

Original Article

COMPARATIVE STUDIES OF EFFICACY AND EFFECTS ON OXIDATIVE STRESS OF AMLODIPINE AND RAMIPRIL IN THE HYPERTENSIVE PATIENTS OF NORTH EAST INDIA

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ABSTRACT

Objective: To compare the efficacy and the effect of the oxidative stress of Calcium Channel Blocker (CCB), Amlodipine and Angiotensin Converting Enzyme (ACE) inhibitor, Ramipril in the hypertensive patients of North East India.

Methods: It is a prospective randomized controlled parallel study of the clinically used antihypertensive drug. Total 75 subjects were included in the study. Out of 75 subjects, 25 numbers were normal healthy subjects and remaining 50 were hypertensive patients. The patients were randomized and divided into two groups; Amlodipine treated group (n=25) and Ramipril treated group (n=25). Amlodipine group received Amlodipine (5-10) mg/day and Ramipril group received Ramipril daily (5-10) mg. All patients were newly detected hypertensive subjects suffering from essential hypertension without any prior antihypertensive therapy. Patients included were either sex (male/female) in age group of 20-65 y with normal fasting blood sugar level (FBS) and free from any other diseases. All groups was subjected to measurement of blood pressure, estimation of blood parameters like FBS, serum lipid profiles, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate (SGOT) before antihypertensive treatment. The height, age, weight, health status and lifestyle pattern of patients were recorded. The patients were followed up during 8 w of antihypertensive treatment. Serum levels of glutathione (GSH), total antioxidant (TAS), superoxide dismutase (SOD) and malondialdehyde (MDA) levels were estimated before and after 8 w of antihypertensive therapy.

Results: The present study indicated that both amlodipine and ramipril have significant effects on normalizing systolic and diastolic blood pressure (BP) after 8 w of treatment. Ramipril significantly reduced malondialdehyde (MDA) level and increased the serum levels of GSH, TAS and SOD during antihypertensive treatment. Ramipril reduces oxidative stress because of its antioxidant activity during antihypertensive therapy.

Conclusion: Amlodipine and Ramipril are effective antihypertensive drugs. ACE inhibitor (Ramipril) has additional benefit of reducing oxidative stress thereby restoring the endothelial dysfunction in the hypertensive patient.

Keywords: Hypertension, Serum levels, Amlodipine, Ramipril, North East patients.

INTRODUCTION

Hypertension is the leading cause of cardiovascular diseases in India and worldwide [1]. Cardiovascular diseases account for a large proportion of deaths and disability all over the world. It has been predicted that by the year 2020, there will be increased by almost 75% in the global cardiovascular disease burden [2]. Hypertension is a common disorder if not effectively treated, results in a greatly increased probability of coronary thrombosis, renal failure, and stroke [3]. Hypertension is an independent risk factor for both coronary heart disease and stroke. High blood pressure is an important public health problem in India. High levels of superoxide anion, the consequent accumulation of hydrogen peroxide and diminished NO bioavailability play a critical role in the modulation of vascular remodeling [4, 5]. The reaction product between superoxide and NO constitutes a strong oxidant molecule which is able to oxidized protein, lipids, and nucleic acid and causing cell damage. These pathological processes are associated with hypertension because of narrowing arterial lumen, consequently to increase peripheral resistance and increase blood pressure (BP).

Renin release from the kidney cortex is stimulated by reduced renal arterial pressure. Renin acts on angiotensinogen to split off the inactive decapeptide, angiotensin-I, which is then converted to angiotensin-II by endothelial angiotensin-converting enzyme [ACE]. Angiotensin-II is a vasoconstrictor and has sodium retention activity. So, peripheral resistance is increased. The ACE inhibitors block the ACE enzyme therefore, reduced peripheral resistance.

Amlodipine is a newer second generation CCB and they have little interaction with other cardiovascular drugs. The intercellular concentration of calcium plays an important role in maintaining the tone of smooth muscle and in the contraction of the myocardium.

