Comparative studies of efficacy and effect on oxidative stress of atenolol and candesartan in the hypertensive patients of North East India

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Abstract

The present study was carried out to investigate the comparative efficacy of β- blockers and Angiotensin receptor blockers. This study was carried out using Atenolol and candesartan which are frequently prescribing to the hypertensive patients of the North Eastern part of India. An attempt was made to evaluate the comparative efficacy and estimated the serum level of superoxide dismutase (SOD), serum glutathione (GSH), lipid peroxidation product, malondialdehyde (MDA) and total antioxidant status (TAS). The study consisted of 60 subjects (20 volunteers and 40 patients). 40 patients were randomized and divided into two groups to receive either Atenolol (50-100mg) or Candesartan (8-16mg) respectively. Patients were examined for blood pressure, fasting blood sugar level (FBS), lipid profiles, serum levels such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate (SGOT) and also estimated enzymatic / non enzymatic antioxidant levels before and after treatment of antihypertensive drugs. The result of the present study shows that both the drugs have significant effect ($P<0.001$) on decreasing systolic blood pressure and diastolic blood pressure. The level of MDA was decreased significantly ($p<0.01$) with Candesartan.The levels of SOD, GSH and TAS were significantly ($p<0.01$) increased after Candesartan therapy. By this study result, it was found that both the drugs possess similar antihypertensive action whereas Candesartan possesses additional benefit in reducing oxidative stress.

Key words: Comparative efficacy, hypertensive status, oxidative stress, serum levels.

1. Introduction

Hypertension is the leading cause of cardiovascular diseases in India and worldwide **[1].** As per 2008 WHO report Global Burden of disease study there were 17.3 million deaths from cardiovascular diseases, representing 30% of all global deaths. Of these deaths, an estimated 7.3 million were due to coronary heart disease and 6.2 million were due to stroke in economically developing countries. However, according to the 1996 health survey, 23% of adults in England had high blood pressure and about one-quarter of all cardiovascular disease deaths occurred in persons who were less than 70 years of age in the developed world. The number of people, who die from CVDs, mainly from heart disease and stroke, will increase to reach 23.3 million by 2030[**2, 3]**. Hypertension is a common disorder if not effectively treated results in a greatly increased probability of coronary thrombosis, renal failure, and stroke **[4].**Hypertension is an independent risk factor for both coronary heart disease and stroke. High blood pressure is an important public health problem in India. In India heart disease occurs 10-15 years earlier than in the west (Simon 2004**) [5].** Over production of oxygen free radicals, which is mediated by super oxide occurs in human hypertension. There are many important enzymatic sources of superoxide production including NADPH oxidase, xanthine oxidase and uncoupled nitric oxide synthase [6].The major vascular reactive oxygen species (ROS) is superoxide anion which inactivates nitric oxide (NO) thus impairing vascular relaxation. High level of superoxide anion the consequent accumulation of hydrogen peroxide and diminished NO bioavailability play a critical role in the modulation of vascular remodeling [7,8]**.**The reaction product between superoxide and NO, peroxynitrite ,constitutes a strong oxidant molecule which is able to oxidized protein, lipids and nucleic acid, causing cell damage. These pathological processes are associated with hypertension because of narrowing arterial lumen, consequently to increase peripheral resistance and increase blood pressure**.**

The acute stimulation of sympathetic nerve system control centers in human activates the heart and causes arteriolar and venous constriction resulting increase in systemic blood pressure **[9].** An excess of renin and angiotensin activity could interact with the sympathetic nerve system over activity in early hypertensive. β-Blockers block the action of endogenous catecholamine on β- adrenergic receptors. Atenolol is a competitive

specific cardio selective β1- antagonist, competitively block β1 receptor in myocardium decreases heart rate and myocardial contraction resulting decreases cardiac output. 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.Angiotensin receptor blockers are nonpeptide; orally active compounds that are extremely potent competitive antagonists of the angiotensin type-1 receptor thereby reduced peripheral resistance.Candesartan is a newer AT1 blocker widely prescribing antihypertensive drug.

2. Materials and methods:

*Study design***.** This study was conducted in the department of Pharmaceutical Sciences, Dibrugarh University, Assam and department of medicine, Assam Medical College & hospital, Dibrugarh, Assam.

The present study comprised of three groups. One group was healthy subject and two groups of essential hypertensive patients. This study included 60 subjects. Out of 60 subjects 20 were normal healthy subjects(NHS) without history of smoking, alcoholism any other diseases taken as control and 40 subjects were untreated hypertensive patient without any other diseases, Out of 40 subjects were randomized,20 subjects were treated with atenolol 50-100 mg and rest 20 subjects treated with candesartan 8-16 mg. The study was conducted after getting approval from the Institutional Ethics Committee. The blood pressure was measured in laying or sitting position at ease, then 4 ml blood was collected with prior consent from patient. The samples were analyzed for estimation of fasting blood sugar, lipid profiles, serum level of SGOT, SGPT and antioxidant status as reported methods. Patient was again checked up and blood samples were collected after 8 weeks during antihypertensive therapy and following parameters were estimated..

