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Comparison of microalbuminuria with biochemical and hematological parameters as a marker for renal involvement in patients at high risk for Chronic Kidney Disease – A pilot study.

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Abstract— Estimation of urine microalbumin is considered as a novel marker for the assessment of early decline in glomerular function compared to the existing biomarkers such us creatinine especially in patients at high risk of developing CKD. Forty One patients (20 diabetic, 12 hypertensive and 9 both diabetic and hypertensive) at high risk for CKD and 19 healthy age and sex matched controls were included in the present study. Conventional renal biomarkers (Urine Protein, Protein Creatinine ratio, Serum Creatinine, Uric acid and Blood Urea), urine microalbumin and a complete blood count were estimated in all the subjects. Creatinine based eGFR using MDRD formulae and CG formula and cystatin C based eGFR were calculated. Microalbuminuria was found to be significantly correlating with serum creatinine, urine protein and urine protein creatinine ratio. Evaluation of the four eGFR equations showed a significant correlation. Among the hematological parameters ESR and the Platelet Count were found to be correlating with microalbuminuria. The study showed that the microalbuminuria is a better marker for the detection of early onset of CKD in high risk population like type 2 diabetes and hypertension.

Index Terms—Chronic Kidney Disease (CKD), Creatinine, Cockcroft-Gault (CG), Cystatin C, Estimated Glomerular Filtration Rate (eGFR), Glomerular Filtration Rate(GFR), Microalbuminuria, Microalbumin, Microalbumin creatinine ratio, Modification of Diet in Renal Disease (MDRD), Cockcroft-Gault (CG).

1 Introduction

Urinary excretion of albumin and lower molecular weight proteins is enhanced by factors that increase glomerular filtration load, either by saturating tubular reabsorptive capacity or as a result of increased tubular volume and flow rate[1],[2]. Under normal physiological conditions approximately 99% of the filtered albumin is reabsorbed in the proximal tubule and only trace of albumin is excreted through urine[3]. In pathological conditions when glomerular capillary wall permeability and/or filtration rate increase, albumin and other macromolecules excretion in urine also increases[4]. Detection of increased urinary albumin excretion is of particular importance in the study of incipient renal disease5. Proteinuria itself causes renal injury by causing mesangial and tubule-interstitial damage[6],[7]. Studies have shown proteinuria itself as a major determinant of progressive renal failure[8],[9].

The term "microalbuminuria" (MAU) describes a urinary albumin excretion that is increased but not to the level of overt proteinuria. MAU is considered to be a risk factor for not only nephropathy and progressive renal insufficiency in type 2 diabetes and hypertension but all cause mortality of the general population[10],[11],[12]. Primary prevention of nephropathy in diabetes and hypertension is feasible if the factors that initiate the change from normal urinary albumin excretion to MAU and from MAU to Nephropathy can be identified and treated[13],[14]. The determinants of microalbuminuria include Blood pressure, Duration of diabetes[15], age[16], genetic factors[17], glycemic status and other micro and macro vascular diseases. Hematological parameters were reported to be useful in predicting renal involvement in hypertension[18].

The established classification of abnormal urine albumin excretion according to the American Diabetic Association (ADA)[10] is given in Table 1.

Table 1. American Diabetic Association10 classification of abnormal UAE

Category	Spot Urine collection (µg/mg creatinine)	24-h Urine collection (mg/24 h)	Timed Urine collection (µg/min)
Normal	< 30	< 30	< 20
Microalbuminuria	30–299	30–299	20–199
Clinical albuminuria	≥ 300	≥ 300	≥ 200

UAE: Urinary Albumin Excretion

Conventional markers like creatinine and urea have been used for years for the diagnosis and management of nephropathy in diabetes and hypertension. Cystatin C and creatinine based eGFR were reported to be useful in predicting renal involvement in high risk population. In the present study an attempt is being made to evaluate the efficacy of urinary microalbumin estimation as a better index for renal involvement in the high

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risk population.

