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Original Research Article

Antioxidant Enzyme Activities (Superoxide Dismutase and Catalase) in Peripheral Blood Mononuclear Cells in Essential Hypertension

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Abstract

Background: Oxidative stress has been implicated in pathogenesis of hypertension, a major cardiovascular risk factor. Peripheral blood mononuclear cells (PBMCs) are primary contributors to systemic reactive oxygen species (ROS). The impact of ROS-products released from PBMCs may contribute to endothelial dysfunction damage in hypertensive subjects. Superoxide Dismutases (SODs) and Catalase comprise first line of defence against ROS.
Aim: To analyse activities of SOD and Catalase in PBMCs of freshly diagnosed essential hypertensive patients as compared to normotensive healthy controls. Further, correlate the antioxidant enzyme activities with oxidative stress in terms of 8-iso-Prostaglandin F2α (8-iso-PGF2α) in both groups.
Methods: Forty-eight patients and age, BMI-matched 48 controls were recruited. Catalase and SOD activities were measured in PBMC-lysates using Enzyme-Linked-Immunosorbent-Assay (ELISA). 8-iso-PGF2α levels in serum and urine of all subjects were also measured using ELISA.
Results: Significantly lower (5.1-fold, $p<0.001$) Catalase activity was found in patients as compared to controls. Similar but non-significant trend (1.3-fold) was observed for SOD.8-iso-PGF2α levels were significantly higher in serum (11.3-fold, $p<0.001$) and urine (4.8-fold, $p<0.001$) of patients as compared to controls. In patients, activity of SOD showed positive correlation with that of Catalase ($rs=0.383$, $p=0.007$) and serum 8-iso-PGF2α levels ($rs=0.459$, $p=0.001$). While in controls, Catalase activity correlated negatively with 8-iso-PGF2α ($rs=-0.344$, $p=0.017$).
Conclusion: This study reports significantly increased oxidative stress in hypertensive patients accompanied by a simultaneous decrease in the antioxidant enzyme activities of Catalase and SOD in their PBMCs. A direct correlation between SOD activity and oxidative stress suggests its defensive position while Catalase seems to adopt a submissive role in defence system in case of essential hypertension.
Keywords: Oxidative stress, 8-iso-Prostaglandin F2α, antioxidant enzyme activity, reactive oxygen species.

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1. Introduction

Hypertension is considered the most important risk factor for the occurrence of cardiovascular disease (CVD) [1]. In recent years, oxidative stress has gained attention as one of the fundamental mechanisms responsible for the development of hypertension [1,2] wherein elevation of blood pressure (BP) by oxidants and its amelioration by antioxidants strongly supports a causal role of reactive oxygen species (ROS) in hypertension [3]. Several studies have provided evidence to support the fact that oxidative stress and ROS are elevated in hypertensive patients and animal models of hypertension, and are linked to its pathogenesis [4].

ROS, such as superoxide (O_2-) and hydrogen peroxide (H_2O_2), are constantly produced during metabolic processes in all living species. They are the normal by-

products of aerobic metabolism and cause extensive cellular damage when in excess. The major consequence of increased ROS is direct oxidative damage to critical biomolecules like DNA, proteins and lipids. The Superoxide Dismutases (SODs) and Catalase comprise the first line of defence system against ROS. SODs catalyse the dismutation of the superoxide radical to oxygen and hydrogen peroxide and Catalase is accountable for converting the hydrogen peroxide to water and oxygen. Impaired activity of SOD and Catalase has been associated with uncontrolled production of ROS and other free radicals [5].

Peripheral blood mononuclear cells (PBMCs) are one of the primary contributors to systemic reactive oxygen species (ROS) [6]. In a previous study, Redon *et al* have suggested that the impact of released ROS products from PBMCs may contribute to the endothelial dysfunction damage to the organs in hypertensive subjects [7]. This study therefore aimed at analysing the antioxidant enzyme activities of SOD and Catalase in PBMCs of freshly diagnosed essential hypertensive patients as compared to normotensive healthy controls.

Due to the complexity involved in measuring free radicals *in vivo* directly, cellular components that react with these free radicals are quantified instead. Measuring lipid peroxidation products is the most effective method for quantifying oxidative stress [8]. Among these, the measurement of isoprostanes, specifically F2-isoprostanes, which are relatively stable and ubiquitous in human plasma and urine, has proven to be among the most sensitive and reliable biomarkers for the investigation of lipid peroxidation and hence oxidative stress *in vivo* [9,10]. The 8-iso-Prostaglandin F2α (8-iso-PGF2α) has been extensively studied in different diseases and is considered the most useful biomarker of oxidative damage [11,12]. Thus, this study further aimed at analysing the serum and urinary levels of 8-iso-PGF2α in patients and controls and correlates them with the PBMC antioxidant enzyme activities, to analyse the extent of oxidative stress and antioxidant defence against it, associated with increased blood pressure.