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 all the samples were analyzed on the same day of collection [**10].**

Blood pressure measurement: Both systolic blood pressure [SBP] and diastolic blood pressure [DBP] was measured in lying down position with the help of mercury sphyagmamometer.

Estimation of Superoxide dismutase: The enzyme SOD level was measured in erythrocytes using photo-oxidation method [**11, 12].** 3 ml packed blood cells were lysed by the addition of equal volume of cold demonized 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 minutes. The supernatant containing SOD was used for measurement of its activity.

0.88 ml riboflavin solution (1.3×10^{-5} M) in 0.01M 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 above cuvette containing reaction mixture was transferred to illuminating box for 4 min. The optical density was again measured. The change in optical density was determined. The SOD content was calculated from 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 minutes. To 0.1 ml of supernatant,1 ml of sodium phosphate buffer (pH. 8) and 0.5ml of 5-5'(Dithiobis-2-nitro benzoic acid) DTNB (39.6 mg in 100 ml of 1% sodium citrate solution to give a concentration of 1 mM) were added. The absorbance of the yellow color developed was measured at 412 nm [**13**].

Total anti-oxidant status: Total anti-oxidant status in serum was determined by the method of using a stable, free radical, α, α-diphenyl-β-picrylhydrazyl (DPPH) at a concentration of 0.2 mM in methanol [**14**].

Lipid peroxides: The amount of lipid peroxidation products present in the serum samples were estimated by the thiobarbituric acid reactive substances (TBARS) method which measures the malondialdehide (MDA) reactive products by using spectrophotometer method [**15**].

Estimation of SGOT and SGPT: SGOT and SGPT levels were assayed by colorimetric method [**16**].

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***$P<0.001$

3. Results and Discussion

Demographic data and clinical characteristics of pretreated atenolol and candesartan groups were shown in Table 1. Demographic data like age, weight, and height were not significantly differing from atenolol and candesartan groups.

Both Atenolol and Candesartan significantly decreased systolic blood pressure [SBP]. SBP reduced in candesartan group **(**$P<0.001$**)** as compared to before antihypertensive treatment. Diastolic blood pressure was

reduced significantly by the both drug treated groups than pretreatment values, Table 2**.**

Serum levels of triglycerides were significantly increased ($p<0.01$) and HDL levels were decreased ($p<0.05$) after 8 weeks of Atenolol treatment, but there was no significant variation in during treatment with Candesartan. Serum level of SGPT and SGOT were not changed in both the drug treated groups Table 3**.**Total antioxidant levels were significantly increased with Candesartan treatment ($P<0.001$) as compared to pretreatment values. Candesartan increases total antioxidant levels. GSH levels were significantly increased ($P<0.01$) during Candesartan treatment. MDA levels were significantly decreased during Candesartan treatment ($P<0.05$) as compared to pretreatment values. Antioxidant levels were not changed in atenolol therapy Table 4.

High blood pressure is chronic illness, If remain untreated, sustained hypertension is a risk factor for the development of cardiovascular diseases. Oxidative stress mediated by free radicals reactive oxygen species.RNS is a primary or secondary cause of many chronic diseases heart failure, stroke, coronary heart disease along with consequences and the impairment of renal function**[**17,18**].**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.

Antioxidants decrease the incidence of diseases however more human studies are required to establish the efficacy and safety of these agents in various chronic or acute oxidative stress-related diseases e.g. cardiovascular diseases[**19**].The role of antioxidants in CVDs is based on the premise that free radicals can injure arteries, also induce atherosclerosis by inducing fatty streaks resulting in atheroma By oxidation of LDL can injure myocardium during reperfusion in myocardial infarction. Hypertension occurs due to deregulating of nitric oxide production. The antioxidants can prevent most of these above processes. Several factors such as low food intake, nutrients malabsorption and inadequate nutrient release from the liver, acute phase response, infection and an inadequate availability of carrier molecules may influence circulating antioxidant concentrations[**20**].In present study the total antioxidant levels were found to be significantly reduced in all hypertensive patients without antihypertensive treatment compared to NHS. Total antioxidant activity were significantly increased ($P<0.01$) with clinical improvement during Candesartan treatment.

Glutathione peroxidase appears to have a major role in the prevention of oxidative stress; it may also be an important antiatherogenic antioxidant. Glutathione (GSH) is a tripeptide comprised of glutamate, cysteine and glyceine. GSH is present in mast cells, where it functions as an antioxidant protecting cells from toxic effects of[**21**] .Glutathione peroxidase deficiency has endothelial dysfunction combined with structural vascular abnormalities, such as increased periadventitial 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. Present study it was observed thatGHS levels were very low in all untreated hypertensive patients. Glutathione levels were significantly increased ($P<0.01$) during treatment with candesartan but not with atenolol.

