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Research Article

EFFECT OF OLMESARTAN AND LABETALOL ON OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN SOUTH INDIAN HYPERTENSIVE PATIENTS

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ABSTRACT

Objective: To evaluate the glutathione (GSH), lipid peroxidation, total antioxidant status levels (TAS), and liver function parameters such as serum aspartate transaminase (AST) and serum alanine transaminase (ALT) in South Indian hypertensive patients before and after olmesartan and labetalol treatment.

Methods: Total 69 subjects were selected for the study. Out of 69 subjects, 29 were healthy volunteers (HVs) and 40 subjects were hypertensive patients. The patients both male and female within the age group of 20-65 years were selected. GSH, lipid peroxidation, TAS, AST, and ALT levels in serum were estimated using reported methods. Individual methods were standardized, standard graphs were plotted, and the parameters were measured.

Results: Significantly fewer levels of GSH and TAS and more levels of malondialdehyde were observed in untreated hypertensive patients as compared with HVs. After drug treatment, there was significant raise in the levels of GSH and TAS as compared with untreated patients. No significant changes in AST and ALT were observed in both olmesartan and labetalol group as compared with HVs.

Conclusions: The antioxidant supplementation is warranted to protect from oxidative stress attack, and the drugs such as olmesartan and labetalol were showing the significant protection against oxidative stress which can significantly reduce blood pressure and prevent possible complications in hypertensive patients.

Keywords: Hypertension, Oxidative stress, Olmesartan, Labetalol, Antioxidant.

INTRODUCTION

The main characteristic of arterial hypertension is a systolic pressure over 140 mm Hg and a diastolic pressure over 90 mm Hg. Hypertension is the leading cause of cardiovascular diseases (CVDs) worldwide. CVDs account for a large proportion of deaths and disability all over the globe. High blood pressure (BP) is an important public health problem in India [1]. Hypertension is estimated to cause 7.5 million deaths, about 12.8% of the total deaths [2]. There will be increase by almost 75% in the CVD burden globally by the year 2020. Hypertension is an independent risk factor for both stroke and coronary heart disease. The "Framingham Heart Study" results suggest that 78% of hypertension in men and 65% in women are directly attributed to obesity and increased oxidative stress as the possible cause of complications that follow hypertension [3]. Oxidative stress occurs as a consequence of a disturbed balance between over free radical production and antioxidant insufficiency. Hypertension is a condition followed by severe oxidative stress proven in the human population [4] and experimental conditions [5]. Oxidative stress is mainly mediated through reactive oxygen species (ROS). Excess production of oxygen free radicals, which is in the form of superoxide (O_2^-), occurs in human hypertension [6]. The reaction product between O_2^- and nitric oxide (NO), peroxynitrite ($ONOO^-$) produces a strong oxidant molecule, which oxidizes proteins, lipids, and nucleic acids, causing cell membrane damage, these pathological processes contribute to the narrowing of the arterial lumen, consequently to increase peripheral resistance and increase BP [7]. Superoxide levels are maintained through endogenous antioxidant systems. Superoxide dismutase is the principal antioxidant in the vascular system [8], which is often associated with endothelial dysfunction and hypertension.

Olmesartan is an angiotensin II sub-type 1 (AT1) receptor antagonist and inhibits the effects of AT II on the renin-AT-aldosterone system, which mediates a key role in the pathogenesis of hypertension. Currently, 10-40 mg of olmesartan (peroral) once daily is recommended for the treatment of adult patients. It is a prodrug that is completely metabolized to the pharmacologically active moiety, olmesartan, by esterase enzymes in the gastrointestinal mucosa, portal blood, and liver after oral administration. The absolute bioavailability of olmesartan after a single oral dose of 20 mg in healthy people was 26%, and peak plasma levels were reached after 2 hrs and reached to steady state after around 5 days of once-daily administration. It is highly bound to plasma proteins and has a low volume of distribution [9,10]. Labetalol is a mixed α_1- and β-receptor blocking drug [11]. Labetalol is completely absorbed (100%) from the gastrointestinal tract (GIT) with peak plasma levels occurring 1-2 hrs after oral administration. The absolute bioavailability is 26%; this is due to "first-pass" metabolism. The extent of bioavailability is influenced by the presence of food in the GIT.

The oxidative stress indicators such as glutathione (GSH), total antioxidant status (TAS), and malondialdehyde (MDA) play a very key role in the pathophysiology of hypertension. By considering these, we have studied the oxidative stress status in hypertensive patients before and after treatment compare to the healthy volunteers (HVs). We studied the effect of labetalol and olmesartan on systolic BP (SBP), diastolic BP (DBP), endogenous antioxidant GSH, lipid peroxidation (MDA), TAS, and the liver function parameters aspartate transaminase (AST) and alanine transaminase (ALT) in South Indian hypertensive patients.

