

ORIGINAL ARTICLE

Protein Thiols in Essential Hypertension Patients

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Abstract:

Background: Essential hypertension is one of the most prevalent diseases of the world and is an unequivocal risk factor for cardiovascular morbidity and mortality. Several previous studies have shown that increased free radical activity is suggested to play an important role in the lipid peroxidation and protein oxidation of cellular structures causing cell injury and is implicated in the pathogenesis of vascular disease and essential hypertension is one of them. The thiols groups on proteins are known to play a major role in maintaining the antioxidant status of the body. *Aims and Objectives:* The current study was designed to find out the levels of such protein bound thiols in essential hypertension patients. *Materials and Methods:* The study was conducted on 45 essential hypertension patients and 25 healthy subjects without any underlying medical disorder as controls. Patients were divided into three groups according to the 2003 European Society of Hypertension-European Society of Cardiology (ESH/ ESC) guidelines (grade I, II, III). Serum and urine protein thiols were determined by spectrophotometric methods using dithionitrobenzoic acid (DTNB) and correlated with blood pressure (BP). *Results:* There was a significant decrease in serum protein thiols in essential hypertensive patients compared to healthy controls ($p < 0.01$) and

correlated positively with grade II and grade III essential hypertension. In urine samples there was a significant increase in protein and a decrease in protein bound thiols ($p < 0.01$) which also correlated positively with grade II and grade III essential hypertension. *Conclusions:* Protein bound thiols; the major antioxidants in the body are decreased in essential hypertensive patients. Due to increased consumption of protein bound thiols in such oxidative environment, there was a significant decrease in protein bound thiols in urine.

Key Words: Microalbuminuria, Urine Protein Thiols, Essential Hypertension

Introduction:

Hypertension is associated with an increased vascular oxidative stress; however, there is still a debate whether this oxidative stress is a cause or a result of hypertension. Animal studies have generally supported the hypothesis that increased blood pressure is associated with increased oxidative stress; however, human studies have been inconsistent [1]. Oxidative stress promotes vascular smooth muscle cell proliferation and hypertrophy and collagen deposition, leading to thickening of the vascular media and narrowing of the vascular lumen. In addition, increased oxidative stress may damage the endothelium and impair the endothelium-dependent vascular relaxation and

increase vascular contractile activity [1]. All these effects on the vasculature may explain how increased oxidative stress can cause endothelial dysfunction. The –SH (reduced thiol) groups that exist 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 [2]. The thiols are the major antioxidants in body fluids which are known to reduce highly reactive free radicals thus protecting the biomolecules [2]. Such thiols have been studied and determined in different disease conditions and found to be decreased in different diseases compared to healthy controls [2, 3]. The current study was designed to find out the levels of such protein bound thiols in essential hypertension patients.

Materials and Methods:

Patients included in the study were selected from an outpatient clinic of Basveshwara Hospital, Gulbarga, over a period of 1 year from 2009– 2010.

Following were the inclusion criteria:

1. Essential hypertension defined according to the 2003 European Society of Hypertension-European Society of Cardiology (ESH/ESC) guidelines (grade 1–3).
2. Never previously treated for hypertension
3. If treated no medication for at least 1 month before the beginning of the study.

The study was conducted on 45 essential hypertension patients and 25 healthy subjects who were not suffering from any type of

disease or nor taking any type medications. The patients were further sub-divided into three groups according to their systolic blood pressure (SBP) and diastolic blood pressure (DBP). This study was approved by institutional review board and informed consent was obtained from all subjects involved in the study. Blood pressure (BP) was measured with a mercury sphygmomanometer, with the patient in the sitting position after 5 min of rest in a quiet environment, following the recommendations of the British Hypertension Society. SBP, DBP (Korotkoff phases I and phase V, respectively), and mean blood pressure (MBP) were averaged by using three readings measured at 5-min intervals. Differences of 05mmHg were allowed.