2 Materials And Methods

The study is conducted in 60 subjects (41 patients and 19 Controls) after getting informed consent. In the patient group 20 were diabetic 12 were hypertensive and remaining where both diabetic and hypertensive. All the patients were on treatment for a period of five years and were well controlled. Patients with possible causes of proteinuria other than by CKD were excluded. All the investigations were performed at Medivision Scan and Diagnostic Research Centre Pvt Ltd., Cochin. Study questionnaire and Proforma were filled for each patient including ID, name, age, sex, present complaints, past history, family history of illness. The BMI and blood pressures of all the patients were recorded and urine samples and 5ml of fasting venous blood were collected. The complete blood cell count was analysed using Sysmex KX-21 analyser. Blood urea, creatinine, uric acid, CRP and urine microalbumin were estimated in Beckman AU 480 analyser. 2 mL of blood sample was collected from all the subjects 2 hours after food for PPBS. Quality controls were monitored using Bio-Rad quality control material. The eGFR were calculated using CG formula and MDRD and Abbreviated MDRD formulae[19]

The results were presented as mean ± standard deviation. The correlations between microalbumin and other parameters were done using Pearson's correlation analysis. $P < 0.05$ was defined as the limit of statistical significance. All statistical analyses were performed using SPSS software version 17.0 and Minitab 16.0.

Table 2. Physical and anthropometric data of the study population.

Parameter	Category			
	Normal (n=19) Mean ± SD	Diabetes Mellitus (n=20) Mean ± SD	Hypertension (n=12) Mean ± SD	Both DM & HT (n=9) Mean ± SD
Systolic BP(mmHg)	108.89 ± 8.5	114.2 ± 5.16	148.33 ± 21.18	150.78 ± 15.43
Diastolic BP(mmHg)	73.00 ± 4.85	75.55 ± 3.68	93.25 ± 9.72	94 ± 6.54
Height (cm)	168.42 ± 7.96	164 ± 7.69	160.92 ± 7.72	160.67 ± 10.76
Weight (Kg)	69.63 ± 9.47	63.6 ± 7.56	65.5 ± 9.76	71.33 ± 8.76
BMI (Kg/m2)	24.46 ± 2.01	23.6 ± 1.68	25.12 ± 1.31	27.64 ± 2.34
FBG (mg/dL)	93.11 ± 8.31	182.7 ± 64.67	100 ± 6.99	153.33 ± 52.9
PPBS (mg/dL)	119.58 ± 9.38	271.6 ± 68.67	113.58 ± 13.24	158.67 ± 48.43
HbA1c(%)	5.55 ± 0.49	10.24 ± 1.42	5.73 ± 0.21	8.51 ± 2.23
MBG (mg/dL)	112.63 ± 14.11	247.3 ± 40.91	117.67 ± 6.26	197.44 ± 64.08

HT: Hypertension, BP: Blood Pressure, BMI: Body Mass Index, FBG: Fasting Blood Glucose, PPBS: Post Prandial Blood Glucose, MBG: Mean Blood Glucose calculated from HbA1c.

Table 3. Correlation between microalbuminuria and renal biomarkers

N= 60	Pearson Correlation coefficient (r)	Sig. (2-tailed)
Urine PC Ratio	0.966	<0.001
Urine Protein (mg/dL)	0.998	<0.001
Serum Creatinine (mg/dL)	0.547	<0.001
Blood Urea (mg/dL)	0.238	0.068
Uric Acid (mg/dL)	0.055	0.677

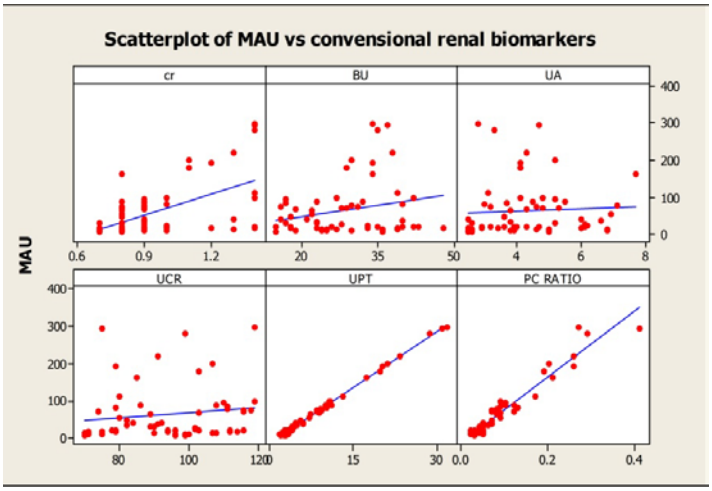
PC Ratio: Protein Creatinine Ratio,
There is a significant correlation between microalbuminuria with Urine protein creatinine ratio, Urine protein and serum creatinine.

Table 4. Correlation of microalbuminuria with eGFR calculated using creatinine and cystatin C.

