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Original Research Article

Cystatin GFR better marker than creatinine GFR for accurate prediction of renal dysfunction in diabetic patients: A tertiary care centre study

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ABSTRACT

Introduction: Diabetic and hypertensive patients gradually gets decreased glomerular function. Creatinine starts rising and exhibits decreased kidney function when more than 50% of glomerular function is lost. Cystatin C, a parameter which accesses kidney function accurately predicts GFR.
Materials and Methods: GFR is calculated by measuring Cystatin C and Creatinine. Groups were normal patients, diabetics patients, Comparison of changes of pre and post treatment GFR by Creatinine and Cystatin.
Results: Total 57 patient studied 1) Cystatin C GFR is lower than creatinine GFR in 20 normal patients with P value with paired t test is 0.0032 hence prompts early renal evaluation whereas creatinine GFR overestimates renal function. 2) cystatin C GFR in 37 patients with kidney dysfunction and diabetes is less than Creatine GFR with p-value < 0.05 suggests more accurate prediction for renal injury. 3) Cystatin GFR is affected by age and gender 4) Change in Pre and Post treatment of 9 patients with creatinine GFR and cystatin GFR with p-value = 0.47657 > 0.05 but these patients high Creatinine levels on admission which gets normalised with remarkable rise in GFR whereas Cystatin C levels gets marginally decrease suggests renal recovery ongoing.
Conclusions: Clearly exhibits Creatinine GFR is overestimates renal function. Patients with normal GFR by Creatinine having raised Cystatin C levels prompts early renal evaluation. Cystatin C is accurately estimating, less affected by variables and predicting severity of renal dysfunction. Thus, Cystatin C GFR better diagnostic and sensitive maker than creatinine GFR for accurate prediction of renal dysfunction in Diabetic patients.

1. Introduction

Glomerular filtration rate estimation is essential to access kidney function as it facilitates the detection, evaluation and management of chronic kidney disease.[1] Creatinine is a normal product of muscle metabolism (breakdown)and is eliminated through urine. The level of creatinine in blood depends on persons muscle mass and the quality of renal function.

However, on account of variation in creatinine generation due to diet, physiologic or clinical condition that affects muscle mass, the GFR (Glomerular filtration rate) estimates based on serum creatinine may be inaccurate in healthy people with high or low meat intake, building muscles, and in patients with illness complicated by malnutrition,

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inflammation or critically ill patients. MDRD (Modifying diet in renal disease) studies are equations which estimate GFR, based on serum creatinine and they include variables like age, sex, race as surrogates for creatinine generation by muscles.[2,3]

2. What is Cystatin C?

It is a non-glycosylated, endogenous 13 KD Protein filtered by glomeruli. The epithelial cells of the proximal tubules reabsorb and catabolise it and only very small amounts are excreted in the urine. It is replacing serum creatinine and is less affected by the muscle mass[4] When GFR decreases cystatin C levels start rising proportionately. Subjects who have liver cirrhosis, are very obese, are malnourished, practice a vegetarian diet, have amputed limb or reduced muscle mass (elderly or children) creatinine measurements may not be reliable. The delay that exists between decline in GFR and rise in creatinine makes creatinine a poorly reliable test for making a therapeutic decision in critically ill like change of nephrotoxic drug or increase renal perfusion.

3. What is GFR?

It is a test used to check how well the kidneys are working. Specifically, it estimates how much blood passes through the glomeruli each minute. Glomeruli are tiny filters in the kidney that filters waste from the blood. GFR 70 ml or more then serum creatinine is normal and GFR < 50 ml indicates kidney dysfunction. GFR of 20ml or less means ESRD (End Stage Renal Disease). GFR in critically ill can change rapidly due to renal hypo perfusion, shock or use of nephrotoxic drugs. Measuring GFR by exogenous markers like inulin, iothalamate, iohexol are time consuming and tedious. However, measures of Cystatin C and Serum creatinine are more convenient for evaluation of acute and chronic decline of kidney function.

