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CYSTATIN C: AN IMPROVED ESTIMATOR OF MODERATELY IMPAIRED GLOMERULAR FILTRATION RATE

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ABSTRACT

Diabetic nephropathy[1-2] is a clinical syndrome characterised by persistent albuminuria (>300mg/24hr), a decrease in glomerular filtration rate (GFR), raised arterial blood pressure and enhanced cardio vascular morbidity and mortality. It is an important micro vascular complication of long standing non insulin-dependent diabetes mellitus (NIDDM) as well as insulin dependent diabetes mellitus (IDDM).Initially there is hyper filtration which declines to return to a normal at approximately 10years[3,4] . After 10 years there is sustained proteinuria and by 14years it reaches the nephritic range. Detection of diabetic nephropathy as early in the disease process as possible currently offers the best chance of delaying or possibly preventing progression to end stage disease[5].Screening for microalbuminuria and proteinuria in a structured, regular manner is recommended[3,4].The present study was conducted to detect the correlation between Glomerular filtration rate determined by creatinine clearance and serum Creatinine, serum Urea, Urea clearance, Cockcroft Gaolt formula, MDRD formula, Cystatin C and Microalbuminuria in diabetic patients with nephropathy and to study the correlation between glomerular filtration rates in diabetic patients at various levels of glomerular filtration rate and to compare ability of various parameters to detect mildly impaired (70-90ml/min) glomerular filtration rate.

KEY WORDS: **Diabetes Mellitus, Cystatin C, G.F.R.**

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INTRODUCTION

Monitoring trends in renal function via Glomerular filtration rate (GFR) is of critical importance in managing patients with potential of developing renal damage. Individuals with moderate or mildly decreased renal functions are at increased risk for chronic kidney disease[5, 6]. Adverse outcomes of renal failure can be prevented or delayed through early detection and treatment. Therefore the routine estimation of glomerular filtration rate (GFR) are strongly recommended for patients at high risk for kidney failure such as diabetic patients. The "gold standard" for determining GFR is to measure the clearance of exogenous substances such as inulin, iohexol, 51Cr-EDTA, 99mTc-labeled diethylenetriamine pentaacetic acid (DTPA), or 125I-labeled iothalamate. These techniques, however, are time-consuming, labour intensive, expensive, require administration of substances and not entirely free of risk for the patient that make them incompatible with routine monitoring. Blood urea nitrogen was the first endogenous substance measured in serum or plasma to assess renal function. Urea is freely filtered by the glomerulus and not secreted by the tubules. However, a large portion (40–70%) is passively reabsorbed from the renal tubules; thus its concentration will underestimate GFR in settings of decreased renal perfusion because some of the urea that is filtered will return to the bloodstream. Furthermore, its concentration in the blood can vary with diet, hepatic function, and numerous disease states. In the last 40 years, serum or plasma creatinine (SCr) has become the most commonly used serum marker of renal function. However, SCr blood concentrations are affected by age and gender. As plasma concentrations increase, tubular secretion of SCr increases, leading to an overestimation of GFR in patients with moderate to severe decreases in GFR (≤50 ml/min). SCr is also insensitive for detecting small decreases in GFR because of the nonlinear relationship between plasma concentration and GFR. Finally, the most common method (picric acid) for analyzing SCr is subject to analytic interferences from substances such as glucose, uric acid, ketones, plasma proteins and cephalosporins.Calculation of creatinine clearance (CrCl) by determining its concentration in timed urine collections and simultaneously in blood correlates with gold standard exogenous[1], however, collection of timed urine is cumbersome and prone to error in the outpatient setting. To circumvent these limitations of serum creatinine and to discover a less cumbersome method than creatinine clearance several formulas have been developed to estimate creatinine clearance from serum creatinine concentration, age, sex, and body size[6] for example Cockcroft Gaolt formula and Modified Diet in Renal Disease formula (MDRD). The formula is also inaccurate in patients with liver disease, muscle wasting, oedema or extreme adiposity. Thus, despite their common use, all the above renal markers have limitations as renal markers, and the search for an ideal endogenous marker of GFR continues. Low-molecular weight proteins have been suggested to likely replace all the above traditional markers.