Calcium enters muscle cells through special voltage sensitive calcium channels. This triggers the release of calcium from the sarcoplasmic reticulum and mitochondria, which further increases the cytosolic level of calcium. Calcium channel antagonists block the inward movement of calcium by binding to L-type calcium channels in the heart and smooth muscle of coronary as well as peripheral vasculature. These cause the vascular smooth muscle to relax, dilating mainly arterioles.

Hypertension is a disease which continuously increasing in the population in India and worldwide. The study shows that genetically Indians are prone to develop hypertension much earlier than the western countries [6]. There is a diverse ethnic population with different lifestyle in North East Region. A very few studies are available about the prevalence of hypertension and control of hypertension in this northeast India [7, 8]. This present study was undertaken to investigate the efficacy of antihypertensive drug suitable for the patient suffering from essential hypertension of this region. Antihypertensive drug should have better efficacy and minimum side effect. The objective of the present study was to compare the efficacy of currently used antihypertensive drugs in the hypertensive patients of this region and also to investigate the effect of such agents on antioxidant status, serum lipid profiles and liver enzymes. Such clinical studies are very important to access the safety as well as better patient compliance.

MATERIALS AND METHODS

Study design

It is a prospective randomized controlled parallel study of the clinically used antihypertensive drug. This study was conducted in the Department of Pharmaceutical Sciences, Dibrugarh University,

Assam and Department of medicine, Assam Medical College & Hospital, Dibrugarh, Assam. The study was conducted after getting approved by the Institutional Ethical Committee; Assam Medical College with the approval No.09/IP/475 dated 20.04.2009.

Population

This study population included male and female hypertensive patients in the age group of 20-70 y from the OPD of Assam Medical College & Hospital, Dibrugarh, Assam. The following data was obtained for each participant.

1. Demographic data (weight, height, age, ethnicity)
2. Taking history of patient
3. Pre-existing medical conditions (history of hypertension, diabetes, or other chronic conditions)
4. Social History (smoking, alcohol consumption, food habit, occupation)

The health information and demographic were collected. Past medical history, social history and pre-existing medical conditions data were self-reported by the study participants. These data were also verified against information from the participant's medical records to ensure the accuracy of the data.

The present study comprised of three groups. One group was healthy subject and other two groups were essential hypertensive patient groups. This study included 75 subjects. Out of 75 subjects 25 were normal human healthy volunteers (NHV) without history of smoking, alcoholism any other diseases taken as control and 50 subjects were untreated hypertensive patients without any other diseases. Out of the 50 subjects, 25 subjects were treated with Ramipril 5 mg/day and rest 25 subjects were treated with Amlodipine 5 mg/day. The blood pressure was measured in lying down or sitting position at ease and then 4 ml blood was collected with prior consent from the patient. The samples were analyzed for estimation of serum level of lipid profile, SGOT, SGPT and total antioxidant status as reported methods. Patients were again checked up after 8 w during antihypertensive therapy and above tests were repeated.

Method of blood collection and processing

Venous blood was collected from the subjects under aseptic condition by venipuncture using 5 ml sterile disposable syringe and needle. About 4 ml of blood was collected. Serum was separated by centrifugation at 2000 rpm for 10 min at room temperature. The samples were stored at 4 °C before analysis and samples were analyzed on the same day of collection [9].

Blood pressure measurement

Blood pressure was recorded by the auscultatory method by using sphygmomanometer in the left arm in sitting position or lying down position.

