EVALUATION OF CYSTATIN-C AS A BIOMARKER OF RENAL FUNCTION IN TYPE-2 DIABETES MELLITUS

INTRODUCTION

The American Diabetes Association (ADA) defined diabetes as “a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both”. Diabetes prevalence is increasing in every country with a rise in its term of claiming of human lives as well as the costs to society. Estimated surveys show that, in 2011, 366 million people of the world's adult population lived with diabetes and it has risen to 371 million in 2012. By 2030 this number is expected to grow to 552 million.1

The term diabetic nephropathy was suggested to describe the clinical manifestations of renal disease in patients with diabetes. Diabetes mellitus is one of the systemic diseases affecting the kidneys.2 Diabetic nephropathy is the leading cause of end stage renal disease and a leading cause of DM related morbidity and mortality. Like other microvascular complications, the pathogenesis of DN is related to chronic hyperglycemia. The mechanism by which chronic hyperglycemia leads to ESRD, involve the effects of soluble factors (growth factors, angiotensin II, endothelin, advanced glycation end products), hemodynamic alterations in the renal microcirculation and structural changes in the glomerulus.3

GFR is a useful index to assess kidney function. GFR is best measured by injecting compounds such as inulin, radioisotopes such as 51Cr-EDTA, 113I-iothalamate, 99mTc-DTPA or radio contrast agents such as iohexol. These tests are complicated, costly, time-consuming and have potential side-effects.

Serum creatinine is the most widely used parameter for everyday assessment of GFR, but it has poor sensitivity & specificity in acute renal failure (ARF) because serum creatinine lags behind both renal injury and renal recovery. It is an insensitive indicator of diminished GFR because its concentration is affected by meat intake, gender, muscle mass, malnutrition and aging. Serum creatinine is freely filtered by glomerulus, not reabsorbed by the proximal tubules but is secreted in small amounts leading to over-estimation of GFR.4

Hence, there is a need to estimate GFR as accurately as possible. Recently, another novel biomarker known as cystatin-C (CC) which can be of use to estimate early decline in GFR in diabetes. It is freely filtered across the glomerular membrane and is almost completely reabsorbed and catabolised in proximal renal tubular cells. Unlike serum creatinine, cystatin-C is not influenced by age, muscle mass, exercise or diet. Therefore, the serum cystatin-C level is a superior marker for the evaluation of renal function compared to other markers such as serum creatinine or creatinine clearance. Serum cystatin-C level has also been reported to have a higher sensitivity and accuracy than serum creatinine for detecting changes in GFR in diabetic patients.5

As there are very few studies on cystatin-C in the Indian population, this study is being undertaken to determine the utility of serum cystatin-C in predicting the decline of renal function in diabetes. Thus, appropriate & timely interventions can be instituted to delay or arrest the progression of diabetic nephropathy.

AIM AND OBJECTIVES

AIM: The aim of this study is to evaluate Cystatin-C as a biomarker of renal function in type-2 diabetes mellitus.

OBJECTIVES

To estimate the concentrations of fasting blood glucose, Creatinine, Cystatin-C and eGFR in Type-2 diabetics and healthy controls.

METHODOLOGY

A case-control study was taken up in group of Type-2 diabetic patients with age and sex matched healthy controls selected from the outpatient and inpatient departments of medicine in the S.S Hospital attached to SSIMS & RC, DAVANGERE during the study period from November-2013 to August-2015. The study was approved by the ethical and research committee of SSIMS &RC, Davangere to use human subjects in the research study. Written informed consent was taken from the study subjects. A total of 50 Type-2 diabetics and an equal number of age and sex matched healthy controls were selected. All patients suffering from Type-2 diabetes diagnosed and confirmed by physician with FBG (fasting blood glucose) and PPBS (post prandial blood sugar) according to American Diabetes Association criteria (FBG ≥126 mg/dL and 2 hour PPBS ≥200 mg/dL). The cystatin-C was analysed by using Agappe kit in Nephelometer, Creatinine and Fasting blood glucose were analysed by using Erba kit in Semi Auto Analyzer. eGFR was calculated by MDRD equation.