CysC[5] is a 122-amino acid, 13-kDa protein that is a member of the family of cysteine proteinase inhibitors. It is the product of a "housekeeping" gene expressed in all nucleated cells and is produced at a constant rate[1]. It is not secreted, but is reabsorbed by tubular epithelial cells and subsequently catabolised so that it does not return to the blood flow. This latter property negates calculation of a CysC clearance using urine concentrations of CysC. The use of serum CysC to estimate GFR is based on the same logic as the use of blood urea nitrogen and creatinine, but because it does not return to the bloodstream and is not secreted by renal tubules, it has been suggested to be closer to the "ideal" endogenous marker. In this study we compare the utility of the new endogenous substance, Cystatin C (CysC), as a marker of GFR to the traditional markers along with other supportive parameter like microalbumin

in diabetic patients and the correlation between Glomerular filtration rate determined by creatinine clearance and serum Creatinine, serum Urea, Urea clearance, Cockcroft Gaolt formula, MDRD formula, Cystatin C and Microalbuminuria in diabetic patients with nephropathy. This study also determines the correlation between Glomerular filtration rate determined by creatinine clearance and serum Creatinine, serum Urea, Urea clearance, Cockcroft Gaolt formula, MDRD formula, Cystatin C and Microalbuminuria in diabetic patients at various levels of Glomerular filtration rate and compares ability of various parameters to detect mildly impaired (70-90ml/min) Glomerular filtration rate.

MATERIALS AND METHODS

SUBJECTS

The subjects for the study were chosen from the patients attending Goa Medical College & Hospital Bambolim – Goa, Medicine OPD, during the year 2008 to 2010. The total numbers of subjects involved in the study were 210.The study group comprised of 180 were diabetics in different stages of diabetic nephropathy and the control group comprised of healthy controls, who were non-diabetic-normoalbuminuric, non hypertensive, with no past or family history of renal failure mainly consisted of hospital doctors and staff. All the participants were informed and explained about the study being undertaken; in detail and their informed consent was taken. Patients were full explained bout the procedure of collection of 24 hour urine and the volume was measured in the laboratory. A fasting sample of blood was collected along. The Institution's ethical committee approved the study. Since the study was a type of cross-sectional study; there was no follow up. If patients were on ACE-inhibitor or Angiotensin receptor blocker, then it was omitted for one week, and changed to other anti-hypertensive .This was done because, ACE-Inhibitors and ARBs decreases proteinuria, which will interfere with determination of stage of diabetic nephropathy. Exclusion criteria were with clinical features suggestive of thyroid dysfunction, subjects with clinical features suggestive of Cushing's syndrome and those on medications which are likely to interfere with the Cystatin C levels e.g. Corticosteroids, probenecid, trimethoprim, cyclosporine and nephrotoxic drugs e.g. cyclosporine, NSAIDS. Also subjects with those conditions which can give rise to transient proteinuria e.g. Fever, congestive cardiac failure, urinary tract infection, heavy exercise, fever, or with extremes of weight or body mass index, hypoalbuminaemia, age more than 60 yrs which can decrease the reliability of Cockcroft – Gault formula were also excluded in this study. Subjects with strong suspicion of malignancy based on clinical and radiological findings, on and with clinical evidence of dehydration which may produce short term changes in GFR were also excluded. Patients with minimum output of >800ml were selected

SAMPLE COLLECTION

24-hour urine collection

On day 1, patient was asked to urinate into the toilet when he/she gets up in the morning. Time was noted. The urine was collected in a special container/ wide mouth bottle for the next 24 hours. On day 2, patient was asked to urinate into the container when he/she gets up in the morning preferably at the same time. Container was capped and kept in the refrigerator or a cool place during the collection period. Container was labelled with name, date and time of completion. Patient was asked to get the full volume on day 2 which was measured in the laboratory using measuring cylinder to note the exact volume. A sample of urine was centrifuged to carry out estimations of below mentioned analytes.