METHODS

This study was done in the Pharmacology Department, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, India. The study comprised three groups: one group of HVs and two groups of essential hypertensive patients. The study was conducted in 69 subjects. Out of 69 subjects, 29 were HVs and 40

subjects were hypertensive patients. Out of 40 patients, 20 were treated with olmesartan and 20 were treated with labetalol. The patients within the age group of 25-65 years were selected. They were clinically newly diagnosed for hypertension. The 29 HVs having no history of smoking, alcoholism, and any diseases were considered as controls. The secondary hypertensive patients, diabetic patients, and patients with renal disease were excluded from the study. The Institutional Ethics Committee (Letter No: UCPSc/KU/BA/03/2013) approval was taken before the study. The patients were treated with either olmesartan 20 mg or labetalol 100 mg for at least 10 days. After 10 days of treatment by taking the prior consent, venous blood was collected in lithium heparin vacutainer and immediately centrifuged at 3000 ×g for 8 minutes at room temperature. The samples were stored at 4°C, and all the samples were analyzed on the same day of collection [12]. All the methods were standardized, and standard graphs were obtained. Serum GSH, MDA, AST, and ALT were measured using standard methods.

GSH in blood

About 0.5 ml of 5% trichloroacetic acid solution was added to 0.5 ml of citrated blood to precipitate the proteins and centrifuged for 20 minutes at 3000 rpm. To 0.1 ml of supernatant, 1 ml of sodium phosphate buffer (pH 8) and 0.5 ml of 5-5[1](dithiobis-2-nitrobenzoicacid) reagents (39.6 mg in 100 ml of 1% sodium citrate solution to give a concentration of 1 mM) were added. The developed yellow absorbance was measured at 412 nm [13].

TAS

Antioxidant activity status was determined by the Blois [14] method using α, α-diphenyl-β-picrylhydrazyl (Sigma, St. Louis, USA), at a concentration of 0.2 mM in methanol.

Lipid peroxidation

The lipid peroxidation products present in serum samples was estimated by the thiobarbituric acid reactive substances (TBARS) method, which estimates the MDA reactive products using spectroscopy [15].

BP

The patient should be sit upright or lying at ease. Both the SBP and DBP were measured using the digital BP apparatus (Omron, USA).

AST and ALT

Both AST and ALT in human serum were assayed by Reitman and Frankel colorimetric method [16].

Statistical analysis

All the values were expressed as mean±standard error of mean. The ANOVA followed by Student's t-test was used for analyzing the data. In tests, the criteria for statistical significance were at $p<0.05$.

RESULTS

Demographic parameters such as bodyweight and height were not significantly different among the three groups (Table 1).

BP

The mean±standard deviation (SD) levels of BP are shown in Table 2. There was a significant decrease in BP is observed in both olmesartan and labetalol groups as compared to pretreatment values. Olmesartan ($p<0.01$) reduced BP more effectively than labetalol ($p<0.05$). It was seen that the both treatment groups having the BP values higher than the HVs/control group.

GSH

The mean±SD levels of GSH expressed as nmol are shown in Fig. 1. GSH levels were significantly increased in both olmesartan ($p<0.01$) and labetalol ($p<0.05$) treatment groups as compared to their pretreatment values. Olmesartan group was more effective in increasing GSH levels as compared to labetalol group. However, in both groups, the GSH levels are lower than the HVs.

TAS

TAS levels were significantly increased with olmesartan ($p<0.05$) treatment as compared to pretreatment values. Labetalol seems to be not significant in increasing total antioxidant levels as compared to olmesartan group. In both drug treatment groups, the TAS levels were lower than the HVs (Fig. 1).

Lipid peroxidation

MDA levels were decreased in labetalol ($p<0.05$) as compared to pretreatment values. MDA levels were not significantly decreased with olmesartan as compared to pretreatment values (Fig. 2).

AST and ALT

No significant changes in AST and ALT were observed in both olmesartan and labetalol group as compared with control group (Table 2).

