



Original Article:

Urinary Peptide Levels in Patients with Chronic Renal Failure

Mungli Prakash,

Department of Biochemistry and Genetics, School of Medicine, St Matthews University, Grand Cayman, British West Indies

Nagaraj M Phani,

Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal 576104, India,

Kavya R,

Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal 576104, India

Supriya M,

Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal 576104, India.

Address For Correspondence:

Dr. Mungli Prakash,

Associate Professor in Biochemistry and Genetics,

St Matthews University, School of Medicine,

Grand Cayman, British West Indies.

E-mail: prakashmungli@yahoo.co.in

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Abstract:

Introduction: Peptide levels in urine are found to be decreased in renal failure. In the current study urinary peptide levels were determined in chronic renal failure (CRF) patients. **Method:** 86 CRF patients and 80 healthy controls were selected for the study. Urinary proteins and peptide levels were determined by spectrophotometer based Lowry and Bradford methods. Urinary creatinine levels were determined by clinical chemistry analyzer. **Results:** There was significant decrease in urinary peptide levels in CRF patients and Urinary % peptides were significantly decreased in CRF patients as compared to healthy controls. Urinary % peptides correlated negatively with proteinuria. **Conclusion:** we have found decrease in urinary peptides and % urinary peptides in CRF patients and possibly measurement of % urinary peptides may possibly serve as better indicator in early detection of impairment in renal function.

Key Words: Peptiduria; Proteinuria; Chronic renal failure; Urinary peptides

Introduction:

Chronic renal failure (CRF) encompasses a spectrum of different pathophysiologic processes associated with abnormal kidney function, and a progressive decline in glomerular filtration.(1) The kidneys attempt to compensate for renal damage by hyperfiltration within the remaining functional nephrons and over a time period this hyperfiltration causes further loss of function. End stage renal disease represents a stage of CRF where the accumulation of toxins, fluid, and electrolytes normally excreted by the kidneys results in uremic syndrome.(1)

Proteinuria is common finding in CRF patients and current evidence indicates that the presence of proteinuria is an early marker of an increased risk of progressive kidney disease, poor cardiovascular outcome and death.(2-4) Proteins are too large to pass through the glomeruli into the urine, but the low molecular weight proteins (less than 1000 kD) are freely filtered by the glomerulus and this depends upon their size, configuration, electrical charge.(5) Until recently, it had been believed that the proteins reaching the renal tubules had been

completely reabsorbed.(6) But, recently it had been postulated that the filtered albumin is taken up by HK-2 cells via a receptor mediated process and it is degraded by the lysosomal enzymes and the resulting peptides were exocytosed to the basolateral sides of the cells.(7) The exact anatomic location of this pathway has not been determined, it likely takes place in cells distal to the glomerular basement membrane, most probably in tubular epithelial cells.(8-11) In the current study we have measured urinary peptides in CRF patients compared them with that of the healthy individuals to see the difference in excretion of these peptides.

Materials and Methods:

Subjects: Eighty six CRF patients admitted to the nephrology ward and 80 healthy controls were included in the study. The urine sample bottles were stored at 4°C during the period of collection. Samples were centrifuged at 3000 rpm for 10 min and analyzed immediately after the collection period. Informed consent was taken from the subjects involved in the study followed by ethical clearance from the institutional review board.

Reagents: Special chemicals such as Bradford reagent and bovine serum albumin (BSA) were obtained from Sigma Chemicals, St Louis, MO, USA. All other reagents were of analytical grade.

Protein stock: BSA was dissolved in phosphate buffered saline (PBS). Standard curves were prepared by dissolving BSA to get the following final concentrations; for Bradford assay: 2, 4, 6, 8, and 10 µg/mL; for Lowry assay: 50, 100, 150, 200, and 250 µg/mL.