Blood collection

Patient is called fasting on day 2. Fasting blood samples were collected in fluoride bulb for fasting blood sugar levels, in EDTA bulb to estimate HbA1c and in plain bulb to estimate other parameters

Following parameters were estimated in the above subjects.

	BLOOD		24-HOUR URINE		CALCULATED PARAMETERS
1	Cystatin C	6	Urinary Creatinine[8]	11	Creatinine Clearance[1,8]
2	Serum Urea[7]	7	Urinary Urea[7]	12	Urea Clearance[1]
3	Serum Creatinine[8]	8	Urine dipstick protein[9]	13	Cockcroft-Gaolt formula [5,10]
4	Fasting blood sugar levels	9	Urine Protein [9]	14	MDRD formula[3,4,5,11]
5	Glycosylated haemoglobin (HbA1c) [12.]	10	Microalbuminuria [13]		

METHODOLOGY

CYSTATIN C was estimated by turbidimetric immunoassay for the quantitative determination in human serum which is based on the principle of agglutination reaction. The test specimen was mixed with Cystatin C latex reagent (R2) and activation buffer (R1) and allowed to react. Presence of Cystatin C in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at wavelength of 546 nm. The extent of turbidity corresponds to the concentration of Cystatin C in the specimen. Serum and urine urea estimations were carried out on Abbott Architect ci 8000 fully automated analyser by the urease method[7].

Serum and urine creatinine were estimated by the Kinetic alkaline picrate on Abbott Architect ci 8000[1, 8].

MICROALBUMINURIA[13]

Microalbuminuria was detected by indirect latex slide kit, based on principle of agglutination inhibition. It is not detected by routine dipstick method. Three early morning urine samples were tested for microalbuminuria in the 6 months period to confirm microalbuminuria[1, 6].

MACROALBUMINURIA

Macroalbuminuria was detected by routine urine reagent dipstick method where 0.3% w/w tetrabromophenol blue is used; 99.7% w/w buffer and non reactive ingredients. Macroalbuminuria was graded as negative, trace, [+] as >30mg/dl, [++] as >100mg/dl, [+++] as >300mg/dl and [++++] as >2000mg/dl.

CALCULATED PARAMETERS

1] CREATININE CLEARANCE

$$C_{Cr} = \frac{U_{Cr} \times V}{P_{Cr}}$$

Where Ccr creatinine clearance in ml/min
Ucr urinary creatinine in mg/dl
Pcr serum creatinine in mg/dl
V can be denoted as vol/min = $\frac{\text{24-hour volume}}{24 \times 60mins}$

$$C_{Cr-corrected} = \frac{C_{Cr} \times 1.73}{BSA}$$

Where BSA body surface area, which was calculated using Du Bois Formula

REFERENCE RANGES

Males 100-130 ml/min
Females 90-120 ml/min

COCKROFT-GAULT FORMULA[1, 10]

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times Constant}{\text{Serum Creatinine (in } \mu\text{mol/L)}}$$

MDRD formula

$$\text{eGFR} = 186 \times \text{Serum Creatinine}^{-1.154} \times \text{Age}^{-0.203} \times [1.210 \; if \; Black] \times [0.742 \; if \; Female]$$

After determining the GFR of the patient by creatinine clearance the patients were classified into
seven groups based on their GFR, GROUP I were GFR <60ml/min/1.73sqm; GROUP II were GFR 60-70ml/min/1.73sqm; GROUP III were GFR 70-80ml/min/1.73sqm; GROUP IV were GFR 80-90ml/min/1.73sqm; GROUP V were GFR 90-100ml/min/1.73sqm; GROUP VI were GFR >100ml/min/1.73sqm [Diabetic Controls] and GROUP VII were Non Diabetic Healthy Controls. Statistical analysis were performed using SPSS software 14.0 version [USA].Data with normal distribution was expressed as mean -+ std. differences in the continuous variables were investigated using one way ANOVA test. Post hoc test using Bofererroni's correction for multiple comparision was used to determine significance between the four groups. The limit of significance was <0.05.Pearson's correlation test was applied to determine correlation between various parameters and GFR.