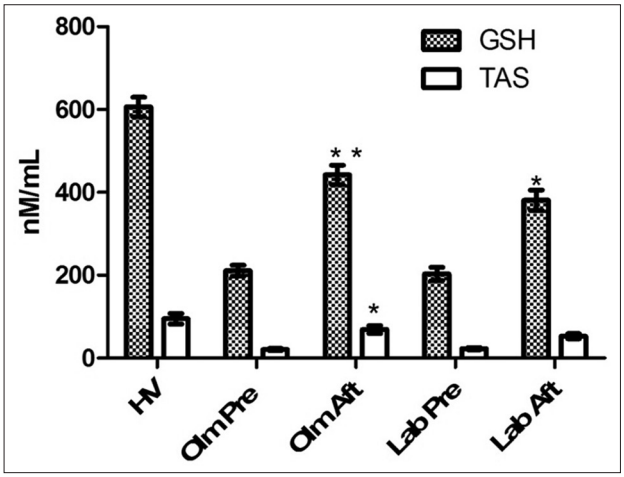

Fig. 1: Effect of olmesartan and labetalol on the levels of glutathione and total antioxidant status of hypertensive subjects

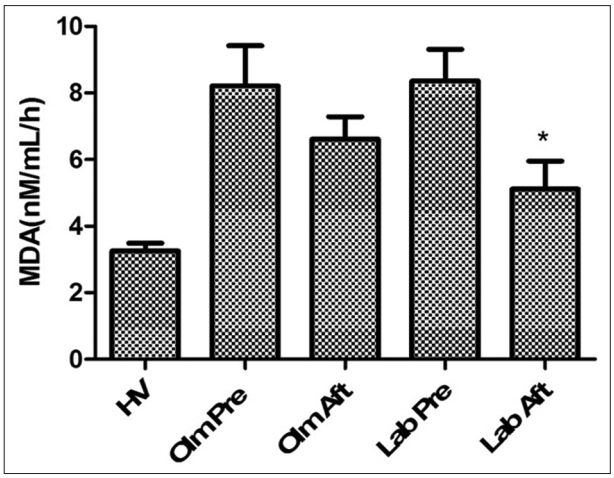

Fig. 2: Effect of olmesartan and labetalol on the levels of malondialdehyde of hypertensive subjects

Table 1: Demographic data of HVs and patients

Parameter	HVs	Olmesartan	Labetalol
Age (years)	31±7.0	45.52±6.23	50.52±7.25
Height (cm)	158±12.58	153±11.65	151±9.07
Weight (kg)	58.35±5.37	62.35±7.36	61.35±7.37

Values are represented as mean±SEM. HV: Healthy volunteer, SEM: Standard error of mean

Table 2: Effect of olmesartan and labetalol on BP, AST, and ALT of hypertensive patients

Parameters	HV	Olmesartan		Labetalol	
		Pre	After	Pre	After
SBP (mmHg)	124.8±4.23	163.7±9.47	141.1±7.51**	166.3±8.23	148.3±5.72*
DBP (mmHg)	81.2±2.46	96.3±5.81	85.9±3.65**	98.46±6.04	86.1±37*
AST (IU/L)	22.7±5.28	36.1±6.92	29.31	34.4±23	28.72
ALT (IU/L)	29.7±7.39	39.8±7.31	31.87±4.67	37.8±8.37	31.9±8.75

Significance was indicated by *p<0.05; **p<0.01. SBP: Systolic blood pressure, DBP: Diastolic blood pressure, AST: Aspartate transaminase, ALT: Alanine transaminase, HV: Healthy volunteer, BP: Blood pressure

DISCUSSION

Free radicals are very reactive unstable species which can alter the cellular antioxidant defense system. This includes antioxidants (such as GSH, ascorbate, and α-tocopherol) and antioxidant enzymes (superoxide dismutase, catalase, and GSH peroxidase). Under the conditions of excessive oxidative stress, cellular antioxidants are depleted.

ROS may play a critical role in the pathophysiology of hypertension. The studies in experimental hypertension in humans have demonstrated increased generation of ROS [17]. The reports suggest that essential hypertension associated with increased pro-oxidants such as O_2^- and H_2O_2 production, as well as decreased antioxidant capacity [18,19]. The involvement of reactive oxygen intermediates in hypertension is also suggested by the increased level of lipid peroxides and decreased concentrations of antioxidant α-tocopherol in plasma of essential hypertensive patients [20].

The results of the present study indicating that the SBP levels were significantly higher (Table 2) in hypertensive patients before treatment compared with HVs. Olmesartan significantly reduced SBP than labetalol treated group. Labetalol has also shown a significant decrease in SBP, but it is less significant than olmesartan in reducing SBP.

DBP is controlled by renin-AT system which causes the peripheral resistance by vasoconstriction. In the overall population, mean DBP increases progressively throughout adult life in men and women. Throughout the adult life, men have a slightly higher mean DBP than women.

In this study, DBP levels were significantly higher in hypertensive patients without antihypertensive treatment compared with healthy subjects. Olmesartan treated group had shown more significant results in reducing DBP than labetalol treated group.