4. Aim and Objective of the Study

Its prospective observational study to compare cystatin GFR with creatinine GFR in Diabetic and non-diabetic patients. To follow up GFR changes in patients with kidney dysfunction post treatment. There are 1) Non Diabetic 20 patients without kidney dysfunction 2) Diabetic 37 patients without follow up and with kidney dysfunction 3) follow up of 9 patients with kidney dysfunction to see response to treatment in the form of improvement in GFR.

5. Materials and Methods

Total 57 patients studied. Control 20 patients, cases 37, follow up 9 patients. Values of sr. cystatin and sr.creatinine obtained. 5 ml of whole blood was collected in red topped plain vacutainer from BD. Post phlebotomy the sample was kept in incubator at 37 degrees for around 15 minutes for separation of serum. The vacutainer was centrifuged for 10 minutes and serum separated. Serum creatinine was analysed on VITROS 4600 clinical chemistry analyser or Vitros 350 clinical chemistry analyser using the Vitros Dry slide technology. The principle used is enzymatic measurement. Serum Cystatin C was measured on the Vitros 4600 clinical chemistry analyser using the CYSTATIN C kit, which is turbidimetric immunoassay based on the principle of agglutination reaction. The test specimen is mixed with Cystatin C latex reagent and activation buffer and allowed to react. This is Prospective observational study. For the study ethical committee approval is taken. All appropriate consents taken and data collected accordingly.

5.1. Reference ranges

Serum creatinine = 0.5 - 1.2 mgs/dl in females and 0.5 -1.5 mgs/dl in males. Serum Cystatin = 0.6 – 1.0 mgs /l. Renal dysfunction was defined as creatinine clearance below 70 ml/min/1.73 m square.

6. Results

Total 57 patient studied. Serum Creatinine with GFR and Cystatin C with GFR was estimated in 20 healthy controls 12 females and 8 males who were non diabetic and non-hypertensive. These patients had normal creatinine with normal GFR as exhibited. Cystatin C and GFR was also normal as indicated in the Figure 1. Considering the data of 20 observations, we found that p-value for (paired t test) pair-wise difference is 0.0032 which is less than <0.05. Hence, Cystatin GFR is lower than Creatinine GFR for those normal patients which prompts early renal evaluation.

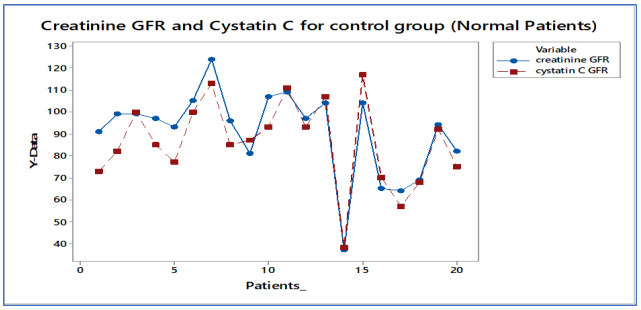

Fig. 1: Comparison of cystatin GFR with creatine GFR in control group, non-diabetic patients without kidney dysfunction

Elderly diabetic patients 24 males, 13 females who had kidney dysfunction but the severity was not exhibited by the creatinine levels or the calculated GFR since creatinine levels were only marginally increased and the GFR was not visibly decreased. However, cystatin C levels were considerably increased with reduced GFR as demonstrated in Table 2. Considering the data of 37 observations, we found that p-value for (paired t test) pair-wise difference

Table 1: Characteristic feature of study with age, gender, control group, diabetic patients with kidney dysfunction and follow up patients with cystatin and creatinine GFR

