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International Journal of Clinical Biochemistry and Research

Journal homepage: www.innovativepublication.com

Original Research Article

Estimated protein output a novel reliable index in early prediction of renal failure

K. Alamelu Mangai[1], Vijaya Srinivasan[1,*], Santhi Silambanan[1]

[1] *Dept. of Biochemistry, Sri Ramachandra Institute of Higher Education and Research. SRU (DU), Chennai, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 15-10-2019
Accepted 30-11-2019
Available online 13-03-2020

Keywords:
Sensitivity
Specificity
Renal failure
Urine protein
Creatinine ratio
Urine albumin
Creatinine ratio
Estimated protein output (EPO)

ABSTRACT

Introduction: Assessment of proteinuria and albuminuria are the major core elements in diagnosis and management of renal failure. Measurement of albumin: creatinine ratio has been chosen as the golden standard for the determination of kidney failure.
Objective: The objective of the present study was to determine whether urine protein – creatinine ratio and estimated protein output(EPO) can be use d as significant indices to identify kidney injury at an earlier stage. To achieve this goal t he sensitivity, specificity and predictive values of urinary protein-creatinine ratio were compared with urinary albumin: creatinine ratio and EPO.
Materials and Methods: Random urine samples were collected from 154 individuals aged between 25-65 years who attended the outpatient clinic at the hospital.
Results: The urinary protein - creatinine ratio had a sensitivity of 87% and specificity of 78% respectively.
Conclusion: The results substantiate that the urinary protein: creatinine ratio can be used as a reliable and non-expensive marker to screen individuals a t risk of chronic kidney failure and quantification by determining EPO is an accurate method to detect kidney failure at an earlier stage.

1. Introduct ion

The incidence and prevalence of kidney failure is increasing alarmingly worldwide. The global burden of renal failure has steeply elevated from 4,26,000 in 1990, to 1,490,000 in 2000 and is expected to be 2,500,000 subjects by 2020.[1,2] This poses an important healthcare problem. As this is a growing global health issue, a larger proportion of health care resources are spent in both developed and developing countries to overcome this clinical problem.

Based on the current population of India is 137.168 crores,[3] even approximate estimate of end stage renal disease (ESRD) burden in India would suggest that about 1,650,000 to 2,200,000 people develop ESRD every year.[4–6] The major reason is that there are not enough financial resources for health care in the developing countries like India for expensive and chronic treatment like renal replacement therapy. Management of this public health problem therefore requires methodological strategies to prevent the adverse outcomes of the disease.

However, prevention requires a clear understanding about the outcome of the disease and the appropriate screening test to detect the population at risk. By the time the kidney disease becomes symptomatic the individual would have lost 70% of the kidney function. However, if it is detected in time, the kidney function can be improved which will avoid dialysis or renal transplantation therapy for patients. The recent advancements in the techniques available to analyse urinary protein level makes it possible to determine protein concentration of less than 2mg/dl.

Under normal physiological circumstances the amount of albumin leaking into the urinary space is minimal. Increased urinary albumin excretion is the consequence of increased glomerular permeability due to renal failure. Urinary albumin to creatinine ratio(uACR) is considered as the gold standard for the quantification of proteinuria[6] but the major limitation is it is costlier compared to urine protein-creatinine ratio(uPCR). But till date there is no

* *Corresponding author*.
E-mail address: avijaya2009@gmail.com (V. Srinivasan).

https://doi.org/10.18231/j.ijcbr.2020.014

sufficient data to determine whether uPCR is relatively reliable method in comparison to uACR for assessing proteinuria. Further, if estimated protein output (EPO) is a better method to quantify proteinuria.

The study is therefore undertaken to find the reliability of uPCR by which a large population of the people could be screened and detected for renal failure in a non-invasive manner before reaching End Stage Renal Disease(ESRD). Based on this rationale the following objectives were envisage sensitivity, specificity and predictive values of uPCR compared to uACR at the same time the cost effectiveness of these tests 3)to determine the association between uACR, uPCR and EPO.

2. Materials and Methods

The cross-sectional study was Sri Ramachandra Medical Centre attached to Sri Ramachandra Medical College & Research Institute, Chennai for a period of 6 months The study Research Ethics Committee of Sri Ramachandra Medical College & research institute.

2.1. Sample population

The study population included individuals of age group within 25-60 years who were willing to participate were enrolled in the study. They were included based on the inclusion criteria and exclusion criteria.