Statistical Analysis

Continuous variables with normal distribution were compared using students t-test. Categorical variables were compared using chi-square test. Statistical analysis was done using Statistical Package for

KEYWORDS

Type-2 DM; Diabetic Nephropathy; Cystatin-C; eGFR

INTRODUCTION

The American Diabetes Association (ADA) defined diabetes as “a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both”. Diabetes prevalence is increasing in every country with a rise in its term of claiming of human lives as well as the costs to society. Estimated surveys show that, in 2011, 366 million people of the world's adult population lived with diabetes and it has risen to 371 million in 2012. By 2030 this number is expected to grow to 552 million.1

The term diabetic nephropathy was suggested to describe the clinical manifestations of renal disease in patients with diabetes. Diabetes mellitus is one of the systemic diseases affecting the kidneys.2 Diabetic nephropathy is the leading cause of end stage renal disease and a leading cause of DM related morbidity and mortality. Like other microvascular complications, the pathogenesis of DN is related to chronic hyperglycemia. The mechanism by which chronic hyperglycemia leads to ESRD, involve the effects of soluble factors (growth factors, angiotensin II, endothelin, advanced glycation end products), hemodynamic alterations in the renal microcirculation and structural changes in the glomerulus.3

GFR is a useful index to assess kidney function. GFR is best measured by injecting compounds such as inulin, radioisotopes such as 51Cr-EDTA, 113I-iothalamate, 99mTc-DTPA or radio contrast agents such as iohexol. These tests are complicated, costly, time-consuming and have potential side-effects.

Serum creatinine is the most widely used parameter for everyday assessment of GFR, but it has poor sensitivity & specificity in acute renal failure (ARF) because serum creatinine lags behind both renal injury and renal recovery. It is an insensitive indicator of diminished GFR because its concentration is affected by meat intake, gender, muscle mass, malnutrition and aging. Serum creatinine is freely filtered by glomerulus, not reabsorbed by the proximal tubules but is secreted in small amounts leading to over-estimation of GFR.4

Hence, there is a need to estimate GFR as accurately as possible. Recently, another novel biomarker known as cystatin-C (CC) which can be of use to estimate early decline in GFR in diabetes. It is freely filtered across the glomerular membrane and is almost completely reabsorbed and catabolised in proximal renal tubular cells. Unlike serum creatinine, cystatin-C is not influenced by age, muscle mass, exercise or diet. Therefore, the serum cystatin-C level is a superior marker for the evaluation of renal function compared to other markers such as serum creatinine or creatinine clearance. Serum cystatin-C level has also been reported to have a higher sensitivity and accuracy than serum creatinine for detecting changes in GFR in diabetic patients.5

As there are very few studies on cystatin-C in the Indian population, this study is being undertaken to determine the utility of serum cystatin-C in predicting the decline of renal function in diabetes. Thus, appropriate & timely interventions can be instituted to delay or arrest the progression of diabetic nephropathy.

AIM AND OBJECTIVES

AIM: The aim of this study is to evaluate Cystatin-C as a biomarker of renal function in type-2 diabetes mellitus.

OBJECTIVES

To estimate the concentrations of fasting blood glucose, Creatinine, Cystatin-C and eGFR in Type-2 diabetics and healthy controls.

METHODOLOGY

A case-control study was taken up in group of Type-2 diabetic patients with age and sex matched healthy controls selected from the outpatient and inpatient departments of medicine in the S.S Hospital attached to SSIMS & RC, DAVANGERE during the study period from November-2013 to August-2015. The study was approved by the ethical and research committee of SSIMS &RC, Davangere to use human subjects in the research study. Written informed consent was taken from the study subjects. A total of 50 Type-2 diabetics and an equal number of age and sex matched healthy controls were selected. All patients suffering from Type-2 diabetes diagnosed and confirmed by physician with FBG (fasting blood glucose) and PPBS (post prandial blood sugar) according to American Diabetes Association criteria (FBG ≥126 mg/dL and 2 hour PPBS ≥200 mg/dL). The cystatin-C was analysed by using Agappe kit in Nephelometer, Creatinine and Fasting blood glucose were analysed by using Erba kit in Semi Auto Analyzer. eGFR was calculated by MDRD equation.

Statistical Analysis

Continuous variables with normal distribution were compared using students t-test. Categorical variables were compared using chi-square test. Statistical analysis was done using Statistical Package for
RESULTS

TABLE 1: Shows the mean serum concentrations of FBG, Creatinine, Cystatin-C and eGFR in Healthy Controls and Type-2 diabetic patients.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>HEALTHY CONTROLS</th>
<th>Type-2 DM</th>
<th>p-Value, Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>&lt;0.001, **</td>
</tr>
<tr>
<td>92.42 ± 13.36</td>
<td>221.48 ± 89.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CREATININE (mg/dl)</td>
<td>0.85 ± 0.43</td>
<td>3.30 ± 1.60</td>
<td>&lt;0.001, **</td>
</tr>
<tr>
<td>CYSTATIN-C (mg/L)</td>
<td>0.64 ± 0.23</td>
<td>2.47 ± 0.82</td>
<td>&lt;0.001, **</td>
</tr>
<tr>
<td>eGFR (mL/min)</td>
<td>102.21 ± 20.71</td>
<td>23.27 ± 13.75</td>
<td>&lt;0.001, **</td>
</tr>
</tbody>
</table>