Characteristic	Control Patients (n-20)	Diabetic with kidney dysfunction (n-37)	Follow up patients (n-9)	Post treatment patients (n-9)
Age	56.3±12.29	68.70±9.17	73.22±19.31	73.22±19.31
Males	8	24	5	5
Females	12	13	4	4
Cystatin C level	0.9±0.2	1.99±0.7	1.89±0.57	1.64±0.63
Cystatin C GFR	86.15±19.7	35.54±15.27	34.22±12.34	43.77±18.65
Creatinine Level	0.82±0.21	1.48±0.65	1.48±0.82	1.18±0.42
Creatinine GFR	90.85±19.77	52.91±23.13	53.55±31.60	60.88±31.03
HbA1C level	5.84±0.64	6.3±0.97	6.3±0.97	6.3±0.97

is less than <0.001 1.3537E-11 which is much less than <0.05. Hence, Cystatin GFR is lower than Creatinine GFR for Diabetic patients (People with kidney dysfunction). (Table 2, Figure 2) This suggests accurate prediction of renal dysfunction by cystatin GFR in diabetic patients.

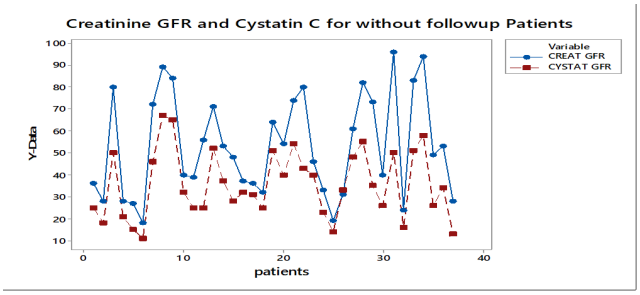

Fig. 2: Comparison of cystatin GFR with creatinine GFR in diabetic patients with kidney dysfunction

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There is a dependence of Sex, Age and Serum Creatinine Level on Cystatin GFR for without follow up patients diabetic patients with kidney dysfunction, Hence, a linear regression analysis was considered and the output is that Adjusted R-Squared is high (0.79908) which indicated a strong relation and also p-value for ANOVA is 3.20435E-12 much lesser than 0.05 which indicates the validity of the linear regression model. (Table 3) The regression equation of this linear regression model can be stated as

$$Cystatine\ GFR = 94.98 + 11.67 * (Sex = M) - 0.53 * Age - 20.58$$

This means that

1. With one unit increase in age Cystatin GFR can decrease by 0.53 units
2. With person being male, his cystatin GFR is expected to be more by 11.67 units than when the person being female

Comparison between normal patients and patients without follow-up Diabetic patients with kidney dysfunction' where hypothesis is usually the GFR levels for normal patients are greater than that of abnormal patients. We can check this hypothesis for both creatinine GFR as well as cystatin GFR Creatinine GFR. Creatinine GFR: We performed two independent samples t-test and got the following result, that p-value = 3.76E-08 which is much less than <0.05. Hence, we can conclude that creatinine GFR is greater for normal patients than for the patients having renal dysfunction.

Cystatin GFR: We performed two independent samples t-test and got the following result. Hypothesis is cystatin GFR in normal patients is greater than that of abnormal diabetic patients. As we see here in Table 4, that p-value = 6.83E-15 which is much less than < 0.05. Hence, we can conclude that cystatin GFR is greater for normal patients than for the patients having renal dysfunction. Hence Cystatin GFR appears better marker than creatinine GFR for accurate prediction of renal dysfunction in Diabetic patients. (Figure 3).

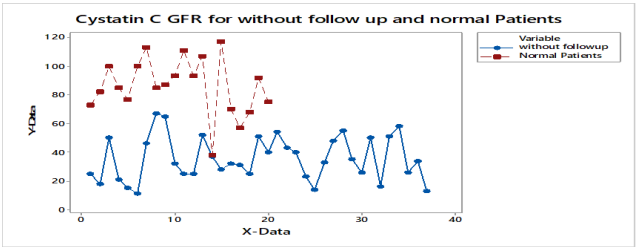

Fig. 3: Cystatin GFR in control and diabetic patients with kidney dysfunction

9 patients were studied for follow up as to what was the effect on creatinine, cystatin C and their calculated GFR pre and post treatment (Tables 1 and 4). Creatinine and Cystatin C were measured and their GFR was calculated at hospitalisation and simultaneously at discharge. The observations were listed as below:

1. All the patients were elderly and above 70 years of age group.
2. Creatinine levels on admission were marginally raised due to the ailment. However, post treatment the

Table 2: Cystatin GFR is lower than creatinine GFR in diabetic patients with kidney dysfunction

	CREAT GFR	CYSTAT GFR
Mean	52.91891892	35.54054054
Variance	535.0765766	233.4219219
Observations	37	37
Pearson Correlation	0.910464615	
Hypothesized Mean Difference	0	
df	36	
t Stat	9.456223198	
P(T<=t) one-tail	1.35E-11	
t Critical one-tail	1.688297714	
P(T<=t) two-tail	2.70757E-11	
t Critical two-tail	2.028094001	

t-Test: Paired Two Sample for Means

Table 3: Age, sex dependence of cystatin C levels

Summary Output

Regression Statistics

Multiple R	0.90323
R Square	0.81583
Adjusted R Square	0.79908
Standard Error	6.84826
Observations	37

ANOVA

	df	SS	MS	F	Significance F
Regression	3	6855.534716	2285.178239	48.725916	3.20435E-12
Residual	33	1547.654474	46.89862041		
Total	36	8403.189189			

	Coefficients	Standard Error	t Stat	P-value
Intercept	94.9876	9.015048418	10.53655601	4.30368E-12
Sex	11.6648	2.450916426	4.75937559	3.74195E-05
AGE	-0.5303	0.125159944	-4.236809361	0.000170981

Table 4: t test for comparison of cystatin and creatine GFR in control and diabetic patients with kidney dysfunction

	Normal	Diabetic patients with kidney dysfunction
Mean	83.5	35.54054
Variance	357.6316	233.4219
Observations	20	37
Pooled Variance	276.3307	
Hypothesized Mean Difference	0	
df	55	
t Stat	10.39533	
P(T<=t) one-tail	6.83E-15	
t Critical one-tail	1.673034	
P(T<=t) two-tail	1.37E-14	
t Critical two-tail	2.004045	

t-Test: Two-Sample Assuming Equal Variances

creatinine levels were normalised with remarkable rise in GFR. (Figure 2).

3. However, post treatment Cystatin C levels decreased but showed marginal decrease and the GFR too marginally increased which signifies that post treatment the kidney function is restored but very slowly and that the treatment has to be continued for a considerable length of time till the GFR is improved. As we can see in the Figure 3 and as per statistics that p-value = 0.47657 > 0.05, hence we can conclude the statement of change (pre and post treatment) in creatinine GFR and change (pre and post treatment) in Cystatin GFR are at 5% level of significance.
4. This clearly exhibits that creatinine GFR is overestimated and that Cystatin GFR predicts the severity of renal dysfunction better compared to creatinine GFR as Cystatin C is less affected by variables.

7. Discussion

Our results and observations are in agreement with Paul Muntner et al.[5] whose findings state that elevated Cystatin C can identify patients with preclinical kidney disease not detected by traditional serum creatinine.

The findings of Herget Rosenthal S et al[6] state that plasma or serum creatinine is the most commonly used diagnostic marker for estimation of GFR in clinical routine. However, they too state that creatinine is affected by preanalytical and analytical interferences. GFR estimating equations like MDRD and Cockcroft and Gault have poor bias and precision. Hence, they too suggest LMW protein Cystatin C to estimate GFR superior to serum creatinine. According to them Cystatin C seems to be sensitive to detect mild GFR reduction between 60-90 ml/min/1.73 m sq. And that cystatin C based equations for GFR calculation were superior to creatinine-based equation. They likewise advice clinicians that they should be aware of limitations and not to make management decisions taking one single analyte like creatinine into consideration.