Individuals suspected for renal disease and included in the study according to the following criteria - Males and female urinary protein was more than 150 mg/day.

Pregnant women, breast feeding women, patients with urinary tract infection, dialysis patients, patients on dialysis or renal replacement therapy were excluded from the study.

2.2. Sample size

Totally 154 individuals were enrolled in the study. The study population was categorized into two groups

Group- *I* – Individuals within age group of 25-65 years, whose urinary albumin: creatinine ratio (u ACR) was less than 30 mg/g.

Group- *I***I** – Patients within age group of 25-65 years, whose uACR was greater than 30 mg/g.

2.3. Sampling technique

As the sample used is the random urine sample from individuals aged between 25-65 years in the hospital, the sampling technique used was that of convenience sample. Within the period of 3 months random urine sample from both case and control groups aged 25-65 years of age who were under the out-patient service and were willing to participate in the study were included in the study. Serum sample of the patients was collected to estimate the serum urea and creatinine levels.

2.4. Data collection

The routine biochemical parameters were tested in the hospital's central clinical laboratory using standard protocols. The random urine sample from 154 individuals were collected in a clean dry plastic container without any preservatives and assayed within an hour. The blood samples were collected in plain vacutainers and assayed.

2.5. Biochemical assays

Within an hour urine protein concentration, urine albumin concentration and urine creatinine concentration were measured using the standard kits in ADVIA 1800 chemistry system.

2.6. eGFR calculation

The eGFR is determined by serum creatinine (SCr) and the preferred method for estimating GFR is the body surface area normalized, 4-variable. Modification of diet in renal disease study (MDRD)equation based on SCr, age, gender, ethnicity.

eGFR (ml/min/1.73m^2) = 186 x $(SCr)^{-1.154}$ x$(Age)^{-0.203}$ x (0.742, if female)

2.7. Statistical analysis

The data were analysed using standard statistical methods SPSS version 18. The results were expressed as mean $\pm$ S.D or as the median (range). The ACR and PCR data were evaluated following a log transformation of the values due to the non-normal distribution. The inter relationship between the PCR and the ACR was examined by Pearson's correlation coefficient.

3. Results

The demographic data and the biochemical findings for the 154 patients enrolled in the study are listed in Table 1 and 2.

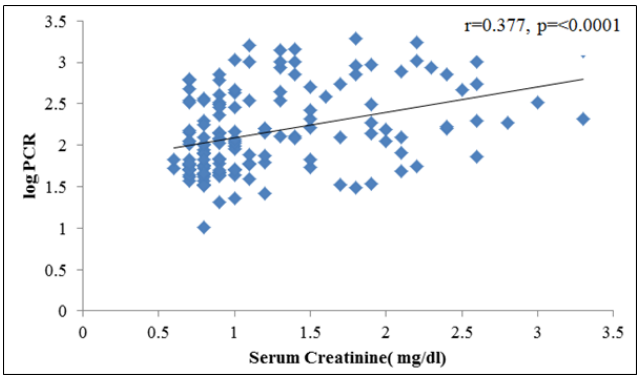

Fig. 1: Scatter plot of urine protein creatinineratio vs serum creatinine.

Table 1: Demographical data and biochemical parameters of study group.

S. No	Variables	Group-*I* (n=78)	Group-*II* (n = 76)
1	Age (Years) mean ± S. D	48.62 ± 14.28	47.31 ± 11.37
2	Serum u rea (mg/dl)	11.78 ± 5.47	13.83 ± 7.01
3	Serum c reatinine (mg/dl)	1.16 ± 0.57	1.33 ± 0.63
4	eGFR (ml/min/1.73m^2)	72.5 ± 27.68	63.17 ± 26.08
5	PCR (mg/ g) median, range	61.9 [45.8-123.28]	352.9 [125.5-722.3]
6	ACR (mg/g) median, range	10 [6.14-15.86]	100.8 [45.68-462.12]

Table 2: Results of analysis of clinical features using ACR and PCR as dependant variables.