2. Materials and Methods

2.1 Study subjects

Forty-eight patients diagnosed with essential hypertension (SBP/DBP > 140/90 mmHg) and 48 age and body mass index (BMI) matched healthy normotensive (SBP/DBP≤ 120/80 mmHg) controls were recruited for the study. Recruitment of hypertensive individuals was in accordance with the Seventh Joint National Committee (JNC7) [13] report guidelines. Freshly diagnosed individuals (age>18 years) with persistent high BP, and normal fasting blood glucose were enrolled. These patients were monitored (twice daily) for BP and referred to a consultant - immediately if SBP/DBP was more than 160/100 mmHg; and after monitoring for 8 days if SBP/DBP <160/100 mmHg - prior to recruitment. On confirmation of essential hypertension, these subjects were recruited under hypertension group after obtaining written informed consent. Detailed information regarding demographic status, clinical history, family history and medication was obtained from all the subjects recruited in the study. The study protocol was approved by the Institutional Ethics Committee, which follows the ethical standards laid down by the Indian Council of Medical Research's (ICMR) Ethical Guidelines for Biomedical Research on Human Participants.

2.2 Biochemical Analysis

Fasting venous blood samples were collected in plain and K_2-EDTA vacutainers at the time of enrolment (for subjects identified with high BP, this sample was collected prior to starting hypertensive medication). Serum samples were used to perform routine biochemical investigations such as lipid profile, renal profile and liver profile tests. Those healthy individuals with levels beyond normal range were excluded from control group. Serum and urine aliquots were stored at -80˚C until further use.

2.3 8-iso-PGF2α Levels

8-iso-PGF2α levels were detected in serum and urine samples of patients and controls using Enzyme-Linked Immunosorbent Assay (ELISA) method (Immuno-Biological Laboratories Co., Ltd., Hamburg, Germany). Total 8-iso-PGF2α - both free and esterified - was measured by this method. Lipoprotein or phospholipid coupled 8-iso-PGF2α were hydrolysed as per manufacturer's instructions.

2.4 Catalase and SOD activity assays

Enzyme activities of Catalase and SOD were measured in PBMC lysates using commercially available ELISA. PBMCs were isolated from K_2-EDTA anti-coagulated blood. Ficoll- Histopaque®-1077 (Sigma-Aldrich) gradient separation method. PBMCs were lysed according to manufacturer's instructions and the cell lysate was stored at -80°C until further analysis. Catalase and SOD enzyme activity were measured using Oxiselect Activity assay kits (STA-341 and STA-340 respectively, Cell Biolabs, Inc, USA) as per manufacturer's instructions.

2.5 Statistical Analysis

Results are expressed as frequency with percentage and mean ± standard deviation (SD) for parametric variables and median with inter quartile (25^{th}/75^{th}) ranges for non-parametric variables. Student's unpaired t test and Mann–Whitney U test were used to determine the significance of differences between the two study groups for parametric and non-parametric variables respectively. Correlations were evaluated by Spearman's rank correlation test. For all tests p value <0.05 was considered statistically significant. Analyses were performed using statistical software SPSS (version 21.0, Chicago, IL).

3. Results

3.1 Demographic and clinical characteristics

The baseline demographic characteristics of patients and controls are depicted in Table 1. The comparison of lipid profile of patients and controls is given in Table 2, which did not differ significantly between the two groups.

Table 1: Comparison of demographic parameters between patient and control groups

Parameters	Controls (N=48)	Patients(N=48)
Age (years)	45.8 ±6.7	48.8 ±5.7
Male/Female	43/5	45/3
BMI (kg/m^2)	26.3 ± 3.9	27.1 ± 5.77
SBP (mmHg)	124.6 ± 7.7	164.3 ± 14.8***
DBP (mmHg)	79.58 ± 6.0	102.08 ± 6.78***

***p<0.001 compared to respective control groups. Values are expressed as mean ± SD. BMI, Body Mass Index; SBP, Systolic Blood Pressure, DBP, Diastolic Blood Pressure

