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Research Article

Dynamic thiol/disulphide homeostasis in patients with newly diagnosed primary hypertension

Ihsan Ates, MD[a,*], Nihal Ozkayar, MD[b], Bayram Inan, MD[a], Fatma Meric Yilmaz, MD[c,d], Canan Topcuoglu, MD[c], Salim Neselioglu, MD[d], Ozcan Erel, MD[d], Fatih Dede, MD[b], and Nisbet Yilmaz, MD[a]

[a]*Internal Medicine Department, Ankara Numune Training and Research Hospital, Ankara, Turkey;*
[b]*Nephrology Department, Ankara Numune Training and Research Hospital, Ankara, Turkey;*
[c]*Biochemistry Department, Ankara Numune Training and Research Hospital, Ankara, Turkey; and*
[d]*Department of Biochemistry, Yildirim Beyazit University Medical Faculty, Ankara, Turkey*

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Abstract

We aimed to investigate the thiol/disulphide homeostasis in patients with newly diagnosed primary hypertension with a novel and automated method. Blood thiol/disulphide homeostasis, which consists of native thiol/disulphide exchanges, was investigated in 45 patients with primary hypertension and 45 healthy controls. The levels of native thiol, total thiol, and native thiol/total thiol ratio were lower while the disulphide level and disulphide/native thiol and disulphide/total thiol ratios were higher in patients with primary hypertension when compared with those in the control group. Positive correlation was detected between 24-hour systolic and diastolic blood pressure levels and disulphide/native thiol ratio. With reference to the stepwise multiple linear regression model; increase in disulphide/native thiol ratio and log(24-hour urine microalbumin) and decrease in native thiol/total thiol ratio are independent predictors of 24-hour systolic and diastolic blood pressure. This study demonstrated that thiol/disulphide homeostasis was shifted toward disulphide formation in patients with primary hypertension. J Am Soc Hypertens 2016;10(2):159–166.

Keywords: Blood pressure; oxidative stress; oxidized thiol; sulfur bonds.

Introduction

According to the data of the World Health Organization, hypertension (HT) is the most common chronic disease (cardiovascular disease, chronic kidney disease, and cerebrovascular disease) that leads to death.[1] Uncertainity of the etiopathogenesis of HT has continuously been a topic of interest for researchers. Recently, one of the topics on HT etiopathogenesis that has received great attention is; oxidative stress.

Oxidative stress occurs as a result of excessive production of free oxygen radicals and insufficiency of the antioxidant defense systems in comparison to the oxidant radicals.[2] Reactive oxygen species (ROS) are the primary molecules responsible for oxidative damage.[3] To protect the organism against the harmful effects of ROS, various enzymatic and nonenzymatic antioxidant mechanisms come into action. One of these antioxidant molecules are the thiols and these are the compounds containing sulfhydryl group which plays a critical role in preventing oxidative stress in cells.

The primary target of ROS is the thiol groups of sulphur containing amino acids (cysteine, methionine…) of proteins. The thiol groups in the media get oxidized by ROS forming reversible disulphide bonds. This conversion is the earliest sign of radical-mediated protein oxidation.[4] The disulphide bonds so formed can again be reduced to thiol groups by

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*Corresponding author: Ihsan Ates, MD, Internal Medicine Department, Ankara Numune Training and Research Hospital, 06100 Ankara, Turkey. Tel: 0903125084666; Fax: 0903123113958.

E-mail: dr.ihsanates@hotmail.com

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a number of antioxidants. And in this way, thiol/disulphide homeostasis is maintained.

A number of experimental studies have demonstrated that abnormal thiol/disulphide homeostasis causes cellular proliferation or apoptosis.[5,6] Thiol/disulphide homeostasis has been measured since 1979 only in one direction,[7] but with the novel method developed by Erel and Neselioglu, the levels of both variables can be measured separately as well as individually and collectively.[8]

To the best of our knowledge, thiol/disulphide homeostasis has not been measured by this novel method in HT patients in any study.