RESULTS

The results of the study conducted in Goa Medical College during the year 2009 – 2010; in Diabetics and Non diabetics (control group) are as follows. There were 110 (52.38%) males and 100 (47.62%) females, who participated in the study.

Table 1

Distribution of Fasting blood sugar levels and HbA_{1c} levels in various groups.

PARAMETERS		N	MEAN	STD. DEVIATION	MINIMUM	MAXIMUM
FBSL (mg/dl)	I	30	178.07	24.526	136	231
	II	30	142.10	20.451	110	188
	III	30	110.07	12.558	91	137
	IV	30	97.50	13.338	76	120
	V	30	92.79	13.211	73	115
	VI	30	92.77	14.017	74	123
	VII	30	86.15	10.536	71	106
Total	210	114.59	35.030	2.417	71	231
HbA1c (%)	I	30	14.32	35.447	59	200
	II	30	13.19	62.814	170	400
	III	30	12.09	57.581	59	400
	IV	30	10.95	0.3754	3.6	4.7
	V	30	7.97	0.2854	3.6	4.5
	VI	30	7.57	0.2520	3.5	4.3
	VII	30	5.2	0.2743	3.5	4.4
Total	210	10.440	3.0462	0.2102	4	16

Table 2
Macroalbuminuric and microalbuminuric patients in various groups.

GROUPS	MICROALBUMINURIC	MACROALBUMINURIC
I	30	30
II	30	30
III	30	29
IV	30	8
V	27	1
VI	10	0
VII	0	0

Table 3
Mean values of blood urea, serum creatinine and Cystatin C in various Groups.

		N	MEAN	MINIMUM	MAXIMUM
Blood Urea (mg/dl)	I	30	52.21	29	74
	II	30	35.31	26	47
	III	30	33.79	26	42
	IV	30	28.10	20	38
	V	30	26.96	18	38
	VI	30	25.79	18	34
	VII	30	24.85	18	34
		210	32.24	18	74
		N	MEAN	MINIMUM	MAXIMUM
Serum Creatinine (mg/dl)	I	30	1.69	136	231
	II	30	1.12	110	188
	III	30	0.98	91	137
	IV	30	0.90	76	120
	V	30	0.82	73	115
	VI	30	0.72	74	123
	VII	30	0.77	71	106
		210	1.00		
Serum Cystatin C (mg/dl)	I	30	1.8010	1.49	2.72
	II	30	1.3680	1.29	1.53
	III	30	1.2247	1.13	1.43
	IV	30	1.0870	1.03	1.23
	V	30	0.9577	0.88	1.03
	VI	30	0.7063	0.52	0.89
	VII	30	0.7490	0.51	0.90
		210	1.1277	0,51	2.72

Table 4
Tests of significance: ANNOVA TEST OF SIGNIFICANCE

		Sum of Squares	df	Mean Square	F	Sig.
SERUM CREATININE	Between Groups	20.174	6	3.362	88.820	0.000
	Within Groups	7.685	203	0.038		
	Total	27.858	209			
BLOOD UREA	Between Groups	16,553.001	6	2,758.833	74.297	0.000
	Within Groups	7,537.904	203	37.133		
	Total	24,090.904	209			
CYSTATIN C	Between Groups	26.160	6	4.360	281.604	0.000
	Within Groups	3.143	203	0.015		
	Total	29.303	209			
FBSL	Between Groups	2,03,422.198	6	33,903.700	128.138	0.000
	Within Groups	52,388.364	198	264.588		
	Total	2,55,810.562	204			
HbA1c	Between Groups	1,787.531	6	297.922	452.115	0.000
	Within Groups	129.813	197	0.659		
	Total	1,917.344	203			

The mean difference is significant at the .05 level.

