BRIEF COMMUNICATION

URINARY PROTEIN THIOLS IN DIFFERENT GRADES OF PROTEINURIA

Mungli Prakash, Jeevan K Shetty, Sambit Dash, Bijay K Barik, Abhirup Sarkar, Sharanabasappa M Awanti and Ravindra Prabhu*

Department of Biochemistry and *Nephrology, Kasturba Medical College, Manipal, India.

ABSTRACT

Total thiol status of plasma, especially thiol groups over protein contributes maximum to the plasma antioxidant status of the body. Serum protein thiols were found to be decreased in various disease conditions including chronic renal failure patients. Only few studies determined the levels of urinary protein thiols in disease conditions. The current study was designed to know the levels of urinary protein thiols in patients with different grades of proteinuria. The study was conducted on urine of 40 healthy controls and 61 cases with proteinuria. Based on proteinuria cases were further divided into two groups; group I - microproteinuria (150-300 mg protein/d), 32 cases, group II - frank proteinuria (>300 mg protein/d), 29 cases. Urinary thiol levels were determined by spectrophotometric method using dithionitrobenzoic acid. A significant decrease ($p < 0.01$) in urinary thiol in group I and group II cases was observed in present study and this decrease was associated with proteinuria.

KEY WORDS

Urinary thiols, Proteinuria, Chronic renal failure, Diabetic nephropathy.

INTRODUCTION

The total thiol status in the body, especially thiol (-SH) groups present on protein are considered as major plasma antioxidants *in vivo* and most of them are present over albumin (1) and they are the major reducing groups present in our body fluids (2). Contribution of glutathione to total thiol status is minor, hence majority of total thiol status is contributed from albumin bound thiol groups (3). The levels of protein SH in the body indicate antioxidant status, and low levels of protein SH correlated with increased levels of lipid hydroperoxides (4) and advanced oxidation protein products (AOPP) (1).

Albumin, a major plasma protein which maintains the colloidal osmotic pressure in blood vessels and acts as carrier for many

compounds in plasma, is an important extra cellular antioxidant. Albumin contains an exposed -SH group over cysteine-34 residue and provide the bulk of total plasma thiol pool (2). Loss of proteins in different disease conditions like chronic renal failure (CRF), long standing diabetes and diabetic nephropathy, patients with sepsis, may lead to decreased functional capacity of major plasma protein albumin. Several previous studies indicated increased production of reactive oxygen species in CRF (4,5,6), diabetes mellitus (7) and sepsis (8). Prakash et al found decreased protein thiols in serum of uremia patients and such decreased protein thiols were correlated positively with decrease in albumin levels (4).

In the current study, we have determined the levels of protein thiols in patients with different grades of proteinuria and compared them with that of normal healthy controls.

Address for Correspondence :

Dr. Mungli Prakash,
Department of Biochemistry,
Kasturba Medical College,
Manipal 576104, INDIA.
Tel: 91- 98448 96961
E-mail: prakashmungli@yahoo.co.in

MATERIALS AND METHODS

The study was carried out on 61 proteinuria cases and 40 healthy controls from October 2006 to July 2007. Mean age and sex of patients was 36 ± 8 years and 43males/18 females, and that of controls was 34 ± 8 years and 29 males/11 females,

respectively. Cases were further classified into two groups based on protein excreted in urine per day. Group I – microproteinuria with 150-300 mg protein per day (32 cases), group II – frank proteinuria with >300 mg protein per day (29 cases). None of the patient received any form of antioxidant medication. The healthy controls were not on any kind of prescribed medication or dietary restrictions. This study was approved by institutional review board and informed consent was obtained from all subjects involved in the study. Twenty four hour urine samples were collected from Department of Nephrology in bottles containing toluene as preservative and assayed for urine total protein, creatinine and protein bound thiols. Special chemical 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), was obtained from Sigma chemicals, St Louis, MO, USA. All other reagents were of analytical grade. Urine protein thiols were measured by a spectrophotometric method using DTNB (3,9). The levels of urine total proteins and urine creatinine were determined by using automated clinical chemistry analyzer (Hitachi 911).