N=60	Pearson Correlation coefficient (r)	P value
eGFR (CG Formula)	-.340**	0.008
eGFR (MDRD formula)	-.383**	0.003
eGFR (Abbreviated MDRD Formula)	-.385**	0.002
eGFR using CysC	-.577**	<0.001

Key: eGFR: estimated Glomerular Filtration Rate, CG: Cockcroft-Gault, MDRD: Modification of diet in renal medicine, CysC: Cystatin C. ** High Significance (<0.01).

Figure 1: Scatter plot of Microalbuminuria against Conventional renal markers.



Key: Cr: Creatinine (mg/dL), BU: Blood Urea(mg/dL), UA: Uric acid(mg/dL), UCR: Urine Creatinine (mg/dL), UPT: Urine Protein (mg/dL), PC Ratio : Urine Protein Creatinine ratio.

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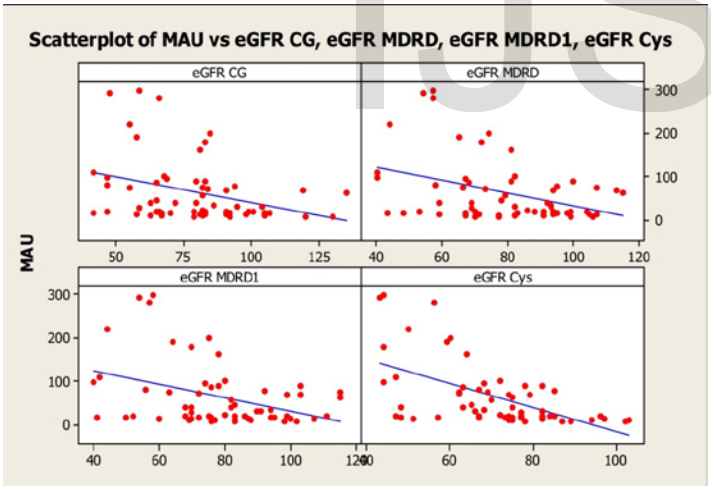
3 Results

A comparative evaluation of microalbuminuria and conventional renal markers like creatinine urea and uric acid was carried out. Microalbuminuria was significantly correlating with urine protein, PC Ratio and Creatinine. There was no significant correlation between microalbuminuria and urea and uric acid (Table 3 and Figure 1). Correlation of microalbuminuria with estimated Glomerular Function tests eGFR is shown in Table 4 and Figure 2. There was a significant correlation of microalbuminuria with all eGFR equations under consideration. Cystatin C based eGFR was found to be a better index(r=-0.577) among the eGFR equations.

The physical and anthropometric data of the study population are summarized in Table 2. The systolic and diastolic blood pressure, BMI, fasting and post prandial blood sugar glycated hemoglobin and mean blood glucose of the test subjects were studied and compared with the controls. All the above values were found to be elevated in the test subjects compared to the normal controls.

Correlation of microalbuminuria with RBC indices is shown in table 5 and Figure 3. There was no significant correlation between the microalbuminuria and the hematological parameters except for ESR and platelet count.

Figure 2 : Scatter plot of Microalbuminuria against estimated Glomerular Filtration rate based on creatinine and cystatin C.



Key: eGFR : Estimated Glomerular Filtration rate, CG: Cockcroft-Gault, MDRD : Modification of diet in renal disease, Cys: CystatinC

4 Discussion

Diabetes and hypertension are the most common diseases which adversely affect the renal function[8],[20]. Early detection of renal injury will help in the better management and prevention of progression of CKD to ESRD[21]. Many markers are being employed worldwide to detect renal involvement in these conditions[22].

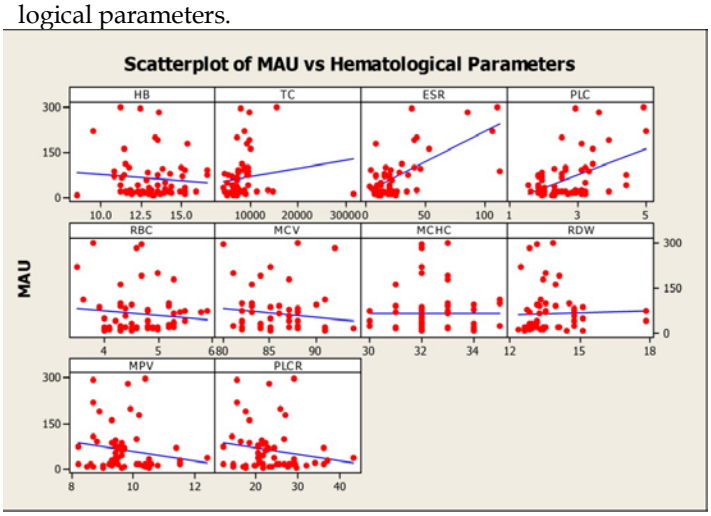
Creatinine and CystatinC based eGFR are extensively employed as markers of renal injury[23]. It was shown earlier that the MDRD and CG formulae were better creatinine based eGFR equations for the assessment of renal involvement in high risk population[24],[25]. The former lacks sensitivity and specificity and the latter is not cost effective. It was also reported that there is uncertainty in eGFR reported based on creatinine and cystatin C26 Hence in the study we have tried to evaluate the efficacy of urine microalbumin as predictive marker for renal involvement in the high risk population.