Table 5
Significance between groups – BONFERRONS TEST

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.
Serum Creatinine (mg/dl)	**1**	2	.571(*)	0.050	0.000
		3	.716(*)	0.050	0.000
		4	.789(*)	0.050	0.000
		5	.879(*)	0.050	0.000
		6	.971(*)	0.050	0.000
		7	.929(*)	0.050	0.000
	2	1	-.571(*)	0.050	0.000
		3	0.146	0.050	0.087
		4	.219(*)	0.050	0.000
		5	.308(*)	0.050	0.000
		6	.400(*)	0.050	0.000
		7	.358(*)	0.050	0.000
		1	-.716(*)	0.050	0.000

		2	-0.146	0.050	0.087
		4	0.073	0.050	1.000
	3	5	.163(*)	0.050	0.030
		6	.255(*)	0.050	0.000
		7	.212(*)	0.050	0.001
		1	-.789(*)	0.050	0.000
		2	-.219(*)	0.050	0.000
		3	-0.073	0.050	1.000
		5	0.090	0.050	1.000
	4	6	.182(*)	0.050	0.008
		7	0.139	0.050	0.127
		1	-.879(*)	0.050	0.000
		2	-.308(*)	0.050	0.000
		3	-.163(*)	0.050	0.030
		4	-0.090	0.050	1.000
	5	6	0.092	0.050	1.000
		7	0.050	0.050	1.000
		1	-.971(*)	0.050	0.000
		2	-.400(*)	0.050	0.000
		3	-.255(*)	0.050	0.000
		4	-.182(*)	0.050	0.008
	6	5	-0.092	0.050	1.000
		7	-0.042	0.050	1.000
		1	-.929(*)	0.050	0.000
		2	-.358(*)	0.050	0.000
		3	-.212(*)	0.050	0.001
		4	-0.139	0.050	0.127
	7	5	-0.050	0.050	1.000
		6	0.042	0.050	1.000
		2	16.903(*)	1.573	0.000
		3	18.423(*)	1.573	0.000
		4	24.112(*)	1.573	0.000
		5	25.254(*)	1.573	0.000
	1	6	26.422(*)	1.573	0.000
		7	27.367(*)	1.573	0.000
		1	-16.903(*)	1.573	0.000
		3	1.520	1.573	1.000
		4	7.208(*)	1.573	0.000
	2	5	8.351(*)	1.573	0.000
		6	9.519(*)	1.573	0.000
Blood Urea (mg/dl)		7	10.463(*)	1.573	0.000
		1	-18.423(*)	1.573	0.000
		2	-1.520	1.573	1.000
		4	5.689(*)	1.573	0.008
		5	6.831(*)	1.573	0.000
	3	6	7.999(*)	1.573	0.000
		7	8.944(*)	1.573	0.000
		1	-24.112(*)	1.573	0.000
		2	-7.208(*)	1.573	0.000
		3	-5.689(*)	1.573	0.008