S. No	Analytes compared	R-value	t-value	p-value
1	S. Cr Vs PCR	0.377151	4.92	<0.0001
2	S. Cr Vs ACR	0.269259	3.38	0.0009
3	EPO Vs PCR	0.716525	12.41	<0.0001
4	EPO Vs ACR	0.660383	10.63	<0.0001
5	eGFR Vs PCR	-0.35611	4.6	<0.0001
6	eGFR Vs ACR	-0.30157	3.82	0.0002
7	ACR Vs PCR	0.810593	16.73	<0.0001

Table 3: Comparison of sensitivity and specificity ofurine total protein and albumin in control and CKD patients.

	Albuminuria		
Proteinuria		Positive	Negative
	Positive	40.25%	5.84%
	Negative	11.68%	42.20%

Albuminuria - urine albumin: creatinineratio ≥ 30 mg/g
proteinuria - urine protein creatinineratio ≥ 150 mg/g.
Sensitivity 78 %, Specificity 88 %, Positivepredictive value 87 %, Negative predictive value 78 %.

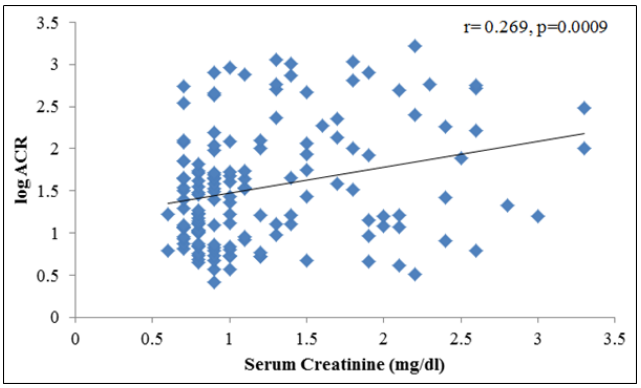

Fig. 2: Scatter plot of albumin creatinine ratiovs serum creatinine.

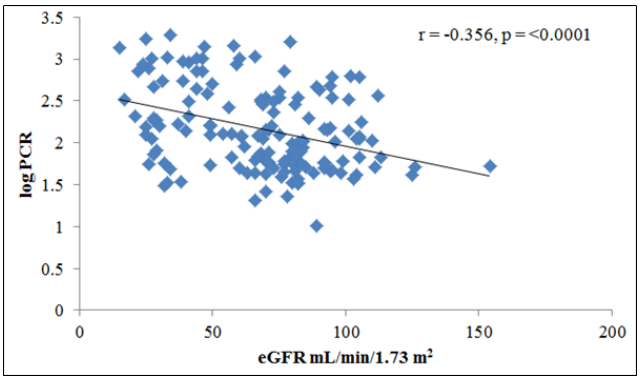

Fig. 3: Scatter plot of protein creatinine ratioVs eGFR.

Considering all inclusion and exclusion criteria our cross-sectional study included a total of randomly selected 85 females and 63 males. Mean age of all the 154 subjects was 48±12.8 years. In present study, based on albuminuria 49.32% of study population were categorized in proteinuria group and 51.04 % as non-proteinuria group.

4. Discussion]=

Quantitative analysis of urinary protein excretion is an independent risk factor for various clinical outcomes hence it is used to identify and determine the progression of renal failure, diabetes mellitus and cardiovascular diseases. Simple and inexpensive method to detect proteinuria is essential in the management of these clinical outcomes.

Estimation of serum creatinine (SCr) is usually considered as inadequate method for early intervention of kidney damage. Mild to moderate kidney failure is unrecognised by the serum creatinine values and by the time the creatinine levels increase considerably the kidneys would have lost more than 50% of its function.[7] So far there

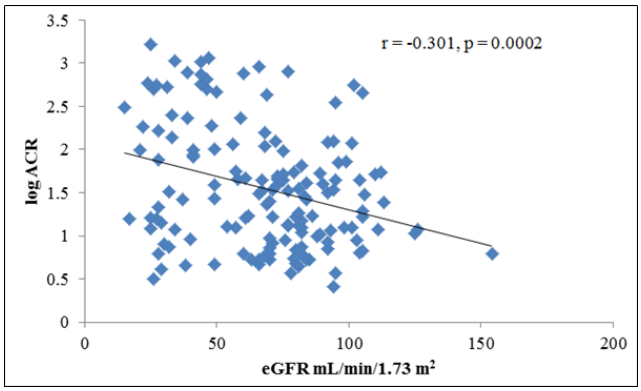

Fig. 4: Scatter plot of albumin creatinine ratio VseGFR.