Table 2 shows the Karl Pearson's correlation coefficient (r) matrix of FBG, Creatinine, Cystatin-C and eGFR in Type-2 diabetic patients.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>FBG</th>
<th>Creatinine</th>
<th>Cystatin-C</th>
<th>eGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>r value</td>
<td>1</td>
<td>0.169</td>
<td>0.283</td>
<td>-0.209</td>
</tr>
<tr>
<td>p-value</td>
<td>0.240</td>
<td>0.047</td>
<td>0.145</td>
<td></td>
</tr>
<tr>
<td>Creatinine r value</td>
<td>1</td>
<td>0.670</td>
<td>-0.768</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cystatin-C r value</td>
<td>1</td>
<td>-0.745</td>
<td>-0.209</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR r value</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 shows the Karl Pearson's correlation between eGFR v/s Creatinine and Cystatin-C in all 100 study subjects including healthy controls and Type-2 diabetic patients. The serum concentrations of Creatinine and Cystatin-C were significantly related to eGFR. The correlation between Cystatin-C v/s eGFR (r = -0.852, -0.854) was significantly stronger than that between the creatinine v/s eGFR (r = -0.800) in all study subjects. However, although there was no significant relationship between eGFR v/s Creatinine and Cystatin-C healthy controls.

TABLE 4: Shows comparison of diagnostic validity of Cystatin-C and Creatinine in Type-2 diabetic patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cystatin-C</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-value</td>
<td>&lt;0.001 HS</td>
<td>&lt;0.001 HS</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>98%</td>
<td>76%</td>
</tr>
<tr>
<td>Specificity</td>
<td>97%</td>
<td>97%</td>
</tr>
<tr>
<td>PPV</td>
<td>98%</td>
<td>97%</td>
</tr>
<tr>
<td>NPV</td>
<td>97%</td>
<td>94%</td>
</tr>
</tbody>
</table>

DISCUSSION

Diabetic nephropathy is the most common cause of chronic kidney disease which is widely prevalent in developing countries. Diabetic nephropathy develops due to complex interaction between metabolic and haemodynamic pathophysiological factors, which lead to renal damage. It presents with microalbuminuria in the earliest stage. This may progress to macroalbuminuria and later renal insufficiency and ESRD.

In this study, we have evaluated the serum concentrations of FBG, Creatinine, Cystatin-C and calculated eGFR in healthy controls and Type-2 DM.

Cystatin-C:

Cystatin-C production rate is constant and is unaffected by inflammatory process, gender, age, protein intake and muscle mass. Due to its small size, it is freely filtered by the glomerulus and not secreted but is fully reabsorbed and broken down by renal tubules. Thus makes cystatin-C as an excellent marker of GFR.

The mean serum concentrations of cystatin-C in healthy controls and Type-2 diabetic patients were in the range of 0.64 ± 0.23 mg/dL and 2.47 ± 0.82 mg/L, respectively. The mean concentrations of serum cystatin-C in Type-2 diabetic patients were higher when compared to healthy controls. Cystatin-C positively correlated with creatinine which was statistically highly significant (p < 0.001). CC also negatively correlated with eGFR which was statistically highly significant (p < 0.001). Diagnostic validity tests revealed a sensitivity of 98%, a specificity of 97%, a PPV of 98% and a NPV of 97% for cystatin-C.

Serum cystatin-C level is significantly increased in Type-2 DM. The cause for this rise is appears to be mainly related to extracellular matrix accumulation, which occurs in the glomerular basement membrane and in tubular basement membrane and is the principal cause of mesangial expansion and contribute to interstitial expansion late in the disease leading to decreased excretion of cystatin-C.

Our findings of the study is in accordance with several other studies,
creatinine in detecting mild acute renal insufficiency in diabetic patients.

Jan Kyhse-Andersen, et al., demonstrated that the serum concentration of cystatin-C is a better marker for GFR than that of creatinine. The overall correlation between cystatin-C and GFR was significantly stronger than that between creatinine and GFR.18

Richard J Mac Isaac, et al., found out in their study that measurement of serum cystatin-C concentration provides a simple and accurate method for detecting early renal impairment in subjects with diabetes.20

Radovan Hojnos, et al., found out that serum cystatin-C is a reliable marker of GFR in patients with mild to moderately impaired kidney function and has a higher diagnostic accuracy than serum creatinine and calculated creatinine clearance from the Cockcroft and Gault formula in female patients.21

Oddoze C, et al., on the other hand, demonstrated serum creatinine and serum cystatin-C to be equal in diagnostic accuracy in microalbuminuric and proteinuric diabetics. Oddoze, et al., examined a low number of Type-1 and 2 diabetic patients, 15 and 34, respectively, and used a modified Jaffe method for serum creatinine.22

CREATININE:
Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). Elevated levels are found in renal dysfunction, reduced blood flow (shock, dehydration and congestive heart failure), diabetes and acromegaly. Decreased levels are found in muscular dystrophy. Glomerular filtration rate is considered the best marker of renal function and serum creatinine is the most commonly used biochemical parameter to estimate GFR in routine practice. Although the best measure for GFR is obtained by techniques that involve infusion of either endogenous or exogenous substances, GFR is usually estimated in clinical practice by various formulae based on serum creatinine concentration.