Hypertension is a state of increased free-radical activity which oxidative stresses or injuries the endothelium conjugated dienes.Lipid peroxidation is thought to be involved in a number of pathological processes.ROS have been implicated in the pathogenesis of various conditions including cardiovascular diseases, MDA is an end product of fatty acid oxidation, and is often used as an indicator of lipid peroxidation [**22]**. In present demonstrated that MDA levels were found to be higher in all hypertensive patients without antihypertensive treatment compared to healthy subjects.MDA levels were decreased with Candesartan therapy.

Cholesterol is a fatty substance found in blood, bile and brain tissue. It is mainly found in esterifies form. It serves as a precursor of bile acids, steroids and vitamin D [**23**]. Triglycerides are a family of lipids absorbed from the diet and produced endogenously. Measurement of triglycerides is important in the diagnosis and management of hyperlipidaemia. Triglyceride levels were changed significantly in subjects during treated with Atenolol.High density lipoprotein (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. It is a good cholesterol.Atenolol reduced the HDL cholesterol after 8 weeks therapy which may have negative impact in long term antihypertensive treatment.

4. Conclusion:

Both the drugs i.e atenolol and candesartan have shown significant effect on reducing target blood pressure. Candesartan improves antioxidant status during therapy may rectify endothelial dysfunction in vascular system and provide additional benefits to hypertensive patient.

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6. References:

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Table 1: Demographic data: Clinical characteristics of pretreatment groups of atenolol and candesartan

Demographic data	NHV	Atenolol	Candesartan
Age (years)	42.3±.1.93	46.85 ± 2.765	47.35 ± 2.534
Height (cm)	151.4±1.45	154.25 ± 1.525	158.2± 1.167
Weight (kg)	57.20±2.79	60.80 ± 2.086	59.250± 2.998

Table 2: Effect of atenolol and candesartan on the systolic blood pressure, diastolic blood pressure and blood glucose levels of hypertensive subjects

Parameter	NHS	**atenolol**		**candesartan**	
		Pre	after	Pre	after
SBP (mmHg)	121.0±0.42	161.7 ±2.476	132.8 ± 1.41***	165.4 ± 3.71	139.1± 1.69***
DBP (mmHg)	80.55 ±0.41	95.25 ± 1.14	85.0± 0.76***	97.55 ± 1.43	87.30 ±1.42***
FBS (mg/dl)	86.55 ±2.18	95.45 ± 2.13	91.0 ± 1.365	99.55 ±1.86	91.9 ±1.753

Table **3** Effect of atenolol and candesartan on the total cholesterol, Triglycerides, HDL, LDL, SGOT and SGPT of hypertensive subjects:

Parameter	NHS	**atenolol**		**candesartan**	
		Pre	after	Pre	after
TC (mg/dl)	145.9±2.34	159.65± 2.21	166.6 ± 1.74	155.95±2.80	161.95 ± 2.69
Trig (mg/dl)	149.6±1.87	110.9 ±7.32	146.2 ± 8.21**	108.95 ±5.77	114.2 ± 5.98
HDL(mg/dl)	37.55±0.52	29.25 ± 0.823	24.2 ± 0.763*	30.8 ± 0.997	31.95 ± 0.974
LDL(mg/dl)	78.44±2.07	88.34 ± 1.974	86.09 ± 1.628	87.95 ± 2.63	85.76 ± 1.73
SGOT (IU/L)	25.35±1.08	32..35 ±1.56	27.50± 1.17	31.45± 2.002	27.9 ±1.761
SGPT (IU/L)	26.75±1.42	33.25 ± 1.481	25.20± 1.15	31.35 ± 2.08	27.65 ± 1.46

Table: 4 Effect of atenolol and candesartan on the TAS, GTH and MDA levels of hypertensive subjects

Parameter	NHV	**atenolol**		**candesartan**	
		Pre	after	Pre	after
TAS(nM/ml)	92.96 ± 3.78	24.27 ±1.97	26.29 ±1.73	22.88±1.18	49.57 ±1.78**
GSH(nM/ml)	636.65± 63.51	247.79±18.61	307±15.78	224.7± 16.41	415.81 ±15.78**
MDA(nM/ml/hr)	3.36 ± 0.226	6.162 ±0.294	5.35±0.276	6.89± 0.309	4.53 ± 0.305**

Pre-pretreatment

In all tables, the values are expressed as Mean ± SEM of 20 subjects. The criteria for statistical significance were *P<0.05, **P< 0.01 and ***P<0.001.

*P < 0.05 atenolol Vs candesartan

**P < 0.01 atenolol Vs candesartan

**P < 0.01 atenolol Vs candesartan