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Biochemistry

STUDY OF COMBINED MARKERS OF DIABETIC NEPHROPATHY

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ABSTRACT **Introduction:** Diabetic nephropathy (DN) is one of the most serious microvascular complications of Type-2 Diabetes Mellitus (Type-2 DM) which significantly impacts morbidity, mortality and quality of life. The onset of DN is signaled by microalbuminuria (Albumin/Creatinine ratio, ACR = 30 to ≤ 300 mg/g) which progresses to macroalbuminuria and eventually lead to an End Stage Renal Disease (ESRD). Cystatin-C has been identified as a promising new biomarker for the detection of renal damage and is more sensitive than creatinine. **Objectives:** To estimate and evaluate the correlation of fasting blood glucose, HbA1c, blood urea, serum creatinine, serum cystatin-C, microalbuminuria & eGFR as biomarkers in detection of renal dysfunction in Type-2 diabetics as compared to healthy controls. **Methodology:** In this study, 50 proven cases of Type-2 Diabetic patients and an equal number of age and sex matched healthy controls were included. Blood glucose, urea, creatinine, cystatin-C, HbA1c and microalbuminuria were measured by GOD, GLDH UREASE, Jaffe's and Nephelometric methods, respectively. eGFR was calculated by using MDRD formula. Appropriate statistical analysis was done. **Results:** The serum concentrations of FBG, HbA1C, Urea, Creatinine, Cyatatin-C, Microalbuminuria and eGFR were found to be significantly increased in Type-2 Diabetic patients as compared to healthy controls ($p, < 0.001$). We also found that there was a significant positive correlation of serum cystatin-C with FBG, HbA1C, Urea, Creatinine and Microalbuminuria and negative correlation with eGFR in Type-2 Diabetes patients. **Conclusion:** This study concludes that the serial measurement of cystatin-C along with microalbuminuria 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 and microalbuminuria can be recommended as routine biomarkers to screen and to monitor the progress of diabetic nephropathy.

KEYWORDS : Type-2 DM; Diabetic Nephropathy; Microalbuminuria; Cystatin-C; eGFR.

INTRODUCTION

Diabetic Nephropathy (DN) is the single most common cause of chronic renal failure (CRF) and is a rapidly growing problem worldwide. It is an acquired sclerotic injury associated with thickening of the glomerular basement membrane secondary to long standing effects of hyperglycaemia, advanced glycation end products and reactive oxygen species.[1]

Diabetic nephropathy can affect 20-30% of the diabetic population, who present with an increase in urinary albumin excretion, microalbuminuria in the earliest stage.[2]

Microalbuminuria is well known as a risk factor resulting in macroalbuminuria in Type-1 and Type-2 diabetic patients which progress to end stage renal disease (ESRD).[3] Type-2 diabetic patients with microalbuminuria showes a rapid decline of glomerular filteration rate (GFR) and also it shows that they had a 1.8 fold increased risk for cardiovascular mortality during 12 years of follow up compared with individuals with normoalbuminuria.[4]

GFR is a useful index to assess kidney function. GFR is best measured by injecting compounds such as inulin, radioisotopes such as ^{51}Chromium- EDTA, ^{125}I-iothalamate, ^{99m}Tc-DTPA or radio contrast agents such as iohexol. These tests are complicated, costly, time-consuming and have potential side-effects.[5]

Endogenous markers like plasma creatinine and urea concentration despite their limitations are commonly used because they are cheap and easily available.[6]

Urea is freely filtered by the glomerulus and not secreted by the tubules. However, a large portion (40-70%) is passively reabsorbed from renal tubules leading to underestimation of GFR and also its concentration in plasma may vary depending on diet, hepatic function and state of numerous diseases.[6]

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.[7] 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.[6]

Hence, there is a need to estimate GFR as accurately as possible. Recently, an 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.[8]

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 & microalbuminuria 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: To evaluate the correlation of fasting blood glucose, HbA_1c, blood urea, serum creatinine, serum cystatin-C, microalbuminuria & eGFR as biomarkers in detection of renal dysfunction in Type-2 diabetics as compared to healthy controls.

OBJECTIVES: To estimate the concentrations of fasting blood glucose, HbA_1c, blood urea, serum creatinine, serum cystatin-C,

microalbuminuria & 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 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.

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 and HbA_1c were analysed by Nephelometry method[9,10,11] by using Agappe kit, Serum Creatinine by Modified Jaffe's method[12], Fasting Blood Glucose by Glucose Oxidase method[13] and Blood Urea by GLDH-UREASE method[14,15] by using Erba kit in Semi Auto Analyzer and Microalbuminuria was analysed by using Turbilyte kit in Semi Auto Analyzer which employs Turbidimetric Immunoassay technique.[16] eGFR was calculated by MDRD equation.[17]

Statistical Analysis

Results were subjected for appropriate statistical analysis by using SPSS version 17 software. Student's unpaired t-test were carried out for comparison of mean difference between two groups. Relationship between measurements was assessed by Karl Pearson's coefficient of correlation. Diagnostic validity test such as specificity, sensitivity, positive predictive value, negative predictive value was used to assess the utility of these parameters as biomarkers in diabetic nephropathy. For all the tests, a p-value of 0.05 or less was considered for statistical significance.

RESULTS

Table 1: Shows The Mean Serum Concentrations Of FBG, HbA_1c, Blood Urea, Creatinine, Cyatatin-C, Microalbuminuria And eGFR in Healthy Controls and Type-2 Diabetic Patients.