Antioxidants reduce the prevalence of diseases, however, more human studies are required to establish the efficacy and safety of these agents in chronic or acute oxidative stress-related diseases [21], e.g. CVDs. Free radicals can damage arteries and can induce atherosclerosis by triggering fatty streaks resulting in atheroma; hypertension occurs due to impair of NO production. The antioxidants can prevent some of these processes. Several factors such as low diet intake, nutrients malabsorption, and scarce nutrient release from the liver and an inadequate availability of carriers may influence circulating antioxidant concentrations [22]. Olmesartan protects the vascular endothelium against free radical-induced functional injury [23].

In this study, the TAS levels were found to be significantly reduced (Fig. 1) in all hypertensive patients before antihypertensive treatment compared with HVs. TAS levels have been significantly increased with clinical improvement in olmesartan treated group ($p<0.05$) as compared to labetalol treated group.

GSH peroxidase has a major role in the prevention of oxidative stress; it is also be an important antiatherogenic antioxidant. GSH is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) present in mast cells, where it functions as an antioxidant protecting cells from toxic effects of ROS [24]. GSH peroxidase deficiency leads to endothelial dysfunction combined with structural vascular abnormalities such as collagen deposition surrounding the coronary arteries [25]. Chronic vascular oxidative stress promotes immune activation and leads to vascular stiffening, ultimately resulting in renal dysfunction and hypertension, according to the findings of a recent study [26]. GSH can regulate immune cell function. In this study GSH levels were significantly low in all untreated hypertensive patients than in treated patients (Fig. 1).

Considering the various data and reports about the presence of oxidative stress in hypertension, it remains unclear whether oxidative stress is the cause/consequence of hypertension. Hypertension is joined with adhesion of leukocytes, accumulation of macrophages, migration, and proliferation of vascular smooth muscle cells into blood vessel intima [27]. During these events, a large quantity of free radicals is produced. The sources of free radicals can be also the enzymes such as NADPH oxidase and xanthine oxidase, activated in response to a salt-rich diet [28]. Our results show that if BP is not drug and diet controlled a significant production of free radicals and lipid peroxidation occur. These findings are in accordance with the study of Wen *et al*., [29] who showed an increase of TBARS concentration in serum of patients with essential hypertension and reduced concentration of ascorbate in plasma. MDA concentration in plasma of the labetalol treated group is significantly reduced related to the olmesartan treated group. MDA levels were found to be higher in all untreated hypertensive patients compared with HVs (Fig. 2).

AST is an enzyme found in heart muscle, liver cells, skeletal muscle, and kidneys. Injury to these tissues results in the leakage of the enzyme into the blood. No significant changes were observed in both the treated groups. ALT is found in a variety of tissues but is mainly present in liver; increased levels are found in hepatitis, cirrhosis obstructive jaundice, and other liver diseases. In this study, no significant change in AST and ALT was observed with the both of the drugs (Table 2).

Hypertension associated with long-standing infusion of AT II is linked to the upregulation of vascular p22phox messenger RNA, a constituent of the oxidative enzyme NAD(P)H oxidase [30]. The AT II receptor-dependent activation of NAD(P)H oxidase is linked with increased formation of the oxidant superoxide anion (O_2^-). Superoxide readily reacts with NO to form the peroxynitrite ($ONOO^-$) oxidant. A reduction in NO bioactivity may thus give another mechanism to explain the enhanced vasoconstrictor effect to AT II in hypertension [31]. It has been shown that AT1 antagonists and AT converting enzyme (ACE) inhibitors limit oxidative reactions in the vasculature by blocking the activation of NAD(P)H oxidase [32]. These findings have led to the hypothesis that the AT1s antagonists and ACE inhibitors may have clinically important vasoprotective effects beyond lowering BP. Our results are also in accordance with these findings.

It has been reported that antihypertensive drugs, reduce production of ROS, which is confirmed in our and also in other studies [33,34]. In the present study, it was observed that the GSH and TAS (both enzymatic and non-enzymatic) was significantly decreased in hypertensive patients as compared to HVs indicate that patients were unable to produce adequate amounts of antioxidants to cope up with the augmented oxidative stress in them. The decrease was more evident in untreated patients implying

that the antioxidants were nearly completely utilized to scavenge the superoxide free radicals.

CONCLUSION

The strength of the work lies in the fact that alterations in the levels of GSH, TAS, and MDA before and after treatment. The results clearly indicate that there was a significant decrease in GSH and TAS in untreated patients and a significant increase in the same after treatment. Lipid peroxidation decreased after drug treatment. Hence, it appears that antioxidant supplementation is warranted to protect from ROS attacks and the drugs such as olmesartan and labetalol were showing the significant protection against oxidative stress which can significantly reduce BP and prevent possible complications in hypertensive patients.

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