Table 5. Correlation of Microalbuminuria with hematological parameters

N=60	Pearson Correlation coefficient (r)	P value
Hb(g/dL)		
TLC(Cells/Cumm)	0.134	0.307
ESR (mm/hr)	0.676	<0.001
PLC (Lakhs/cumm)	0.458	<0.001
PCV(%)	-0.132	0.315
RBC (millions/cumm)	-0.115	0.380
MCV(fL)	-0.125	0.340
MCH (Pg)	-0.114	0.387
MCHC (%)	0.000	1.000
RDW (%)	0.024	0.854
PDW (fL)	-0.131	0.318
MPV (fL)	-0.184	0.159

Key: Hb: Hemoglobin, TLC: Total Leukocyte Count, ESR: Erythrocyte Sedimentation Rate, PLC: Platelet Count, PCV: Packed Cell Volume, RBC: red blood cells count, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin MCHC: Mean Corpuscular Hemoglobin concentration, RDW: Red cell distribution Width, PDW: platelet distribution width MPV: mean platelet volume

Figure 3 : Scatter plot of Microalbuminuria against hematological parameters.



Key: HB: Hemoglobin, TC: Total Leukocyte Count, ESR: Erythrocyte Sedimentation Rate, PLC: Platelet Count, RBC: red blood cells count, MCV: Mean Corpuscular Vol-

ume, MCHC: Mean Corpuscular Hemoglobin concentration, RDW: Red cell distribution Width, PDW: platelet distribution width, MPV: mean platelet volume

In the present study MAU is found be a better marker for predicting the progression of CKD which is well in agreement with the earlier studies by Powrie et al[13]. More over ESR which is reported to be a general health marker for all-time morbidity and mortality[27] is found to be correlating well with MAU. The non invasive nature and cost effectiveness makes urine microalbumin a better indicator for renal involvement in high risk population. This study clearly indicates the importance screening urine for microalbumin in all subjects with hypertension and type 2 diabetes Mellitus.

5 References

[1] Haraldsson, Börje, Jenny Nyström, and William M. Deen. "Properties of the glomerular barrier and mechanisms of proteinuria." Physiological reviews 88, no. 2 (2008): 451-487.

[2] Metcalfe, Wendy. "How does early chronic kidney disease progress? A background paper prepared for the UK Consensus Conference on early chronic kidney disease." Nephrology Dialysis Transplantation 22, no. suppl 9 (2007): ix26-ix30..

[3] Mogensen, CE. "Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes." New England journal of medicine 310, no. 6 (1984): 356-360.

[4] Mogensen, CE., and CK. Christensen. "Predicting diabetic nephropathy in insulin-dependent patients." New England Journal of Medicine 311, no. 2 (1984): 89-93.

[5] Mogensen, CE. "Microalbuminuria as a predictor of clinical diabetic nephropathy." Kidney int 31, no. 2 (1987): 673-689.

[6] D'amico, Giuseppe, and Claudio Bazzi. "Pathophysiology of proteinuria." Kidney international 63, no. 3 (2003): 809-825.

[7] Eddy, Allison A. "Proteinuria and interstitial injury." Nephrology Dialysis Transplantation 19, no. 2 (2004): 277-281.

[8] Keane, William F., Barry M. Brenner, Dick De Zeeuw, Jean-Pierre Grunfeld, Janet Mcgill, William E. Mitch, Artur B. Ribeiro. "The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL study." Kidney international 63, no. 4 (2003): 1499-1507.

[9] Bernard, Alfred, and Robert Lauwerys. "Epidemiological application of early markers of nephrotoxicity." Toxicology letters 46, no. 1 (1989): 293-306.

[10] American Diabetes Association: Diabetic nephropathy (Position Statement). Diabetes Care 2000; 23 (Suppl. 1):S69 –S72.