Estimation of superoxide dismutase

The SOD enzyme level was measured in erythrocytes using photo-oxidation method [10, 11]. 3 ml packed blood cells were lysed by the addition of equal volumes of cold de-ionized water. Hemoglobin was precipitated by the addition of chloroform: ethanol (1:5). This was diluted with 500 µl water, centrifuge at 3000 rpm for 15 min. The supernatant containing SOD was used for measurement of its activity. 0.88 ml riboflavin solution (1.3×10^{-5} M) in 0.01 M potassium phosphate buffer pH 7.5 was added to 66 µl O-dianisidine and 100 µl of clear supernatant, optical density was measured at 460 nm. Then cuvette containing reaction mixture was transferred to the illuminating box for 4 min. The optical density was re-measured. The change in optical density was determined. The SOD content was calculated from the standard graph.

Estimation of glutathione

0.5 ml of 5% tricarboxylic acid solution was added to 0.5 ml of citrated blood to precipitate the proteins and centrifuged at 3000 rpm for 20 min. To 0.1 ml of supernatant, 1 ml of sodium

phosphate buffer (pH 8) and 0.5 ml of 5, 5-Dithiobis-2-nitro benzoic acid (DTNB) (39.6 mg in 100 ml of 1 % sodium citrate) were added. The absorbance of the yellow color developed was measured at 412 nm [12].

Total antioxidant status

Total antioxidant status in serum was determined by the method of a stable, free radical, 1, 1-diphenyl-β-picrylhydrazyl (DPPH) at a concentration of 0.2 mM in methanol [13].

Lipid peroxidation activity

The amount of lipid peroxidation products present in the serum samples were estimated by the thiobarbituric acid reactive substances (TBARS) method. The reactive malondialdehyde products were measured by using spectrophotometer method [14].

Estimation of SGOT and SGPT levels

SGOT and SGPT levels were assayed by a colorimetric method [15].

Statistical analysis

All the values were expressed as mean±SEM. The data were analyzed using ANOVA, Newman Koel method. In tests, the criteria for statistical significance were* P<0.05, **P<0.01 and***P<0.001.

RESULTS

Demographic data and clinical characteristics of pretreated ramipril and amlodipine groups were shown in table 1. Demographic data and clinical characteristics were not significantly differing from pretreated ramipril group from amlodipine pretreated group.

Levels of SBP were much higher in all pretreated patient groups. After antihypertensive treatment for a period of 8 w, SBP were significantly reduced both antihypertensive drugs. The SBP were reduced more significantly (P<0.001) from 168.4±4.80 mmHg to 146.8±3.44 mmHg in the ramipril-treated group and 160.7±3.38 mmHg to 136±1.84 mmHg in amlodipine-treated group (table 2).

DBP significantly reduced after antihypertensive treatment as compared to pretreatment values. DBP reduced significantly (P<0.001 from 95.9±1.64 mmHg to 82.95±2.66 mmHg in ramipril-treated group and 95.25±1.14 mmHg to 83.0±0.74 mmHg in amlodipine-treated group (table 2).

HDL levels were significantly (P<0.05) increased from 33.92±1.61 mg/dl to 43.16±1.37 mg/dl after amlodipine treatment. Serum levels of total cholesterol, triglyceride, HDL and LDL were not significantly altered during the treatment with ramipril (table 3).

SOD levels were very low in pretreated hypertensive groups. This indicates the antioxidant levels of SOD were utilized to scavenge the superoxide radicals in hypertensive condition. The SOD levels were significantly (P<0.001) increased during treatment of ramipril. The level of SOD was increased with clinical improvement ramipril treated group from 16.15±1.80 IU/ml to 33.12±2.66 IU/ml (table 4).

Total antioxidant levels were low in pretreated patient groups but gradually increased with clinical improvement during treatment. Total antioxidant levels were significantly (P<0.001) increased from 24.07±2.02 nmol/ml to 65.13±3.01 nmol/ml after treatment with ramipril (Table.4).

GSH levels were very low in all untreated hypertensive patients. GSH levels were significantly (P<0.01) increased from 235.9±13.07 nmol/ml to 472.49±21.80 nmol/ml after ramipril therapy for a period of eight weeks (table 4).

