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Cystatin is Superior to Creatinine in Diabetic Patients in the Measure of GFR

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Abstract: ***Background: Glomerular Filtration Rate (GFR) has been known to be one of the best measures to assess renal functions and Creatinine levels. However more recently serum Cystatin C (Cys-C) has emerged as an alternative to serum Creatinine. Objective: To study the benefits of using Serum Cystatin C (Cys-C) over serum Creatinine and assessif Cys-C can be a better marker to depict GFR in patients with diabetes mellitus (DM). Methods: Data was collected from 174 patients in this clinical trial. From the blood and urine samples collected, Cys-C, SC, MAU, and ACR tests were made and values calculated from them. A data comparison between these tests will be made and GFR will be calculated from (Cys-C) and comparison was made with (SC). Results: Cystatin C possessed high area (AUC: 0.990) compared to other bio markers namely, MAU (AUC: 0.806) and ACR (AUC: 0.799). This shows that, Cys-C is the better marker of renal disease compared to SC, MAU and ACR. Conclusion: It can beconcluded that Cys-C is a better option for a biomarker for screening early renal impairments in patients with diabetes, while Microalbuminuria and Albumin/creatinine ratio are also powerful diagnostic tools. The implication for clinical practice is that Cys-C may be employed as an alternative to current investigations to measure GFR, however research on a larger scale is still warranted.***

Keywords: cystatin

1. Introduction

Glomerular Filtration Rate (GFR) is considered as the best index for depicting renal functions and Creatinine is the most widely used marker to assess the GFR.While Serum Creatinine (SC) has its own merits, there are also certain limitations to using (SC) as a GFR marker(1,2).

Creatinine remains the widely used marker in healthcare sectors to assess GFR in patients till date(1). While Creatinine is relatively specific, it is not very sensitive as its levels significantly increase when 50% of Glomerular Filtration is decreased. (3) Due to tubular secretion and reabsorption, serum creatinine may estimate GFR inaccurately (4). Creatinine can also be inadequately sensitive to detect renal impairment especially in the beginning stages (5). Concentration of creatinine can also depend on many extra renal factors such as tubular secretion, muscle mass, food intake and so on. Secretion of serum creatinine is proportional to age and muscle mass and therefore in elderly patients who have low muscle mass, are chronically ill and are malnourished, serum creatinine values may appear in normal range. Although SC has been standardized, the estimates of GFR measured can be relatively imprecise (5,6).

Serum Cystatin C(Cys-C) on the other hand is considered a potential alternative to SC as amarker for measuring GFRand Cys-C could be used as an adverse prognosis for patients with Chronic Kidney disease (7–9). The advancement of laboratory assays and certified reference material has improved the accessibility and validity Cys-C testing (10,11). Cys-C is a low molecular weight protein (13,359 Da) that is produced by all human nucleated cells and is hence unique (12). The structure of the human Cys-C gene and its promoter shows that the gene is a house keeping gene type.

Since Cys-C has low molecular weight, they usually almost freely pass the glomerular membrane and when kidney functions are normal, this protein is then reabsorbed and degraded by the proximal tubular cells. Studies on human kidneys using northern blot strongly indicated this(13,14).

Another marker for diagnosing early signs of kidney damage is Albumin Creatinine Ration (ACR). Albumin is a major plasma protein and normally the kidneys filter albumin so albumin presence in urine can be an early sign of kidney damage. Excess albumin excretion (between 30-300 mg) in urine is called Microalbuminuria (MAU). When 24 hour sample collection is not possible, the ACR can be calculated from the urine, which will project if there is excess Albumin orMAU in urine(15,16). When ACR is more than 30 mg/ml of urine it is defined as a sign of Chronic Kidney Disease (CKD) and because ACR must be assessed routinely, it is only recommended for patients with diabetes (9).

Even with the advancements in laboratory assays, SC remains the widely used test for depicting GFR. In this research we aim to study the benefits of using Serum Cystatin C (Cys-C) over serum Creatinine and assessif Cys-C can be a better marker to depict GFR in patients with diabetes mellitus (DM).

2. Methodology

Data was collected from 174 patients in this clinical trial. From the blood and urine samples collected, Cys-C, SC, MAU, and ACR tests were made and values calculated from them. A data comparison between these tests will be made and GFR will be calculated from (Cys-C) and comparison was made with (SC).