The results were expressed as mean $\pm$ standard deviation (SD) and p value of <0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-10, Chicago, USA). One way analysis of variance (ANOVA) followed by multiple comparison by post-hoc test was used to compare mean values among the three groups.

RESULTS

As depicted in Table 1, there was significant increase in total protein in group I cases ($p<0.01$) and group II cases ($p<0.01$) compared to healthy controls. There was significant decrease in urinary protein thiols in group I cases ($p<0.01$) and in group II cases ($p<0.01$) compared to healthy controls. Among the two groups in cases, group II showed significantly reduced

levels of protein thiols as compared to group I (microproteinuria) cases.

DISCUSSION

We have found significant increase in urinary total protein in frank proteinuria cases and microproteinuria compared to healthy controls. Though proteins are excreted in urine, we have found decrease in protein bound thiols in urine, but logically there should be increase in protein bound thiols in urine according to the amount of proteinuria. This indicates there occurs oxidation of these protein bound thiols in the body before excretion in urine. Albumin is an important chain breaking extra-cellular antioxidant which contains an exposed cysteine-SH groups and provides bulk of "total serum thiols" (2). Previous studies have shown significantly decreased protein thiols in serum of uremia cases and correlated positively with serum albumin (4). These findings suggest that excreted albumin in urine is deficient in thiol groups. We speculate that the decreased thiols in urine of proteinuria patients could be because of increased oxidation of albumin bound thiol groups in serum due to already existing oxidative stress. Hence excretion of such albumin deficient in reduced form of thiol groups in urine decreased the levels of protein bound thiols in urine.

However, our data is just an indication of such possibilities, to prove this, one need to undertake well designed studies to understand the molecular details of these findings. In conclusion, our data suggests that there is significant decrease in urinary thiols in patients with proteinuria and it varies with the amount of protein excreted in urine.

REFERENCES

1. Himmelfarb JE, McMonagle E, McManamin. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. *Kidney Int* 2000; 58: 2571-8.
2. Himmelfarb JE, McMonagle E. Albumin is the major plasma protein target of oxidant stress in uremia. *Kidney Int* 2001; 60: 358-63.
3. Hu ML. Measurement of protein thiol groups and glutathione in plasma. In: Parker L (ed), *Methods of enzymology* 1994, vol 233, California: Academic press; pp 380-5.
4. Prakash M, Upadhyaya S, Prabhu R. Protein thiol oxidation and lipid peroxidation in patients with uremia. *Scand J Clin Lab Invest* 2004; 64:599-604.
5. Sezer MT, Akin H, Demir M, Erturk J, Aydin ZD, Savik E, Tunc N. The effect of serum albumin level on iron-induced oxidative stress in chronic renal failure patients. *J Nephrol* 2007; 20(2):196-203.

Table 1 Biochemical parameters of proteinuria cases and healthy controls (Mean $\pm$ SD)

	Healthy controls (n = 40)	Proteinuria group I cases (n = 32)	Proteinuria group II cases (n = 29)
Urine Creatinine (g/day)	0.82 $\pm$ 0.07	0.66 $\pm$ 0.1*	0.44 $\pm$ 0.12*
Urine protein (mg/24 hr urine)	84.8 $\pm$ 31.3	198.1 $\pm$ 32.4*	1542.3 $\pm$ 567.6*
Urinary protein thiols (μ moles/g of protein)	587.23 $\pm$ 142.76	236.54 $\pm$ 81.2*	132.82 $\pm$ 57.2*

*p value < 0.01 compared to healthy controls

6. Ienaga K, Nakamura K, Fujisawa T, Fukunaga Y, Nihei H, Narita M, et al. Urinary excretion of creatol, an in vivo biomarker of hydroxyl radical, in patients with chronic renal failure. *Ren Fail* 2007; 29(3):279-83.
7. Ramakrishna V, Jaikhan R. Evaluation of oxidative stress in Insulin Dependent Diabetes Mellitus (IDDM) patients. *Diagn Pathol* 2007; 2(1):22-30.
8. Huet OR, Obata C, Aubron A, Spraul-Davit J, Charpentier C, Laplace T, et al. Plasma-induced endothelial oxidative stress is related to the severity of septic shock. *Crit Care Med* 2007; 35(3):821-6.
9. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82: 70-7.