[11] Wang, Thomas J., Jane C. Evans, James B. Meigs, Nader Rifai, Caroline S. Fox, Ralph B. D'Agostino, Daniel Levy, and Ramachandran S. Vasan. "Low-grade albuminuria and the risks of hypertension and blood pressure progression." Circulation 111, no. 11 (2005): 1370-1376.

[12] Romundstad, Solfrid, Jostein Holmen, Kurt Kvenild, Hans Hallan, and Hanne Ellekjær. "Microalbuminuria and all-cause mortality in 2,089 apparently healthy individuals: a 4.4-year follow-up study. The Nord-Trøndelag Health Study (HUNT), Norway." American journal of kidney diseases 42, no. 3 (2003): 466-473.

[13] Powrie, J. K., G. F. Watts, J. N. Ingham, N. A. Taub, P. J. Talmud, and K. M. Shaw. "Role of glycaemic control in development of microalbuminuria in patients with insulin dependent diabetes." Bmj 309, no. 6969 (1994): 1608-1612.

[14] Zucchelli, Pietro, and Alessandro Zuccalà. "Hypertension and renal dysfunction." Current opinion in nephrology and hypertension 5, no. 1 (1996): 97-101.

[15] Basi, Seema, Pierre Fesler, Albert Mimran, and Julia B. Lewis. "Microalbuminuria in Type 2 Diabetes and Hypertension A marker, treatment target, or innocent bystander?." Diabetes Care 31, no. Supplement 2 (2008): S194-S201.

[16] Chowta, N. K., P. Pant, and M. N. Chowta. "Microalbuminuria in diabetes mellitus: Association with age, sex, weight, and creatinine clearance." Indian journal of nephrology 19, no. 2 (2009): 53.

[17] Pinheiro, Sérgio VB, Anderson J. Ferreira, Gregory T. Kitten, Kátia D. da Silveira, Deivid A. da Silva, Sérgio HS Santos, Elisandra Gava et al. "Genetic deletion of the angiotensin-(1–7) receptor Mas leads to glomerular hyperfiltration and microalbuminuria." Kidney international 75, no. 11 (2009): 1184-1193.

[18] Jithesh, Tharayil Kattil, Riju Mathew, Vidyadharan Jayapal, and Thankappan Vijayakumar. "Microalbuminuria and Red Cell Distribution Width as Predictive Markers of Renal Involvement in Hypertension." International Journal of Health and Rehabilitation Sciences (IJHRS) 1, no. 2 (2012): 99-106.

[19] Launay-Vacher, Vincent, Hassane Izzedine, and Gilbert Deray. "Using the right MDRD equation." Kidney international 66, no. 5 (2004): 2089-2089.

[20] Levey, Andrew S., Brad C. Astor, Lesley A. Stevens, and Josef Coresh. "Chronic kidney disease, diabetes, and hypertension: what's in a name&quest." Kidney international 78, no. 1 (2010): 19-22.

[21] Levin, Adeera, and Paul E. Stevens. "Early detection of CKD: the benefits, limitations and effects on prognosis." Nature Reviews Nephrology 7, no. 8 (2011): 446-457.

[22] Riju Mathew, Vinitha R Pai and Vijayakumar. T., "Comparative evaluation of conventional and new biomarkers of renal involvement in patients with type 2 diabetes mellitus and hypertension – a pilot study." International Journal of Pharma & Bio Sciences 4, no. 2 (2013). 742 – 750.

[23] Ferguson, Michael A., and Sushrut S. Waikar. "Established and emerging markers of kidney function." Clinical chemistry 58, no. 4 (2012): 680-689.

[24] Jithesh, Tk; Riju Mathew; Jayapal., V; Vijayakumar, T. A comparison of eGFR using serum creatinine and cystatin for the assessment of renal involvement in hypertension. International Journal of Pharma & Bio Sciences 4, no. 1 (2013) 1-9.

[25] Riju Mathew, Vinitha R. Pai, Ramesh K, Vijayakumar .T, Microalbuminuria and estimated glomerular filtration rate (eGFR) using six prediction equations Proceedings in International conference on Proteomics and Genomics. (2011) p221-224.*(Conference Proceedings)*

[26] Badrie T., Turner P., "The Uncertainty of eGFR" Indian Journal of Biochemistry 28 vol 3 (2013) 242-247.

[27] Stevens, D., R. Tallis, and S. Hollis. "Persistent grossly elevated erythrocyte sedimentation rate in elderly people: one year follow-up of morbidity and mortality." Gerontology 41, no. 4 (1995): 220-226.