	4	5	1.142	1.573	1.000
		6	2.310	1.573	1.000
		7	3.255	1.573	0.836
	5	1	-25.254(*)	1.573	0.000
		2	-8.351(*)	1.573	0.000
		3	-6.831(*)	1.573	0.000
		4	-1.142	1.573	1.000
		6	1.168	1.573	1.000
		7	2.113	1.573	1.000
	6	1	-26.422(*)	1.573	0.000
		2	-9.519(*)	1.573	0.000
		3	-7.999(*)	1.573	0.000
		4	-2.310	1.573	1.000
		5	-1.168	1.573	1.000
		7	0.945	1.573	1.000
	7	1	-27.367(*)	1.573	0.000
		2	-10.463(*)	1.573	0.000
		3	-8.944(*)	1.573	0.000
		4	-3.255	1.573	0.836
		5	-2.113	1.573	1.000
		6	-0.945	1.573	1.000
Cystatin C mg/L	**1**	2	.43300(*)	0.03213	0.000
		3	.57633(*)	0.03213	0.000
		4	.71400(*)	0.03213	0.000
		5	.84333(*)	0.03213	0.000
		6	1.09467(*)	0.03213	0.000
		7	1.05200(*)	0.03213	0.000
	2	1	-.43300(*)	0.03213	0.000
		3	.14333(*)	0.03213	0.000
		4	.28100(*)	0.03213	0.000
		5	.41033(*)	0.03213	0.000
		6	.66167(*)	0.03213	0.000
		7	.61900(*)	0.03213	0.000
	3	1	-.57633(*)	0.03213	0.000
		2	-.14333(*)	0.03213	0.000
		4	.13767(*)	0.03213	0.001
		5	.26700(*)	0.03213	0.000
		6	.51833(*)	0.03213	0.000
		7	.47567(*)	0.03213	0.000
	4	1	-.71400(*)	0.03213	0.000
		2	-.28100(*)	0.03213	0.000
		3	-.13767(*)	0.03213	0.001
		5	.12933(*)	0.03213	0.002
		6	.38067(*)	0.03213	0.000
		7	.33800(*)	0.03213	0.000
	5	1	-.84333(*)	0.03213	0.000
		2	-.41033(*)	0.03213	0.000
		3	-.26700(*)	0.03213	0.000
		4	-.12933(*)	0.03213	0.002
		6	.25133(*)	0.03213	0.000

	7	.20867(*)	0.03213	0.000
	1	-1.09467(*)	0.03213	0.000
	2	-.66167(*)	0.03213	0.000
	3	-.51833(*)	0.03213	0.000
	4	-.38067(*)	0.03213	0.000
6	5	-.25133(*)	0.03213	0.000
	7	-0.04267	0.03213	1.000
	1	-1.05200(*)	0.03213	0.000
	2	-.61900(*)	0.03213	0.000
	3	-.47567(*)	0.03213	0.000
	4	-.33800(*)	0.03213	0.000
7	5	-.20867(*)	0.03213	0.000
	6	0.04267	0.03213	1.000

**The mean difference is significant at the .05 level.*

Table 6
Correlation

		CREATININE CLEARANCE
CREATININE CLEARANCE	Pearson Correlation	1
	Sig. (1- tailed)	
	N	210
C&G formula	Pearson Correlation	.938(**)
	Sig. (1- tailed)	0.000
	N	210
Cystatin C	Pearson Correlation	-.982(**)
	Sig. (1- tailed)	0.000
	N	210
MDRD	Pearson Correlation	.903(**)
	Sig. (1- tailed)	0.000
	N	210
SERUM CREATININE	Pearson Correlation	-.849(**)
	Sig. (1- tailed)	0.000
	N	210
BLOOD UREA	Pearson Correlation	-.808(**)
	Sig. (1- tailed)	0.000
	N	210
FBSL	Pearson Correlation	-.839(**)
	Sig. 9 (1- tailed)	0.000
	N	205
HbA_{1c}	Pearson Correlation	-.870
	Sig. (1 – tailed)	0.000
	N	204

*** Correlation is significant at the 0.01 level (1- tailed).*

Table 7
Correlation among groups.