Table 2: Comparison of lipid profile between patient and control groups

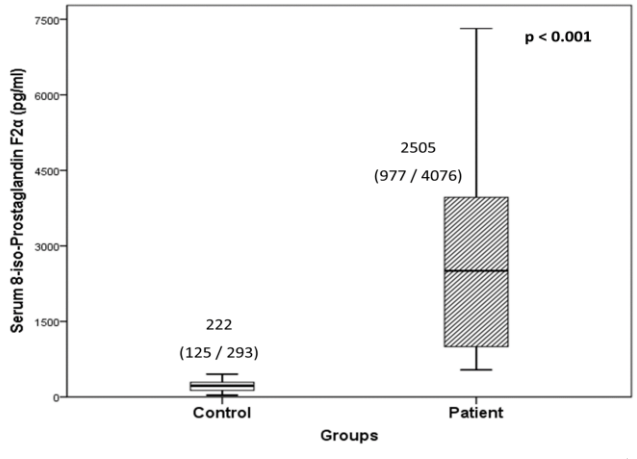
Parameters	Controls (N=48)	Patients (N=48)
TC (mg/dl)	169.6 ± 34.4	172.6 ± 31.6
HDL-C (mg/dl)	38.8 ± 11.6	43.0 ± 11.6
TC/HDL-C	4.6 ± 1.3	4.2 ± 1.0
Triglycerides (mg/dl)	110.9 ± 43.7	126.5 ± 69.7
VLDL-C (mg/dl)	22.2 ± 8.7	25.3 ± 13.9
LDL-C (mg/dl)	108.6 ± 30.2	104.3 ± 29.0
LDL-C/ HDL-C	2.9 ± 1.1	2.6 ± 0.9

Values are expressed as mean ± SD. TC, Total cholesterol; HDL-C, High Density Lipoprotein Cholesterol; VLDL-C, Very Low Density Lipoprotein Cholesterol; LDL-C, Low Density Lipoprotein Cholesterol

3.2 8-iso-Prostaglandin F2α Levels

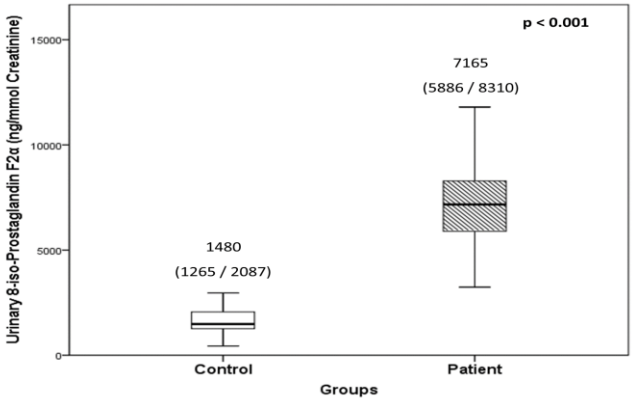
In hypertensive patients, the levels of 8-iso-PGF2α were significantly high p<0.001) in serum (11.3 fold, p<0.001) and urine (4.8 fold, p<0.001) as compared to controls (Figure 1, 2). There was no correlation observed between this oxidative stress marker and lipid parameters.

Figure 1: Serum levels of 8-iso-Prostaglandin F2α in hypertensive patients and controls



Values are expressed as median with interquartile (25th /75th) range

Figure 2: Urinary 8-iso-Prostaglandin F2α levels of hypertensive patients and controls



Values are expressed as median with interquartile (25th /75th) range

3.3 Catalase and SOD activity assays

In patients, the enzyme activity of Catalase was found to be significantly lower (5.1 fold, p<0.001) as compared to controls (Figure 3). Despite a similar trend (1.3 fold), the fall in SOD enzyme activity as compared to controls did not reach statistical significance (Figure 4). In patients, the activity of SOD showed positive correlation with that of Catalase (rs=0.383, p=0.007) and serum levels of 8-iso-PGF2α (rs=0.459, p=0.001). While in controls, Catalase activity correlated negatively with 8-iso-PGF2α (rs= -0.344, p=0.017).