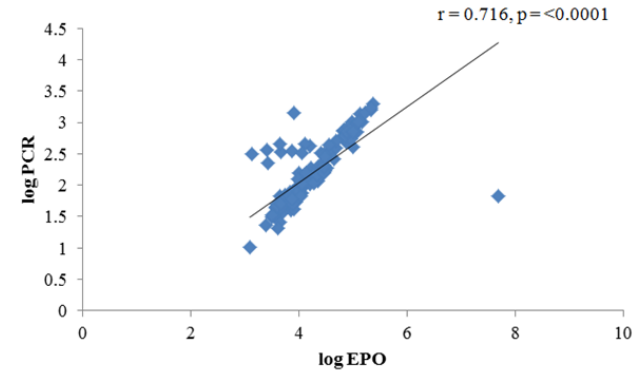

Fig. 5: Scatter plot of protein - creatinine ratio VsEPO.

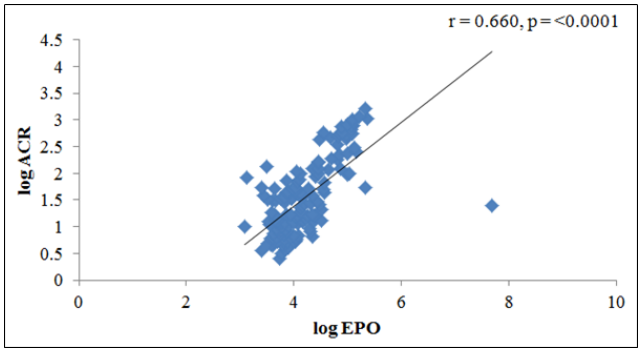

Fig. 6: Scatter plot of albumin -creatinineratio Vs EPO.

are not sufficient reports that show the correlation of serum creatinine values with other clinical parameters reflecting chronic kidney disease.[8] Our study shows a significant positive correlation between P CR and serum creatinine (r = 0.377, p = <0.0001) and a weak correlation with ACR and serum creatinine (r= 0.269, p=0.0009). Our results corroborate with (17) who have also observed similar results on comparison of PCR and SCr.

Our study shows a negative correlatio n between PCR and eGFR (r = - 0. 35, p = <0.0001) and also between

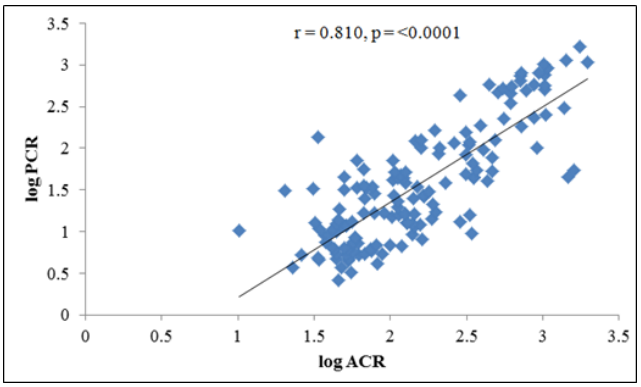

Fig. 7: Scatter plot of protein creatinine ratio Vsalbumin creatinine ratio.

ACR and eGFR (r = - 0.301, p = 0.0002). The result obtained is supported by the study performed by Stevens et al in 2006 to determine the use of proteinuria as a possible marker for GFR decline in earlier stages of CKD which rises early in the course of kidney disease and remains elevated throughout, whereas GFR remains normal unless severe derangement of kidney is observed.

Measurement of albuminuria and total proteinuria are central aspects of the management and prognosis of patients with CKD. However, there is uncertainty regarding the best measure of urinary protein excretion which has clinically important implications from a practical and cost-effectiveness perspective[9] Albumin is a low molecular weight protein, and albuminuria likely is a reflection of early damage to the glomerular vascular endothelium, as well as decreased ability of the tubule to reabsorb urinary albumin.

Urinary measurement of total proteinuria also includes higher molecular weight nonalbumin urinary proteins, which may be tubular as well as glomerular in origin. However, albumin still makes up the majority of total urinary protein in patients with CKD (particularly at higher ranges of proteinuria.[10] Thus, these two clinical measures would be comparable in the general CKD population.