		I	II	III	IV	V	VI	VII
Creatinine Clearance	Pearson Correlation	1	1	1	1	1	1	1
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000
	N	30	30	30	30	30	30	30
Cockcroft Gault Formula	Pearson Correlation	-.851 (**)	.737 (**)	.121	.075	.203	.282	.900 (**)
	Sig. (2-tailed)	.000	.000	.525	.694	.282	.131	.000
	N	30	30	30	30	30	30	30
MDRD Formula	Pearson Correlation	.772 (**)	.224	.025	.061	.264	.502 (**)	.669 (**)
	Sig. (2-tailed)	.000	.234	.894	.749	.158	.005	.000
	N	30	30	30	30	30	30	30
Serum Creatinine	Pearson Correlation	-.816 (**)	.737 (**)	.121	.075	.203	.282	.900 (**)
	Sig. (2-tailed)	.000	.000	.525	.694	.282	.131	.000
	N	30	30	30	30	30	30	30
Blood Urea	Pearson Correlation	-.772 (**)	.224	.025	.061	.264	.502 (**)	.669 (**)
	Sig. (2-tailed)	.000	.011	.010	.931	.273	.296	.605
	N	30	30	30	30	30	30	30
Urea Clearance	Pearson Correlation	.730 (**)	-.119	-.113	.114	.154	.207	.688 (**)
	Sig. (2-tailed)	.000	.530	.551	.549	.415	.272	.000
	N	30	30	30	30	30	30	30
Cystatin C	Pearson Correlation	-.914 (**)	.260	-.298	-.701 (**)	-.921 (**)	-.877 (**)	-.951 (**)
	Sig. (2-tailed)	.000	.165	.110	.000	.000	.000	.000
	N	30	30	30	30	30	30	30
FBSL	Pearson Correlation	-.581 (**)	-.794 (**)	-.010	.109	-.013	.207	-.100
	Sig. (2-tailed)	.001	.000	.957	.567	.951	.281	.599
	N	30	30	30	30	30	30	30
HbA_{1c}	Pearson Correlation	-.570 (**)	-.507 (**)	-.089	-.530 (**)	-.394 (**)	.138	.088
	Sig. (2-tailed)	.001	.004	.639	.003	.047	.484	.645
	N	30	1	30	30	26	28	30

**Correlation is significant at the 0.01 level (2- tailed)*

DISCUSSION

Among the study group individuals, group I had highest levels of mean FBSL and HbA_{1c} values compared to all the other groups. FBSL and HbA_{1c} showed statistical significant difference between groups by ANOVA test (F value = 128.14, sig <0.0001) and (F value = 452.115, sig <0.0001) respectively. However when mean values of FBSL were compared within the groups by using Bonferroni's test there was no statistical difference between group III and IV (0.066), group IV and V, VI, VII (1.00, 1.00, 0.16) , group V and VI, VII (1.00, 0.643), group VI and VII (1.00). Group I has achieved this stage due to uncontrolled diabetes and not the reverse. The serum urea was found to be highest in group I (52.21) whereas it appeared within the normal range between all the other groups. Statistical significant difference between groups by ANOVA test (F value = 74.297, sig <0.0001).However mean values of urea were compared within the groups by using Bonferroni's test there was no statistical difference between group II and III (1.000), group IV and V, VI, VII (1.00, 1.00, 0.86) , group V and VI, VII (1.00, 1.00), group VI and VII (1.00). This showed that traditionally used serum urea significantly increased only when the GFR decreased to < 60ml/min. The mean serum creatinine showed statistically significant difference between the groups by ANOVA test (F value=88.2, sig=<0.0001). However, when mean serum creatinine was compared within the groups by using Bonferroni's test there was no statistically significant difference between Group II & III (p=0.087), III & IV (p=1.00) and IV& V VII (p=1, 0.127), group V & VI, VII (1.00, 1.00) and VI & VII (1.00) .But there was statistically significant between other Group pairs, as shown in the table. Of the 90 diabetics and healthy controls with GFR>90ml/min/1.73m^2 (group V, VI & VII) creatinine was found to be in normal range. In 90 diabetics with GFR between 60-90ml/ min, 82 (91%) had serum creatinine values in the normal range. More of which concentrated in group II (GFR 60-70) and 8 (9%) had serum creatinine levels above the reference range. Whereas in group I (GFR <60ml/min) 28 patients (93%) had serum creatinine levels above the reference range. The serum creatinine did not show any significant change until the GFR was around <60ml/min. This creatinine blind range is due to increased tubular secretion of creatinine with falling GFR[5, 6, 14].