This clinical study was conducted in King Abdul Aziz Medical Hospital over a period of one year and approved by the institutional ethics committee. A total of 174 patients with diabetes mellitus were included in this study out of which 128 were females and 48 were males. All the patients with renal impairment were excluded. Patients were also categorized based on their nationality (98 Saudi and 76 Non Saudi). Table 1 lists the demographics of the patients with Diabetes Mellitus.

Blood samples were collected from the patients and Serum was centrifuged from the blood samples collected and stored for analysis. Urine sample was collected and also stored for analysis. Serum Creatinine (SC) was measured and serum Cystatin C (Cys-C) was measured using the Dimension vista system Albumin Concentrations in the Urine samples was measured using and microalbuminuria (MAU) was calculated from this value.

3. Results

The following statistical tests were used to analyze the data: frequency distribution, descriptive statistics, independent sample t test, correlation analysis and ROC curve.Five percent level of significance was considered as statistically significant.

From the data collected, a descriptive statistics is listed in table 3, which shows the mean, standard deviation, minimum and the maximum value of bio-chemical parameters of the patients. The mean value of Cys-C was 1.15 mg/L and SC was 75.40 µmol/L.

On the basis of standard deviation, the patients' microalbuminuria had more variations because one patient's MAU value was very low (0.43 mg/L) while the other's was very high (3890 mg/L).

Glomerular Filtration Rate (GFR) was calculated from the Serum Cystatin C (Cys-C) values. A cut off value of 90 mL/min/1.73 m^2 was chosen based on previously published literature. Table 4 represents the status of kidney functions of the DM patients based on the GFR. The analysis shows that a maximum number of diabetes mellitus patients had the risk of renal impairment: i.e., around 58 percent of the DM subjects had the risk of renal impairment while 33 percent had normal renal functions. However, 9 percent of the DM patient's reports concerning the kidney function were not available owing to lack of serum cystatin C data.

Also, the patient who had DM together with renal disease, had significantly high Cys-C levelsACR compared to the DM patients without renal disease ($p<0.01$**). Serum Creatinine (SC) level on the other hand was almost similar among the DM patients with and without renal disease showing no significant difference in values ($p>0.05$).

A correlation between the results was made and represented in table 5. The results from the correlation analysis shows that GFR has a strong negative relationship with Cys-C ($r = -0.845$); i.e., if Cys-C increases, then their GFR rate is decreased. This shows that Cys-C can be a good marker for renal disease. Similarly, GFR has a weak negative relationship with SC level ($r = -0.195$), MAU ($r = -0.312$) ACR ($r = -0.445$).Hence creatinine, microalbuminuria and ACR are also a biomarker for kidney disease. Meanwhile, Cys-C as a marker is the better option for renal disease compared to SC, MAU and ACR because it has a strong relationship with GFR. Also, Cys-C has a positive relationship with SC, MAU and ACR.

Table 6 shows the receiver operating characteristic (ROC) curve analysis for Cys-C, SC, MAU and ACR.MAU and ACR were significant biomarkers of renal disease as they had the p-values below 1 percent level. However, creatinine failed to depict the significant biomarkers of renal disease ($p>0.05$).

As per the area under the curve, cystatin C possessed high area (AUC: 0.990) compared to other bio markers namely, MAU (AUC: 0.806) and ACR (AUC: 0.799).This shows that, Cys-C is the better marker of renal disease compared to SC, MAU and ACR. The specificity and sensitivity of all markers used in this study can be found in Figure 1.

4. Discussion

GFR is considered as the best index for depicting renal functions and therefore it is absolutely necessary that the marker used to calculate GFR has high sensitivity and specificity. Many studies have already recognized Cys-C to be a better marker than Serum Creatinine to assess impaired kidney functions. The advantage of using Cys-C to calculate GFR is that the production Cystatin-C is less subjected to the effects of race, sex, age, diet intake, muscle mass, weight height and so on and the concentration of the serum Cys-C remains the same from 1 to 50 years of age(17–20).

The production Cys-C in fact is affected by very few factors. Lower and medium doses of glucocorticoids found to have no influence in the production of Cys-C, while very high doses have been shown to increase its production(21,22).

Thyroid dysfunction has a major impact on Cys-C production levels and this applies for even mild thyroid dysfunction and hence thyroid function needs to be taken into account when using Cys-C as a marker for kidney functions(23,24).

The Sensitivity and specificity of Cys-C as a GFR marker is also higher than that of SC(5). SC can be quite an unreliable marker for early detection of renal failure and the results from the present study support this statement. With the decrease in GFR, Serum Cystatin C increases significantly that serum creatinine. The results from our study also support this claim(4,5).

