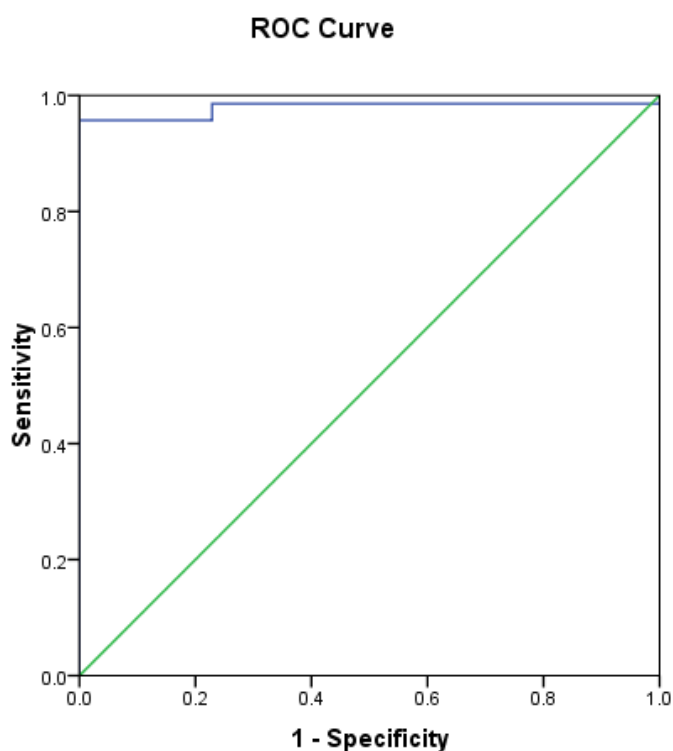


Fig. 2: ROC showing sensitivity and specificity of creatinine for DN

*Dr Rahul Singh. "A Comparison Of Diagnostic Accuracy Of Cystatin C With Creatinine In The Sample Of Patient Of T2 DM With Diabetic Nephropathy." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.7 (2017): 53-57.