MDA levels were significantly decreased during ramipril treatment (P<0.05) as compared to pretreatment values. It remained unchanged in case of amlodipine therapy.

DISCUSSION

High blood pressure is one of the important public health problems in India. If remain untreated, sustained hypertension is a risk factor for the development of cardiovascular diseases. Oxidative stress mediated by free radical reactive oxygen species ROS. It is a primary

or secondary cause of many chronic diseases, heart failure, stroke, coronary heart disease along with the impairment of renal function [16, 17]. The serious complications are not only the consequences of increased blood pressure but also related to the arterial endothelial dysfunction and accelerate the process of hypertension.

SBP is controlled by the stroke volume of the heart and the stiffness of the arterial vessels. BP varies from moment to moment with respiration, exercise, meals, alcohol, tobacco, bladder distension, temperature and pain. It is also influenced by circadian rhythm, age and race. In the overall populations, mean SBP increases progressively throughout adult life in men and women. The present study showed that ramipril comparatively a better antihypertensive agent due to its dual mode of action. BP was also reduced during 8 w of amlodipine treatment (table 2).

SOD is one of the important free radical scavenging enzymes present in the human body. This SOD catalyzes the dismutation of superoxide radicals to O₂ and H₂O₂. Study results showed that the enzyme was decreased in the hypertensive stage. It indicated that the enzyme level was nearly completely utilized to scavenge the superoxide radicals. Prevention of tissue damage due to intracellular superoxide requires elevation of intracellular SOD. ACE inhibitor increases antioxidant enzyme activity [18, 19]. The role of antioxidants in the cardio-vascular diseases is based on the premise that free radicals can injure arteries and also induce atherosclerosis by inducing fatty streaks resulting in atheroma. Several factors such as low food intake, nutrients mal-absorption and inadequate nutrient release from the liver, acute phase response, infection and an inadequate availability of carrier molecules may influence circulating antioxidant concentrations [20]. The previous study indicated that ramiprilat protects the vascular endothelium against free radical induced functional injury [21]. Present study result demonstrated that the total antioxidant levels were found to be significantly reduced in all hypertensive patients without antihypertensive treatment. Total antioxidant activity was significantly (P<0.001) increased with clinical improvement during

ramipril treatment and improved antioxidant activity can correct the restoration of NO bioavailability and endothelial dysfunction.

Glutathione peroxidase (GSH) appears to have a major role in the prevention of oxidative stress; it may also be an important anti-atherogenic antioxidant. Glutathione is a tripeptide comprised of glutamate, cysteine, and glycine. GSH is present in mast cells where it functions as an antioxidant and protects cells from toxic effects of ROS. Glutathione peroxidase deficiency results in endothelial dysfunction combined with structural vascular abnormalities, such as increased peri-adventitial inflammation and collagen deposition surrounding the coronary arteries. Glutathione has been regulated by immune cell function. Glutathione peroxidase with 5-lipoxygenase might constitute a protective function of the enzyme, in addition to its antioxidant activity [22]. Enalapril and captopril enhance glutathione-dependent antioxidant defenses [23]. In the present study, glutathione were estimated in hypertensive patients. GSH levels were very low in all untreated hypertensive patients. But the glutathione levels were significantly increased (P<0.01) in the ramipril-treated group as compared to pretreatment values (table 4).

Hypertension is a state of increased free radical activity which injures the endothelium conjugated dienes and lipid peroxides. MDA is an end product of fatty acid oxidation and is often used as an indicator of lipid peroxidation. Our present study result demonstrates that MDA levels were higher in all hypertensive patients without any treatment when compared to healthy subjects. MDA levels were decreased during ramipril treatment as compared to pretreatment values. It may result to beneficial effects in the restoration of NO bioavailability and endothelial function.