For Lowry assay: We standardized the modified Lowry's assay for determining levels of total urinary proteins; the reagents were prepared as follows: reagent A: 2% sodium carbonate, reagent B1: 1% sodium potassium tartarate, reagent B2: 0.5% CuSO₄ in reagent B1, reagent C: 50 mL reagent A + 1 mL reagent B2, and reagent D: 1N Folin-Ciocalteu reagent.

Methods: Protein and peptide levels in urine were measured using a Genesys 10UV spectrophotometer whereas urine cre-

atinine levels were determined by a Clinical Chemistry Automated Analyzer (Hitachi 912). Both Lowry and Bradford assays were performed after diluting the urine samples suitably. Dilutions were made according to our dilution factors proposed by Prakash et al.(12)

Urinary proteins, together with urinary peptides, were measured using the Lowry assay,(13) whereas urinary proteins were determined using the Bradford assay.(14) Urinary peptide levels were determined by subtracting the Bradford's value from Lowry's value of the same urine sample (Lowry value – Bradford value). All calculations were done using separate calibration curves prepared for each method.

For Lowry estimation, 0.2 mL of the diluted urine sample was taken in two sets of eppendorf tubes (sets 1 and 2) while 0.2 mL of 145 mM NaCl was taken in another tube and labeled as reagent blank (RB). To RB and to set 1, 1 mL of reagent C was added while 1 mL of reagent A was added to set 2 tubes. The tubes were shaken vigorously and incubated for 10 min at room temperature. Reagent D was added to all the tubes at the end of 10min and the tubes were vortexed; this step is crucial for color development. The tubes were incubated at room temperature for 30 min and the absorbance was read at 600 nm. After correcting for respective blanks, absorbance values of set 2 samples were subtracted from their counterparts.

Statistical analysis: Statistical analysis was done using statistical package for social sciences (SPSS) version 16. Inde-

pendent sample t test was used to compare mean values and Pearson's correlation was used to correlate between the parameters. $p < 0.05$ is considered significant.

Results:

As depicted in Table 1, there was no significant difference in urine creatinine levels between healthy controls and CRF patients on conservative management. There was no significant difference in total urinary proteins (determined by Lowry assay) per liter of urine between two groups, however there was significant proteinuria (determined by Bradford's assay) in CRF patients on conservative management when compared to healthy controls ($p < 0.0001$). We have found significant difference in both to total urinary proteins (Lowry assay) gram of creatinine ($p < 0.0001$) and urinary proteins (Bradford's assay) per gram of creatinine ($p < 0.0001$) between both groups. There was significant difference in urinary peptides per liter of urine between CRF patients on conservative management compared to healthy controls ($p < 0.0001$). However, there was no significant difference in urinary peptide levels expressed per gram of creatinine between CRF patients on conservative management and healthy controls. There was a significant decrease in percent urinary peptides in CRF patients on conservative management compared to healthy controls ($p < 0.0001$). On applying Pearson's correlation, percent urinary peptides correlated negatively with the grams of proteins per gram of creatinine ($r^2 = 0.426$, $p < 0.01$) (Figure 1).

Table 1: Independent sample t test for all the determined biochemical parameters in both healthy controls and chronic renal failure cases (values expressed as mean ± standard error of mean).

	Healthy Controls (n = 80)	Chronic Renal Failure Cases (n = 86)
Urinary Creatinine (g/L)	0.81±0.05	0.52±0.08
Gm Proteins/L (Lowry's method)	2.95±0.22	2.86±0.26
Gm Proteins/L (Bradford's method)	0.06± 0.005	1.54±0.17*
Gm Proteins/gm Cr (Lowry's method)	3.85±0.20	6.87±0.55*
Gm Proteins/gm Cr (Bradford's method)	0.09±0.01	3.60±0.33*
Gm Urinary Peptides/L (Lowry – Bradford)	2.88±0.22	1.32±0.14*
Gm Urinary Peptides/gm Cr	4.13±0.25	3.25±0.22
(Lowry – Bradford) x 100 Lowry (% urinary peptides)	97.12±0.45	47.32±2.15*

*P <0.0001 compared to healthy controls.