Parameters	Healthy Controls	Type-2 DM	p -Value,
	Mean ± SD	Mean ± SD	Sig
FBG (mg/dl)	92.42 ± 13.36	221.48 ± 89.62	<0.001, **
HbA_1c	4.49 ± 0.88	8.91 ± 2.20	<0.001, **
Urea (mg/dl)	23.47 ± 8.53	68.84 ± 30.76	<0.001, **
Creatinine (mg/dl)	0.85 ± 0.43	3.30 ± 1.60	<0.001, **
Cystatin-C (mg/L)	0.64 ± 0.23	2.47 ± 0.82	<0.001, **
Microalbuminuria (mg/g)	22.89 ± 6.47	207.75 ± 75.23	<0.001, **
eGFR (ml/min)	102.21 ± 20.71	23.27 ± 13.75	<0.001, **

Student's unpaired t-test,
p > 0.05 NS (Not Significant),
p < 0.05 *S (Significant),
p < 0.001 **HS (Highly Significant)

Table 1: Statistical analysis by student's unpaired t-test showed that the mean concentrations of fasting blood glucose, HbA_1c, blood urea, serum creatinine, serum cystatin-C and microalbuminuria were increased and eGFR was decreased in Type-2 diabetic patients when compared to healthy controls and were statistically highly significant (p, <0.001).

Table 2: Shows Karl Pearson's Correlation Coefficient (r) Matrix of FBG, HbA_1c, Urea, Creatinine, Cystatin-C, Microalbuminuria And eGFR in Type-2 Diabetic Patients.

		FBG	HbA_1c	Urea	Creatinine	Cystatin-C	Microalbuminuria	eGFR
FBG	r value	1	0.212	0.134	0.169	0.283*	0.273	-0.209
	p-value		0.140	0.352	0.240	0.047	0.055	0.145
HbA_1c	r value		1	0.008	0.072	0.064	0.023	0.109
	p-value			0.957	0.621	0.661	0.876	0.450
Urea	r value			1	0.874**	0.627**	0.360*	-0.588**
	p-value				<0.001	<0.001	0.010	<0.001
Creatinine	r value				1	0.670**	0.442**	-0.768**
	p-value					<0.001	<0.001	<0.001
Cystatin-C	r value					1	0.597**	-0.745**
	p-value						<0.001	<0.001
Microalbuminuria	r value						1	-0.536**
	p-value							<0.001
eGFR	r value							1
	p-value							

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is highly significant at the 0.01 level (2-tailed).
r = Karl Pearson's coefficient of correlation

Table 2: Karl Pearson's correlation shows that there is a highly statistically significant positive correlation between urea v/s creatinine and cystatin-C, creatinine v/s cystatin-C and microalbuminuria and cystatin-C v/s microalbuminuria (p, <0.001). There is also a statistically significant positive correlation between FBG v/s cystatin-C (p, 0.047) and urea v/s microalbuminuria (p, 0.010). There is a positive correlation between FBG, HbA_1c, Urea, Creatinine, Cystatin-C and Microalbuminuria.

There is a negative correlation between urea, creatinine, cystatin-C and microalbuminuria with eGFR which is statistically highly significant (p, <0.001). There is also a negative correlation between FBG and eGFR.

Table 3: Shows Comparison Of Diagnostic Validity Of Cystatin-C And Creatinine In Type-2 Diabetic Patients.

Variable	Cystatin-C	Creatinine
p-value	< 0.001 HS	< 0.001 HS
Sensitivity	98%	76%
Specificity	97%	97%
PPV	98%	97%
NPV	97%	94%

P > 0.05 NS (Not Significant), P < 0.05 S (Significant),
P < 0.001 HS (Highly Significant)

PPV- Positive Predictive Value, NPV- Negative Predictive Value

Table 3: It was found from this table that sensitivity, specificity, positive predictive value and negative predictive value of serum cystatin-C was better as compared to serum creatinine in assessing renal impairment in Type-2 diabetic patients.

DISCUSSION

Diabetic nephropathy is the most common cause of chronic kidney disease which presents with microalbuminuria in the earliest stage, may progress to macroalbuminuria and later renal insufficiency and ESRD.

In this study, we have evaluated the serum concentrations of FBG, HbA_1c, Urea, Creatinine, Cystatin-C, Microalbuminuria 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 a excellent marker of GFR.

Creatinine:

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body. Elevated levels are found in renal dysfunction, reduced blood flow, 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.

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 kidney's 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.

Microalbuminuria:

Microalbuminuria typically occurring after 5 or more years of

diabetes. In diabetes, vascular permeability increases and microalbuminuria appears when metabolic regulation is poor, at least in part because of glycosylation and loss of negative charges on the membranes. Compared with normoalbuminuric patients, patients with persistent microalbuminuria have threefold to fourfold greater risk of progression to proteinuria and ESRD. At this microalbuminuric stage, glomerular lesions are generally more severe and blood pressure tends to be increasing, often into hypertensive range. Patients with microalbuminuria showed the fastest GFR decline.

Our findings of the study is in agreement with several other studies like Maryam Nejat, et al in 2012[19], Michael G Shlipak, et al in 2013[20], Mukherjee Brijesh and Patra Saurav in 2015[21] et al.

Strength And Further Scope Of The Study:

The concentrations of cystatin-C, creatinine, urea and microalbuminuria 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 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

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 along with microalbuminuria 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 and microalbuminuria can be recommended as routine biomarkers to screen and to monitor the progress of diabetic nephropathy.

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