In this study, we aimed to investigate the levels of native thiol, total thiol and disulphide, and the ratios of disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol in patients with newly diagnosed primary HT using a novel, automated method that determines dynamic thiol/disulphide homeostasis.

Materials and Methods

Study Population

This study was performed at Ankara Numune Training and Research Hospital, Internal Medicine Clinic, between April and July 2015.

Forty-five patients older than 18 years of age with newly diagnosed primary HT and not yet on treatment, and 45 healthy individuals were enrolled in the study. The secondary causes of HT were eliminated by medical history, physical examination, and laboratory measurements where necessary.

Patients with diabetes mellitus, acute-chronic kidney disease, proteinuria at nephrotic levels, documented coronary artery disease, heart failure, peripheral artery disease, cerebrovascular disease, malignancy, liver diseases, and rheumatological diseases, and those using antioxidant substance, lipid lowering drugs, cigarette, alcohol, and vitamin supplements were excluded from the study.

Body mass index (BMI) was calculated as body weight in kilograms divided by square of height in meters (BMI = kg/m^2).

The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Research Committee. All subjects provided written informed consent before participation in the study.

Ambulatory Blood Pressure Monitoring

Watch Blood Pressure 03 device (Microlife WatchBP AG, Switzerland) was used for ambulatory blood pressure monitoring (ABPM). Blood presssure measurements with ABPM were set at 30-minute intervals. During ABPM, patients were instructed to continue their daily activities and not to bend their arms during blood pressure measurements. Twenty-four–hour (h) systolic blood pressure (SBP) and diastolic blood pressures (DBP) were obtained from these measurements. The method was considered reliable if more than 70% of the measurements were valid. The 24-h average of blood pressure measurements with ABPM being >130/90 mm Hg was accepted significant for primary HT.[9]

Biochemical Parameters

Blood samples for biochemical parameters and thiol/disulphide homeostasis tests were taken after 8 hours of fasting. Collected samples were immediately centrifuged at 1500 rpm for 10 minutes to separate the plasma and serum, and serum was stored at −80°C until analysis. Thereafter, all parameters were analyzed in the same serum sample.

Albumin was measured by bromocresol green method; total protein, total cholesterol, and triglyceride by enzymatic colorimetric method; high-density lipoprotein cholesterol by homogenous enzymatic colorimetric method; 24-h urinary protein by microalbumin turbidimetric method; and C-reactive protein by immunoturbidimetric method using Hitachi Moduler P800 (Roche Diagnostic Corp., Indianapolis, Indiana, USA) autoanalyzer. Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald formula.[10]

Serum Thiol/Disulphide Homeostasis

Thiol/Disulphide homeostasis tests were conducted as described previously.[8] First, reducible disulphide bonds were reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde and after reaction with DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)], all thiol groups including reduced and native thiol groups were determined. Half of the difference between total thiol and native thiol gave the amount of dynamic disulphide. After determining native and total thiols, disulphide level, disulphide/total thiol percent ratios, native thiol/total thiol percent ratios, and disulfide/native thiol percent ratios were calculated.[8]

Catalase Activity Assay

Catalase activity was gauged by Goth's method.[11] Sample (0.2 mL) was propagated in 1.0 mL substrate (65 μmol per H_2O_2 in 60 mmol/L sodium–potassium phosphate buffer, pH 7.4) at 37°C for 60 seconds. The enzymatic reaction was ceased with 1.0 mL of 32.4-mM ammonium molybdate, and the yellow complex of molybdate and H_2O_2 was measured at 405 nm. One unit of catalase dissociates 1 μmol of H_2O_2 min^{-1} under these conditions. Results were expressed in kU/L.

Measurements of Myeloperoxidase

Serum myeloperoxidase activity was measured through a modification of the o-dianisidine method[12] based on kinetic measurement at 460 nm with the rate of the yellow is orange