The findings of the present author are contradicted by Oddoze C et al[7] who stated that Cystatin C was not more sensitive than creatinine for early renal impairment in patient with diabetes. However, Lorenz Risch and Andreas Huber however challenged Oddoze C et al[8] and were in favour of the present author stating that Cystatin C was of higher diagnostic accuracy than serum creatinine in detecting impaired GFR below 60 ml/min/1.73 m. sq.

As per Xia LH et al[9] it is very essential to detect renal dysfunction in diabetic patients. Their research work was based on the experiments to replace Cystatin C as a screening marker in lieu of creatinine. Their findings state that cystatin C reflects reduced GFR more efficiently in diabetic patients compared to creatinine GFR. The findings of present author are in agreement with view of Xia and his associates.

The findings of John Lieske[10] have graded Cystatin C as a very useful tool for evaluating CKD (Chronic Kidney Disease) patients. He has stated that people having cystatin C GFR lower than that of the creatinine GFR appear to have worse prognosis and hence that the nephrologists might try to treat such patients with better available management techniques to reduce their risk factors to a great extent. Thus, J Lieskis findings are in agreement with those of the present author.

Murty et al[11] defined creatinine blind area as a range between 40 and 70 ml/min/1.73 M^2 where a decrease in GFR starts to occur. They mentioned that Cystatin C gives true positive reduction in GFR whereas creatinine gives a false negative result in acute kidney injury and that cystatin C gives real time functional state of the kidney. According to them values of cystatin C in AKI gave ideas of early management for good response. In critically ill patients due to hypoperfusion and shock there is decline in GFR which is not expressed by serum creatinine. This delays therapeutic decision such as decisions to change the dosage of the nephrotoxic drug or to change the drug itself to improve the renal perfusion. Though steady Cystatin C expresses the decrease in GFR and hence by serum creatinine make therapeutic interventions possible. Their ideas do correlate with those of the present author in the existing study.

The study of the present author is in agreement with the findings of Jeon YL et al[12] in whose opinion too cystatin C is elevated with a decrease in GFR in diabetic patients with kidney disease ranging from stages 1-3.

The data of present author matches with Shilpak M G et al,[13] who state that the rise of cystatin C in the elderly is with associated comorbidities. They say that the kidney dysfunction is a risk factor of mortality in the elderly. However, they have shown like the present author that creatinine is an insensitive parameter for detection of reduction in kidney function which may deteriorate > 50% before serum creatinine exceeds the reference range. As per them the kidney function is underestimated by serum creatinine concentration in the elderly. In the present study too all the candidates were 50 years and above. Thus, cystatin is a better parameter to estimate GFR than serum creatinine.

The present author has used the formula to measure e GFR as per National Kidney foundation guidelines and has considered estimated GFR (using creatinine and cystatin C values) much better than measured GFR. This observation of the present author is in alignment with John G Toffaletti[14] who has summarised in his article that e GFR measurement based on serum creatinine and cystatin C along with estimation of proteinuria or albuminuria should be considered as a gold standard for estimating and monitoring kidney dysfunction[14].

8. Conclusion

1. We get a better picture of GFR calculated from cystatin C values predicts early renal dysfunction.
2. Those patients with abnormal creatinine in comparison with high cystatin C exhibit two findings viz.
   (a) Creatinine GFR is overestimated and it is greatly affected by age, weight, gender, antibiotics treatment etc.
   (b) Cystatin C is affected by age and gender.
3. Patients with normal creatinine and normal GFR but having elevated cystatin C levels and decreased GFR suggests renal dysfunction and prompts you for renal evaluation or a nephrologist's opinion. Thus, Cystatin C is a diagnostic and a sensitive marker for early renal injury which is missed out on calculating a creatinine GFR.
4. Cystatin GFR better marker than creatinine GFR for accurate prediction of renal dysfunction in Diabetic patients.

9. Conflict of Interest

The authors declare no conflict of interest.

10. Source of Funding

None.

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