A study by Swedko et al.(1) states that SC can be an inadequate marker for screening renal failure in elderly. They used a cutoff value of 150 µmol/L. The results showed that 87.4% of the elderly patients with renal failure had a SC value of 150 µmol/L or less.

Results from previous studies has shown that in receiver operating curve (ROC) analysis, Cys-c shows more sensitivity and specificity compared to that of SC (5,20). The results obtained from the present study also showed that Cys-C has a larger are under the curve compared to other markers and also showed that it had more sensitivity and specificity than the other markers and thus supports the results obtained from previous studies.

MAU and ACR are other potential markers to detect early renal impairment. They are measured from urine samples and are hence non-invasive methods of sample collection. Although MAU has been accepted as an early marker for

renal impairment, a large proportion of renal impairment occurs at non-albuminuric state (25). Hence it is important to be more diverse in the use of markers for diagnosing early renal impairments. ACR is only recommended for patients with Diabetes Mellitus (DM) since it has to be measured routinely. To study ACR alone for detection of early stages of kidney disease might not be sufficient and hence the GFR. In comparative analysis by the ROC curve, ACR showed a better sensitivity and specificity than SC and hence is also a powerful marker. Since ACR is a non-invasive test, it could be preferred as an initial screening test for early detection of kidney damage.

5. Conclusion

Based on the results obtained, we conclude that Cys-C is a better option for a biomarker for screening early renal impairments in patients with diabetes, while Microalbuminuria and Albumin/creatinine ratio are also powerful diagnostic tools. The implication for clinical practice is that Cys-C may be employed as an alternative to current investigations to measure GFR, however research on a larger scale is still warranted.

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Appendix

Table 1: Frequency distribution of demographic details of the DM patients

Demographic information	Frequency (n)	Percent (%)
Gender		
Male	46	26.4
Female	128	73.6
Total	174	100.0
Nationality		
Saudi	98	56.3
Non-Saudi	76	43.7
Total	174	100.0

Table 2: Difference between Saudi and non-Saudi patients based on their age and diabetes mellitus durations

(in Years)	Nationality		t-value	p-value
	Saudi	Non-Saudi		
	Mean±SD			
Age	58.08±11.40	58.59±12.13	-0.285	0.776
Diabetes mellitus duration	8.18±5.79	9.22±6.09	-1.148	0.252

Table 3: Descriptive statistics for bio-chemical parameters of DM patients

Bio-chemical parameters	Min.	Max.	Mean	SD
Cystatin C (milligram/L)	0.49	4.65	1.15	0.57
Creatinine (micromole/L)	35.00	428.00	75.40	37.23
microalbuminuria (mg/L)	.43	3890.00	117.93	357.27
Albumin/Creatinine ratio (mg/gm)	2.90	2244.80	124.79	309.55

Table 4: Demographic and bio-chemical parameter of the patients based on the Glomerular Filtration Rate (GFR)

S.NO	variables	GFR	
		Renal disease (GFR<90)	Normal (GFR>90)
I	**Gender**		
	Male	21	21
	Female	79	36
II	**Nationality**		
	Saudi	60	29
	Non-Saudi	40	28
III	**Bio-chemical parameter**		
3.1	Creatinine (micromol/L)	79.13±43.51	72.15±25.59
	t-value (p-value)	-1.056 (0.293)	
3.2	Cystatin C (milligram/L)	1.37±0.61	0.75±0.09
	t-value (p-value)	-7.534 (0.001**)	
3.3	Microalbuminuria (mg/L)	153.44±441.35	44.06±136.04
	t-value (p-value)	-1.793 (0.075)	
3.4	albumin/creatinine ratio (mg/gm)	176.85±377.68	32.68±89.84
	t-value (p-value)	-2.626 (0.010**)	

Table 5: Correlation between cystatin C, serum creatinine, microalbuminura / creatinine ratio and glomerular filtration rate

	GFR	Cystatin C	Creatinine	Microalbuminuria	Albumin/creatinine ratio
GFR	1	-.845**	-.195*	-.312**	-.445**
Cystatin C		1	.394**	.377**	.436**
Creatinine			1	.079	.027
Microalbuminuria				1	.740**
Albumin/creatinine ratio					1