There was statistically significant difference between the means of serum Cystatin C of the 7 study groups, analyzed by ANOVA test (F Value =281.60, sig <0.0001). By applying the Bonferroni's test, there was statistically significant difference between all the groups except VI & VII (p=1.000). Cystatin C showed a rising trend with increasing stage of nephropathy. There was no blind range as for creatinine. The mean Cystatin C concentrations showed statistically significant increase as GFR decreased. Although, the GFR is normal or increased in the early stages of diabetic-nephropathy, the serum Cystatin C values in Group V (GFR 90-100ml/min/1.73m^2) were significantly higher than in Group VI and might be those, who have almost reached the stage II of diabetic-nephropathy, which coincides with fall in GFR below 90ml/min. In qualitative assessment of micro and macroalbuminuria in all the diabetic subjects, 81% were found to be positive microalbuminuric. And 51% were found macroalbuminuria positive. Microalbuminuria seemed to occur much before GFR seemed to decrease in diabetic subjects. It was almost 70% in Group V with GFR 90-100 ml/min/1.73m^2. In the later stages, gross proteinuria is seen with further deterioration of renal function. When GFR 70ml/min/1.73m^2 around 80% showed dip stick protein test positive in trace amounts. Incidence of microalbuminuria increases with age as well as with increased duration of diabetes mellitus. Creatinine clearance was within normal range in microalbuminuric patients similar to this study. The presence of microalbuminuria alerts the physician to prevent further renal damage by timely administration of ACE inhibitors and correction of risk factors. When GFR calculated from measured creatinine clearance was correlated with all the other parameters, serum Cystatin C (r-0.986)

showed the best correlation followed by Cockcroft Gaolt formula(r-0.938), MDRD (r -0.903), urea clearance (r 0.898), serum creatinine (r -0.849) and serum urea (r -0.808). From the statistical analysis, one can conclude that serum Cystatin C correlates with GFR better. This strong association of serum Cystatin C with GFR has been shown in multiple studies done in diabetic populations. Stefano et al in his study in diabetic patients with renal impairment noted that Cystatin C correlated better with GFR (r -0.857) than with creatinine (r - 0.772). Many studies had similar findings to this present study which show that Cystatin C is a better parameter as far as diagnostic accuracy is concerned.[15-21,] .However, studies by Grubb et al[22] and Randers et al[23], reported that Cystatin C and creatinine correlated similarly with GFR. This may be attributable at least in part to intra assay variations for creatinine and Cystatin C measurements related to differences in assay techniques. Discrepancies may also rise from different and often arbitrarily chosen cut-points for the definition of abnormalities in renal function. Urea clearance was a poor indicator of GFR compared to Cockcroft Gaolt and Cystatin C. Results were more scattered and showed correlation similar to MDRD. Since blood urea shows lot of variations with protein metabolism, muscle mass and protein intake as in study by Lee E Farr.[24] MDRD was not found to be superior over Cockcroft Gaolt formula. The correlation was found to be r = 0.9 much less compared to Cockcroft Gaolt r = 0.938. This was similar to a study by Sanusi Abukar Abefe et.al, which studied 34 healthy subjects and 32 with established chronic kidney disease and compared Cockcroft Gaolt formula, MDRD formula and 3 other creatinine clearance estimating formulas with measured creatinine clearance[25].

CONCLUSION

From the present study the serum creatinine and urea did not show a significant change until the glomerular filtration rate had dropped to around < 60 ml per min. Thus blood urea and creatinine alone are not very sensitive markers to estimate renal function. Though urea clearance showed significant correlation with GFR, it is obsolete, subjected to lot of variations and cumbersome to be used in clinical practice. In formula derived GFR Cockcroft Gaolt formula was found to be superior over MDRD formula in this study. The serum Cystatin C showed a step wise increase with falling glomerular filtration rate, so there was no blind range. The glomerular filtration rate showed a stronger correlation with serum Cystatin C in health and disease. Cystatin C is more sensitive to detect mild to moderate renal dysfunction. Cystatin C also correlated better with different stages of diabetic nephropathy. The poor control of blood sugar was more evident in Group I which showed that poorly controlled diabetic are more at risk of developing severe renal dysfunction. Creatinine clearance was within normal range when microalbuminuria began to appear in patients. This suggested that if urinary excretion of albumin is monitored routinely in such patients with diabetes mellitus further renal damage can be prevented.

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