The laboratory analysis of a 24-hour(hr) urine sample collection is considered as the gold standard to assess proteinuria as it detects both albumin and globulin. The major limitation is that the 24 hr urine collection is cumbersome and many patients fail to understand the actual procedure. Investigators[11–13] have done a comparative study between random urine protein : creatinine ratio and 24 hours protein excretion rate. From their study it has been concluded that random protein: creatinine ratio has very good correlation with 24 hour urine protein excretion.

The benefits of using random urine PCR as an alternate for 24 hr protein measurement have also been reported however only few of these have involved patients with kidney disease, or have examined the ability of PCR or ACR to rule out abnormal protein loss. Most studies have shown

good correlations between PCR and 24 h protein loss and have demonstrated adequate sensitivities and specificities when PCR was used to predict 24 h proteinuria.[14]

Since, ACR is suggested by various guidelines and considered to be the gold standard ACR is performed routinely. The main problem associated with quantification of urinary albumin concentration for the diagnosis of proteinuria are its high cost and its lack of availability to the general population. A semi-quantitative dipstick analysis of urinary protein concentration, which is not expensive, is routinely used for screening in the general health check-up system. The clinical significance of this method is not well defined and reliability of the method does not fulfill the criteria to accept it as a quantitative test.

Studies[15] have shown that ACR does not increase with PCR. From our data we observed a very strong correlation between ACR and PCR with r value of 0.81, $p< 0.0001$ which indicates that PCR values can be used for the assessment of renal failure. Methven S et al in 2010 reported that PCR (compared with ACR) can be employed as a screening test when proteinuria had protein excretion < 0.5 and <1.0 g/day.

In our study cut off value of 150 mg/g for measuring proteinuria and the cut off values above 30 mg/g for measuring albuminuria was used to determine individuals with proteinuria.[16] The analysis showed that PCR had a sensitivity and specificity of 78% and 88% respectively. 5.84% individuals showed false positive for proteinuria at the cut off value of 150 mg/g. The probability for an individual to have disease with his / her test result positive is 82.54%. The negative predictive value for PCR was observed to be 76.47%.

Therefore, we also determined the relationship between the ACR and the PCR and found that there was a strong positive correlation between these ratios. PCR with this range of sensitivity and specificity could be suggestive for using it for screening individuals at high risk of developing renal damages. A possibility of a better sensitivity would have been obtained with a larger population group.

Estimated Protein Output (EPO) may be an even better method of quantifying proteinuria as it takes lean body weight into consideration. Accuracy of 24 -hour urine collection can be gained by measuring urine creatinine. In 24-hour, urine sample the ratio of measured creatinine (MC) to estimated creatinine (ECE) lies between 0.75 and 1.25 where EC excretion is given by the following formula,

Lean weight = 22.5 * height (m^2)

ECE = (140- age) x lean weight (kg) x 0.2(x 0.85 if female)

EPO can be determined more accurately by calculating it using the formula as published by (Viknesh Selvaraj et al.[17] where ECE is the estimated creatinine excretion

EPO g/24hour = PCR x ECE

To our knowledge, this is the first study to investigate the association between PCR and ACR and EPO. In our study, PCR showed a strong correlation of ($r = 0.716$, $p = <0.0001$) whereas ACR had a moderate correlation of ($r = 0.66$, $p = < 0.0001$) with respect to EPO.

5. Conclusion

Proteinuria being an initial abnormality noted in early stage of kidney derangements, a inexpensive and reliable method is required to screen individuals at increased risk of chronic kidney disease. uACR and uPCR are important indices of kidney failure, however the study of the association of uACR and uPCR with a relatively new parameter Estimated protein output (EPO) is first reported by our team which may help the clinicians to get a clear understanding of the clinical outcome of renal failure without relying on albumin: creatinine ratio alone.

There are few limitations in our study. It is a self-financed study, assessing microalbumin in all individuals was expensive, so the sample size of the study was small. This study confirmed that there is a significant correlation between protein creatinine ratio and albumin creatinine ratio. Protein creatinine ratio can be used to screen individuals at initial stage of Chronic kidney disease. This study can be carried out in larger population to bring out an exact relationship between albumin creatinine ratio and protein creatinine ratio.

6. Source of funding

None.

7. Conflict of interest

None.

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Author biography

Vijaya Srinivasan Assistant Professor

Santhi Silambanan Professor

Cite this article: Mangai KA, Srinivasan V, Silambanan S. **Estimated protein output a novel reliable index in early prediction of renal failure**. *Int J Clin Biochem Res* 2020;7(1):65-70.