**p<0.01, *p<0.05

Table 6: ROC curve for cystatin c, creatinine, microalbuminurea and microalbuminurea/creatinine ratio as biomarkers of renal disease in the DM patients

Area Under the Curve					
Test Result Variable(s)	Area	Std. Error	p-value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Cystatin C(milligram/l)	.990	.006	.000**	.978	1.000
Creatinine (micromol/L)	.554	.055	.321	.447	.661
Microalbuminuria (mg/L)	.806	.046	.000**	.716	.896
Albumin/creatinine ratio (mg/gm)	.799	.043	.000**	.714	.884

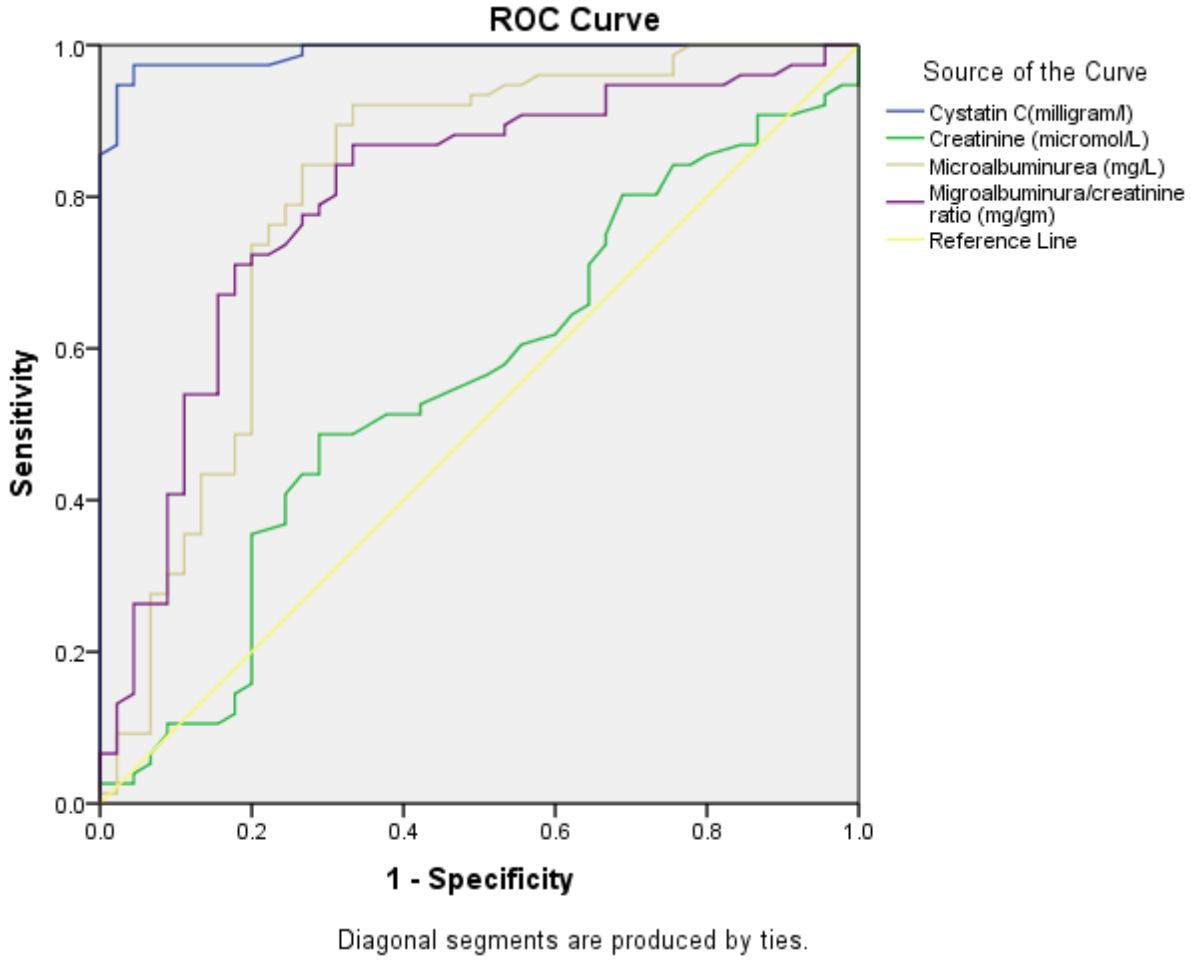

Figure 1: ROC curve for cystatin c, creatinine, microalbuminurea and microalbuminurea/creatinine ratio