The mean serum concentrations of creatinine in healthy controls and Type-2 diabetic patients were in the range of 0.85 ± 0.43 mg/dl and 3.30 ± 1.60 mg/dl, respectively. The mean concentration of serum creatinine in Type-2 diabetic patients was higher when compared to healthy controls. The eGFR in healthy controls and Type-2 diabetic patients were in the range of 102.21 ± 20.71 ml/min and 23.27 ± 13.75 ml/min, respectively. Creatinine correlated positively with cystatin-C but it negatively correlated with eGFR and which were statistically highly significant (p <0.001).

Diagnostic validity tests revealed a sensitivity of 76%, a specificity of 97%, a PPV of 97% and a NPV of 94% for creatinine. These results showed higher sensitivity, specificity, PPV and NPV for cystatin-C when compared to creatinine suggesting superiority of cystatin-C over creatinine in assessing renal impairment in Type-2 diabetic patients.

In diabetic nephropathy, the cause of decreased GFR is due to loss of integrity of the glomerular basement membrane. Estimation of the GFR is the most widely used test of renal function and reflects the kidneys ability to clear a particular substance from plasma. The small molecule creatinine is endogenously produced by muscles and excreted by the kidneys. Therefore, a reduction in GFR leads to an increase in serum creatinine.

Our findings of the study is in agreement with several other studies,23-27

Hany S. Elbarbary, et al., showed that serum creatinine levels were increased in microalbuminuric Type-2 DM patients when compared to normoalbuminuria. There was a significant positive correlation between serum creatinine with cystatin-C and significant negative correlation GFR.23

A G Christenson, et al., found that serum cystatin-C is an interesting alternative to serum creatinine as a GFR marker. Their study infers that serum cystatin-C is valuable in the detection of early or mild diabetic nephropathy. Whereas increased serum creatinine seems to be as good as serum cystatin-C in the diagnosis of severe nephropathy.24

Michele Mussap, et al., concluded that cystatin-C may be considered as an alternative and more accurate serum marker than serum creatinine or the Cockcroft and Gault estimated GFR in discriminating Type-2 diabetic patients with reduced GFR from those with normal GFR.19

Ashwin Kumar A S, et al., showed that serum creatinine as well as serum cystatin-C were significantly elevated in the study group as compared to non-diabetic controls. There was a strong positive correlation of serum cystatin-C with serum creatinine. Serum cystatin-C can be used as an alternative to serum cystatin in determining GFR in Type-2 diabetes mellitus.25

Strength and further scope of the study:
The concentrations of cystatin-C and creatinine were significantly increased in Type-2 diabetic patients as compared to healthy controls except eGFR which was significantly decreased in Type-2 diabetic patients as compared to healthy controls. This study also proved that cystatin-C is a better marker with high sensitivity and specificity in diabetic nephropathy than creatinine.

The diagnosis of diabetic nephropathy can be improved by measuring several new biochemical markers that have the potential to detect early renal impairment in Type-2 DM than the traditional markers. These new markers include Kidney Injury Molecule-1 (KIM-1), N-acetyl-glucosaminidase (NAG), human neutrophil gelatinase-associated lipocalin (NGAL), j2-microglobulin, α1-microglobulin, transferrin, type-IV collagen, interleukin-18 (IL-18), clusterin and ceruloplasmin. The study should have included a larger number of subjects for highly statistically significant data to prove the reliability of serum cystatin-C in Type-2 diabetic nephropathy patients.

CONCLUSION
The inadequacy of the traditional markers in detecting early changes in GFR and particularly in monitoring the course of advanced diabetic nephropathy calls for alternative non-invasive methods in clinical nephrology. Cystatin-C seems to be an alternative and more accurate serum marker than serum creatinine in discriminating Type-2 diabetic patients with a reduced GFR from those with a normal or near-to-normal GFR. The more prominent rise in serum cystatin-C values, as found in this study, allows a more rapid diagnosis of decline in GFR, with an earlier therapeutic intervention.

Our results show that cystatin-C is superior for renal glomerular function assessment in a well-defined patient group and its measurement may be recommended in the routine management of diabetic patients. So serial measurement of cystatin-C would allow to detect and stage the degree of renal impairment in diabetic nephropathy and would indicate how the disease evolves in these patients, allowing us to adopt early measures to control the disease. Thus, cystatin-C can be recommended as routine biomarkers to screen and to monitor the progress of diabetic nephropathy.

REFERENCES