Figure 3: Catalase activity in PBMCs of hypertensive patients and controls

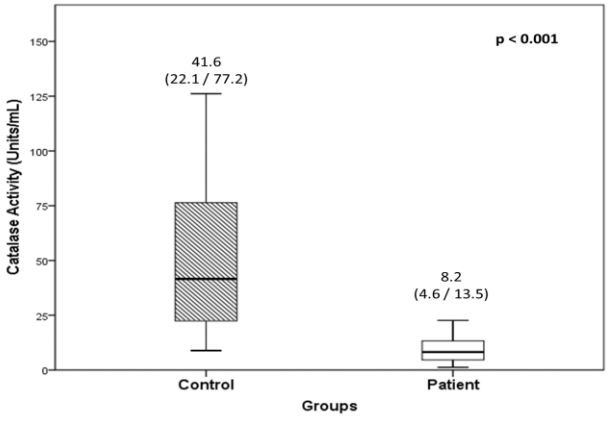
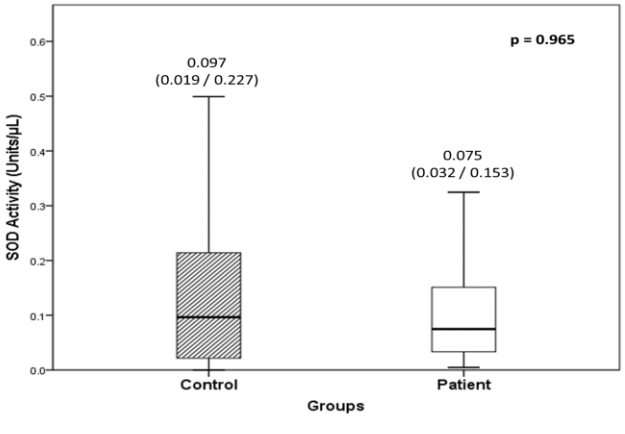


Figure 4: SOD activity in PBMCs of hypertensive patients and controls



4. Discussion

In the present study, Catalase activity in the hypertensive group was remarkably low as compared to controls. Although the SOD activity in patients showed a similar trend, the fall was not significant. Moreover, significantly higher serum and urinary levels of 8-iso-PGF2α in essential hypertensive patients as compared to normotensive healthy controls were observed. These findings support previous reports of decreased antioxidant status in the hypertension [7,14].

The role of antioxidative enzymes in hypertension has been extensively studied by Redon *et al* who have reported a reduction in the activity of antioxidant enzymes such as SOD, Catalase and Glutathione peroxidase in mononuclear cells of hypertensive subjects accompanied by increase in oxidative stress [7]. They suggest that the reduced antioxidant activity observed may be due to their consumption by excess ROS produced in the disease. Evidence in support of their hypothesis was reported by Venkatesan *et al*, who demonstrated that H_2O_2 significantly reduced the abundance of Catalase protein in rat mesangial cells [15]. However, Otitoju *et al* have demonstrated significantly elevated SOD activity in the brains of adult rats exposed to oxidative stress [19]. They propose that the increased SOD activity may suggest a possible survival mechanism. The current study also found a positive correlation between SOD activity in PBMCs and oxidative stress levels in sera of patients in spite of non-significant reduction in SOD activity. However, no such correlation was observed for Catalase. In fact, in the control group there was significant negative correlation observed between PBMC Catalase activity and serum 8-iso-PGF2α levels.

Montuschi *et al*, have reported 15-F2t-Isoprostane (another term for 8-iso-PGF2α) as a potent vasoconstrictor, suggesting that isoprostanes may function as pathophysiologic mediators of oxidant injury [9]. Thus, their elevated presence in hypertension and the accompanying decrease in antioxidant enzyme activities suggest their role in pathogenesis of the disease. Similar to the present finding of elevated levels of 8-iso-PGF2α in hypertensive patients, Cracowski *et al* have also reported increased oxidative stress in patients with pulmonary hypertension [17]. Zhang *et al* have found elevated plasma 15-F2t-isoprostane, concentration in idiopathic pulmonary arterial hypertension. They also found it to be an independent factor associated with mortality [18]. Hozawa *et al* observed plasma 8-isoprostane levels to be elevated in older subjects with severe hypertension [19]. Further, Belch *et al* have reported increased lipid peroxides in patients with congestive heart failure, and proposed that free radicals may be important in heart failure [20].

The measurement of F2-isoprostanes has implicated a role of free radicals and oxidant injury in a wide variety of human diseases, including cardiovascular, pulmonary, neurological, renal, and liver diseases, and therefore has an important impact on clinical medicine [9]. The current study reports significant increase in 8-iso-PGF2α, thus oxidative stress, accompanied by a significant decrease in Catalase activity in freshly diagnosed hypertensive patients as compared to healthy normotensive controls. Though the role of antioxidant-oxidant interplay in essential hypertension has been well established, weather the reduced antioxidant status in hypertension is the cause or consequence of the disease has not yet been resolved. However, a direct correlation in patients between SOD activity and oxidative stress suggests its defensive position in hypertension, while Catalase seems to adopt a submissive role in the defence system.

Conflicts of interest

The authors report no conflicts of interest.

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