Under aseptic conditions blood samples (5 ml) were drawn into plain vacutainers from ante-cubital veins of controls and essential hypertensive patients. The collected blood was allowed to clot for 30 minutes and then centrifuged at 2000 RPM for 15 minutes for clear separation of serum. All assays were performed immediately after serum was separated. 24 hour urine samples were collected from same cases and controls and assayed for urine protein, and 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. Serum and urine protein thiols were measured by a spectrophotometric method using DTNB (4, 5). Briefly, 900 μ L of 0.2 M Na_2HPO_4 containing 2 Mm Na_2EDTA , 100 μ L serum or urine and 20 μ L of 10Mm DTNB in 0.2M Na_2HPO_4 were

taken in an Eppendorf tube and warmed to 37°C. The solution was mixed in a vortex mixer and transferred to a cuvette, and the increase in absorbance was measured at the end of 5 min at 412 nm in UV spectrophotometer 118 (Systronics). Appropriate sample and reagent blanks were prepared and the corrected absorbance values were used to calculate the concentration of protein thiols using calibration curve. Values were expressed in imoles/L for serum protein thiols and in imoles/g of proteins.

The results were expressed as mean \pm 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). One way analysis of variance

(ANOVA) followed by multiple comparison by post-hoc test was used to compare mean values among the groups.

Results:

The study was performed in 45 hypertensive subjects and 25 control volunteers. The characteristics of the patients and control subjects are shown in Table 1. There was significant decrease in serum protein thiols in grade II and grade III hypertensive patients ($p<0.01$) compared to controls. We also found significant decrease in urine protein thiols in grade II and grade III essential hypertensive patients ($p<0.01$) compared to controls. Among the patients groups grade III showed significant reduced levels of protein thiols as compared to grade I and grade II.

Table-1 Demographic and clinical characteristics of the patients with different degrees of essential hypertension and healthy controls (Expressed in Mean \pm SD).

| Parameters | Normotensive (n=25) | Patients with essential hypertension with (ESH/ESC) grade | | |
|-------------------------------|---------------------|---|------------------|------------------|
| | | Grade I (n=15) | Grade II (n=12) | Grade III (n=18) |
| Age | 52.6 \pm 5.1 | 53.2 \pm 4.6 | 54.1 \pm 4.2 | 59.6 \pm 5.1 |
| Sex M / F | 10/15 | 6/9 | 5/7 | 7/11 |
| Mean SBP (mmHg) | 124 \pm 9.1 | 144 \pm 9.8 | 176 \pm 10.9 | 211 \pm 11.8 |
| Mean DBP (mmHg) | 78.8 \pm 8.1 | 95 \pm 6.4 | 105 \pm 7.5 | 122 \pm 10.3 |
| Serum thiols (μ moles/L) | 250.9 \pm 42.2 | 193.9 \pm 51.3 | 165.6 \pm 48.3 | 132.4 \pm 43.3 |
| Urine thiols (μ moles/L) | 310 \pm 251.3 | 174.2 \pm 48.5 | 141.3 \pm 46.1 | 62.42 \pm 45.8 |

Grade I (Mild) – Systolic blood pressure 140 – 159, Diastolic blood pressure 90 – 99 mmHg.

Grade II (Moderate) – Systolic blood pressure 160–179, Diastolic blood pressure 100–109 mmHg.

Grade III (Severe) – Systolic blood pressure \geq 180, Diastolic blood pressure \geq 110 mmHg.

Discussion :

We have found significant microproteinuria in grade II and grade III hypertensive cases and compared to healthy controls. Though microproteins 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" [6]. Previous studies have shown significantly decreased protein thiols in serum of uremia cases and correlated positively with serum albumin [2]. These findings suggest that excreted albumin in urine is deficient in thiol groups. We speculate that the decreased thiols in urine of essential hypertensive 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 which is deficient in reduced form of thiol groups in urine decreased the levels of protein bound thiols in urine. In conclusion, our data suggests that there is significant decrease in urinary thiols in

patients with grade II and grade III essential hypertension and it varies with the amount of micro albumin excreted in urine.

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