Oxidation of LDL can injure myocardium during reperfusion in myocardial infarction. HDL is the smallest lipoproteins. HDL particles synthesize both from the liver and intestine. HDL transport cholesterol from the peripheral tissues to the liver for excretion. The measurement of HDL cholesterol provides valuable information for the assessment of coronary heart diseases. Amlodipine significantly increases the HDL levels of the hypertensive patients after treatment (table 3).

Table 1: It shows the demographic data of pretreatment groups of ramipril and amlodipine

Demographic data	NHV	Ramipril treated	Amlodipine treated
Average age (years)	42.3±1.93	49.3±2.45	46.44±3.03
Average height (cm)	151.4±1.45	147.9±1.63	158.65±2.29
Average weight (kg)	57.20±2.79	53.05±2.87	48.44±1.33

All the values were expressed as mean±SEM (n=25).

Table 2: It shows the effect of ramipril and amlodipine on the systolic blood pressure, diastolic blood pressure and fasting blood glucose levels of hypertensive subjects

Parameter	NHV	Ramipril treated		Amlodipine treated	
		Pre	After	Pre	After
SBP(mmHg)	121.0±0.42	168.4±4.80	146.8±3.44***	160.7±3.38	136±1.84***
DBP(mmHg)	80.55±0.41	95.9±1.64	83.0±0.74***	98.96±1.021	87.04±0.95***
FBS (mg/dl)	86.55±2.18	95.35±2.13	91.0±1.36	86.28±2.10	88.23±1.02

All the values were expressed as mean±SEM (n=25). Statistical significance was ***P<0.00

Table 3: It shows the effect of ramipril and amlodipine on the total cholesterol, triglycerides, HDL, LDL, SGOT and SGPT of hypertensive subjects

Parameter	NHV	Ramipril treated		Amlodipine treated	
		Pre	after	Pre	After
TC (mg/dl)	145.9±2.34	160.25±2.22	157.05±1.61	160.88±5.64	158.9±2.54
Trig (mg/dl)	149.6±1.87	150.65±3.17	151.6±2.68	146.68±3.46	147.7±3.02
HDL(mg/dl)	37.55±0.52	36.25±0.79	39.95±0.69	33.92±1.61	43.16±1.37*
LDL(mg/dl)	78.44±2.07	93.34±1.94	86.09±1.62	88.40±1.33	86.81±1.63
SGOT(IU/l)	25.35±1.08	32.35±1.56	27.50±1.17	27.48±1.57	29.4±1.82
SGPT(IU/l)	26.75±1.42	33.25±1.48	29.20±1.15	28.64±1.67	30.5±1.63

All the values were expressed as mean±SEM (n=25), *P<0.05

Table 4: It shows the effect of ramipril and amlodipine on the SOD, TAS, GSH and MDA levels of hypertensive subjects

Parameter	NHV	Ramipril treated		Amlodipine treated	
		Pre	After	Pre	After
SOD(IU/ml)	69.38±2.47	16.15±1.80	33.12±2.66***	16.93±1.39	22.92±1.40
TAS(nmol/ml)	92.96±3.78	24.07±2.02	65.13±3.01***	27.93±1.16	35.70±1.07
GSH(nmol/ml)	636.65±63.51	235.9±13.07	472.49±21.80**	327.68±17.68	374.48±16.98
MDA (nmol/ml/hr)	3.36±0.22	6.948±1.07	3.72±0.714*	6.66±0.52	5.05±0.49

All the values were expressed as mean±SEM (n=25). Statistical significance were *P<0.05, **P<0.01 and ***P<0.001.

CONCLUSION

Both amlodipine and ramipril are effective antihypertensive drugs to normalize SBP and DBP. ACE inhibitor, Ramipril has a significant effect on reducing oxidative stress. Ramipril seems to be more effective in the management of essential hypertension because of improving endothelial dysfunction for its antioxidant activity. Antioxidants may decrease the incidence of disease. However, more human studies are required to establish the efficacy and safety of these agents.

CONFLICT OF INTERESTS

There is no conflict of interest.

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