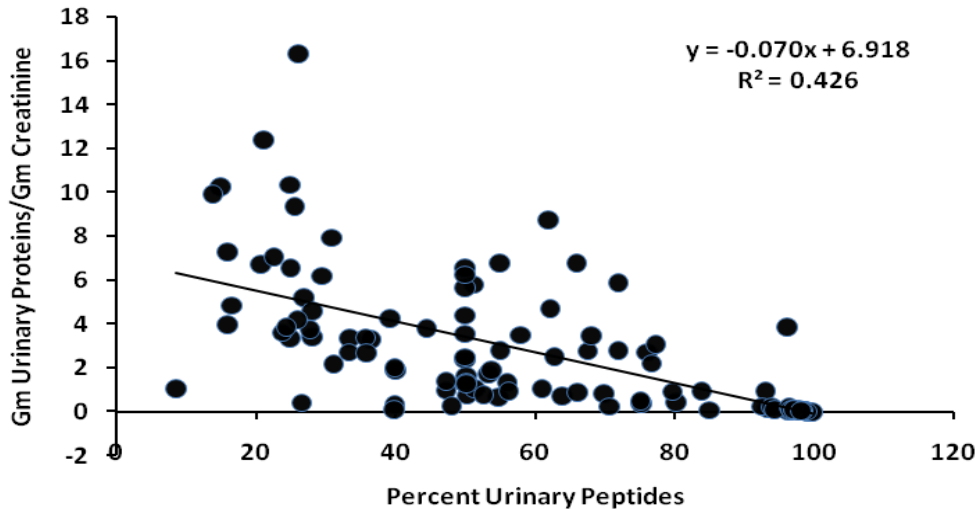


Figure 1. Correlation between percent urinary peptides and urinary proteins per gram of creatinine

Discussion:

We have found significant increase in proteinuria and decrease in peptide excretion in CRF patients indicating increased leakage of intact proteins through the glomerulus and decreased reabsorption from renal tubules. Normally renal tubules will reabsorb the proteins filtered by glomerulus and within the tubules proteins are degraded to peptides and amino acids which will enter into amino acid pool and some amount of peptides and amino acids will be secreted by tubules into urine.(7-11,15) Previous authors have also shown significant decrease in urinary peptides in renal failure cases in different settings. (12,16) We have found significant decrease in % urinary peptides in CRF patients which indirectly indicates decrease in filtration load on glomerulus as there is leakage of intact protein from it. Previous authors have also shown measurement of % urinary peptides is better marker for indirectly measuring filtered load on kidneys.(12,16) In the current study we have found significant decrease in filtered load on glomerulus with % urinary peptides at 47.32 as compared to 97.12 in healthy controls (table 1). This possibly indicates leakage of intact protein from glomerulus their by decreasing the filtration load on it.

We have found significant decrease in urinary peptides along with decrease in % urinary peptides. This can be probably due to decrease in tubular reabsorption and degradation of intact protein possibly due to tubular damage and leakage of lysosomal enzymes into urine. Previous study has supporting finding for this in which they have shown significant tubular damage and leakage of lysosomal enzymes into urine in renal failure cases.(16) The possible leakage and loss of lysosomal enzymes decreases the capacity of functioning tubules there by decreased reabsorption of intact proteins escaped in glomerular filtration there by findings their way into urine. The findings in our study shown similar findings where by CRF patients shown decrease in urinary peptides and increase in urinary proteins and also there was negative correlation between proteinuria and % urinary peptides (Figure 1) supporting the above findings by other authors.

In conclusion, we have found decrease in urinary peptides and % urinary peptides in CRF patients and possibly measurement of % urinary peptides may possibly serve as better indicator in early detection of impairment in renal function.

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