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RESEARCH ARTICLE

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PROTEIN THIOLS IN THE URINE OF PRE-ECLAMPSIA PATIENTS

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ABSTRACT

Hypertensive disorders of pregnancy are important causes of maternal and fetal mortality & morbidity. Pre-eclampsia is one of them. The presence of oxidative stress in pre-eclampsia is well proven fact. The thiol groups contribute most to the antioxidant status of the body. The plasma thiols were found to be decreased in patients with pre-eclampsia. The current study was designed to know the levels of such protein bound thiols in 8,12 hr urine samples and to correlate with 24 hr urine sample in pre-eclampsia patients. Serum and urine protein thiols were determined by Spectrophotometric method using dithionitrobenzoic acid (DTNB).There was significant increase in protein ($p<0.01$) and significant decrease in urine protein thiols ($p<0.01$) in 8, 12 and 24 h urine protein in pre-eclampsia patients. Among 8,12 and 24 hr urine sample in both the groups; 8 and 12 hr urine showed significant decrease in thiols compared to 24 hr urine. These results suggest that determination of total protein of 8 ,12 hour urine samples along with urine protein thiols can be a better alternative for 24 hr urine protein value in the early diagnosis of pre-eclampsia.

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KEY WORDS

Thiols, Pre-eclampsia, proteinuria, serum protein thiols, urine protein

INTRODUCTION

Preeclampsia is a pregnancy complication recognized by new onset gestational hypertension and proteinuria. It is a complex multisystem disorder seen exclusively in the human species and a leading cause of maternal and fetal morbidity and mortality worldwide.

Reduced perfusion as a result of abnormal placentation is thought to lead to ischemia reperfusion injury to the placenta. Placental oxidative stress, which results from the ischemia reperfusion injury, is increasingly reported to be involved in the etiopathogenesis of gestational hypertension/preeclampsia[1]. Diagnostic criteria for preeclampsia include new onset of elevated blood pressure and proteinuria after 20 weeks of gestation. Features such as edema and blood pressure elevation above the patient's base line no longer are diagnostic criteria[2]. Proteinuria is used as criteria in the classification system for hypertensive disorders of pregnancy including preeclampsia[3]. Urinary protein excretion was usually assessed by collecting urine continuously over 24 hour period[4]. Inspite of many efforts for establishment of more rapid method of measurement of urine protein in pregnant women, 24 hour urine protein measurement still remains a gold standard. However, this method is not only onerous for patients; it is also unreliable if urine sample is incomplete. In this regard several previous studies have suggested determination of 8 hour and 12 hour urine protein would be a better alternative in the early diagnosis of preeclampsia[5]. Several previous studies have indicated an increased production of ROS and occurrence of lipid peroxidation in preeclampsia[6,7].

The --SH (reduced thiols) groups that exit both intracellularly and extracellularly either in free form (reduced glutathione) or bound to proteins (protein bound thiols) play a major role in maintaining the antioxidant status of the body[8].The thiols are the major antioxidants in body fluids which are known to reduce highly reactive free radicals thus protecting the biomolecules[8]. Such thiols have been studied and determined in different disease conditions and found decreased in different diseases including preeclampsia[9]. The present study was carried out to know the levels of such protein bound thiols in 8 and 12 hour urine of samples and to correlate with 24 hour urine sample.

MATERIAL AND METHODS

The study was carried out on 60 patients of which 30 had mild and 30 had severe preeclampsia. This study was approved by institutional review board and informed consent was obtained from all subjects involved in the study. Under aseptic conditions blood samples (5 ml) were drawn into plain vacutainers from ante-cubital veins of pre-eclamptic patients. The collected blood was allowed to clot for 30 minutes, and then centrifuged at 2000 g for 15 minutes for clear separation of serum. Serum albumin and serum protein thiols were estimated immediately after serum was separated. The 8, 12 and 24 hour urine was collected in separate bottles (with toluene as preservative) for estimation of urine protein thiols and total protein.

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. Serum and urine protein thiols were measured by a spectrophotometric method using DTNB[10,11]. Urinary total proteins and levels in 8, 12 and 24 hour sample were estimated by spectrophotometric methods using semi-automated analyser (Roche 112).

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation (SD). A p value of <0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-17, Chicago, USA). Chi square, and independent t test was used to compare mean values.

Results: As depicted in table 1, there was significant increase in 8, 12 and 24 hour urine protein in both mild ($p<0.01$) and severe pre-eclampsia ($p<0.01$). There was significant decrease in urinary protein thiols in 8, 12 and 24 hour urine sample in both the groups. Among 8, 12 and 24 hour urine sample in both the groups; 8 and 12 hour urine showed significant decrease in thiols compared to 24 hour urine.

Table 1
Biochemical parameters of mild and severe pre-eclampsia cases.

Variables	Mild Pre-eclampsia	Severe Pre-eclampsia
Serum albumin in m/dl	3.9±0.3	2.5±0.7
Serum protein thiols in µmoles/l	103.4±12.2	74.2±10.6
Urine protein in mg		
8 hour	295.2±35.2	2012.3±714.5
12 hour	412.9±39.6	2656.7±893.1
24 hour	631.4±52.5	3896.2±934.3
Urine protein thiols in µmoles/g protein		
8 hour	68.2±22.8	20.4±9.6
12 hour	89.1±12.8	36.8±12.9
24 hour	120.5±30.9	65.9±22.3

DISCUSSION

The results presented in this study demonstrate that the concentration of protein in 8, 12 and 24 hour urine of both mild and severe pre-eclampsia patients were significantly increased along with significant decrease in serum albumin and serum protein thiols. This decrease in serum protein thiol levels in both the groups may be due to enhanced free radical generation in pre-eclampsia, an inflammatory condition. Albumin is an important chain breaking extra cellular antioxidants which contains an exposed cysteine–SH groups and provides bulk of “total serum thiols”. These reduced thiol groups were oxidized by electron deficient free radicals, in the process there occurs oxidation of albumin

molecule itself. This type of antioxidant property makes albumin a "sacrificial" antioxidant [12].

These findings suggests that excreted albumin in urine is deficient in thiol groups. Then what is the fate of the albumin bound thiol groups? We speculate that the decreased protein thiols in urine of pre-eclampsia patients could be because of increased oxidation of albumin bound thiol groups in serum due to existing oxidative stress. Increased oxidation of reduced SH groups present on albumin, decreased the levels of serum protein thiols. Hence excretion of such albumin deficient in reduced form of thiol groups in urine decreased the levels of protein bound thiols in urine.

In conclusion, our data suggests that measurement of 8 and 12 urine protein thiols along with measurement of urinary protein could serve as a better alternative for 24 hour urine protein estimation and aid in early diagnosis of pre-eclampsia. Consequently, it reduces morbidity and mortality of mother and fetus.

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