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CLINICAL COMPARATIVE STUDY FOR THE EFFECT OF ANTIHYPERTENSIVE DRUGS ON SERUM OXIDANT-ANTIOXIDANTS STATUS

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ABSTRACT

The aim of this study was to investigate the effect of antihypertensive drugs on serum oxidant-antioxidants status. Using the lipid peroxidation marker, malondialdehyde (MDA) and preventative antioxidants ceruloplasmin (Cp) and albumin (Alb), in serum of patients with hypertension. Blood samples were obtained from (160) patients treated with different antihypertensive drugs, as well as (40) healthy subjects as a control group. They divided into five groups as the following: control Group:- Included forty healthy subjects aged (40-70 years). ACEIs Group:- Included forty patients with Angiotensin-Converting Enzyme Inhibitors (ACEIs) drugs aged (40-70 years). ARBs Group:- Included forty patients with Angiotensin-II Receptor Blockers (ARBs) drugs aged (40-70 years). β.blockers Group:- Included forty patients with Beta.blockers drugs aged (40-70 years). CCBs Group:- Included forty patients with Calcium Channel Blockers (CCBs) drugs aged (40-70 years). Results: The results show non-significant differences in serum MDA between each of ACEIs and ARBs drugs groups, in comparison with control group ($P \leq 0.05$). While a significant increase in the levels of serum MDA can be observed in two patients groups with β.blockers and CCBs drugs in comparison with control group when compared with groups of ACEIs and ARBs drugs. Ceruloplasmin (CP) also shown significant increase in all patient groups in comparison with control group, as well as significant increase in the levels of serum CP can be observed in β.blockers and CCBs drugs groups in comparison with groups of ACEIs and ARBs drugs, While serum albumin (Alb) levels have non significantly ($P \leq 0.05$) differences in all patients groups.

KEYWORDS: *Hypertension, Antihypertensive drugs, Oxidant-antioxidants Status.*

INTRODUCTION

Hypertension (HT) is defined as an elevated systolic blood pressure (SBP) (achieved during ventricular contraction of the heart), diastolic blood pressure (DBP) (achieved during ventricular dilatation). or both.[1,2]

Hypertension is a term used to describe high blood pressure. Flow of blood is based on the beat of which the heart pumps blood. The pressure of the heart does not stay at the same level at all times. It varies based on activities at a particular point in time. Hypertension occurs as a result to long duration of abnormal pressure of the main arteries.[3]

Generally ACEIs are commonly used in the management of hypertension, cerebrovascular disease, diabetes-associated nephropathy, heart failure and stable coronary heart disease.[4] ACE inhibitors have been shown to improve the vascular endothelial function.[4,5]

ACE inhibitors inhibit the conversion of (angiotensin I) to (angiotensin II), and the degradation of bradykinin. This inhibition reduces the level of vasoconstrictor peptide angiotensin II, and increases the level of bradykinin in tissues.[6] They reduce the degradation of bradykinin and therefore increase the circulatory bradykinin levels and hence are contraindicated in renal artery stenosis, Captopril, enalapril, fosinopril, lisinopril, ramipril etc. are some of the commonly used ACE inhibitors.[7]

On the other hand ARBs are more specific than ACE inhibitors, since they affect the last step of the renin-angiotensin cascade. There is no bradykinin potentiation in this case. Candesartan, eprosartan, irbesartan, losartan, valsartan etc. are some of the AT1 blockers.[8]
AT1 blockers mechanism of action, inhibit RAAS by selective inhibition of angiotensin II by competitive antagonism of the angiotensin II receptors, specifically blocking the AT-1 receptors. has been speculated to reduce adverse effects and possibly improve clinical efficacy. ARBs displace angiotensin II from the angiotensin I receptor and produce their blood pressure lowering effects by antagonizing angiotensin II–induced vasocon-striction, aldosterone release, catecholamine release, arginine vasopressin release, water intake, and hypertrophic response.[9,10]

Whereas Beta blockers such as (Atenolol, metoprolol, nadolol, oxprenolol, pindolol, propranolol etc). have been used as a first line treatment of hypertension, since last four decades. Apart from anti-hypertensive action, they also have anti-anginal and anti-arrythmic

actions which effectively reduce coronary artery disease and ultimately death.[11,12] Although their specific mechanisms of action are incompletely understood, β-blockers most likely lower blood pressure (BP) and provide target-organ protection by several different mechanisms, including inhibition of the renin-angiotensin system by decreasing renin release by the juxtaglomerular cells of the kidney, central inhibition of sympathetic nervous system (SNS) outflow, and slowing of heart rate with a decrease in cardiac output.[13]

Calcium channel blockers Commonly, they are classified according to chemical structure and site of action as: dihydropyridines and non-dihydropyridines. Dihydropyridines (selective to vascular tissues); include amlodipine, felodipine, nifedipine etc. and nondihydropyridines (relatively selective for myocardium); include diltiazem and verapamil. The efficacy and safety of dihydropyridine calcium channel blockers are well established, but there are also studies supporting the benefits of non-dihydropyridine calcium channel blockers.[14,15,16] Several types of calcium channels occur, with a number of classes of blockers, but almost all of them preferentially or exclusively block the L-type voltage-gated calcium channel.[17]

Lipid Peroxidation (LPO)

Lipid peroxidation or reaction of oxygen with unsaturated lipids produces a wide variety of oxidation products. The main primary products of lipid peroxidation are lipid hydro peroxides (LOOH). Among the many different aldehydes which can be formed as secondary products during lipid peroxidation, malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE) and other aldehyde.[18,19] MDA the products of lipid peroxidation are easily detected in the blood plasma and have been used as a measure of oxidative stress. Approximately 20% of end- products derived from oxidative damage of lipids in vitro are MDA.[20] MDA formation may be the result of the suppression of non-enzymatic lipid peroxidation.[21] Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants are effective because they are willing to give up their own electrons to free radicals which start a chain reaction that damages cells. When a free radical gains the electron from an antioxidant, it no longer needs to attack the cell and the chain reaction of oxidation is broken.[22] To protect the cells and organ system of the body against free radicals, humans have evolved complex antioxidant protection system. It involves a variety of components, both endogenous (such as Albumin and Ceruloplasmin) and exogenous (such as Vitamin C and Vitamin E..etc) in organ.[23]

Ceruloplasmin (Cp) is a glycoprotein with a polypeptide chain including 1046 amino acid residues.[24] Cp is a circulating multicopper oxidase that contains 95% of copper in the plasma[25], Cp is synthesized mainly in the liver as a single chain polypeptide, and after the incorporation of six atoms of copper in the biosynthetic pathway, it is secreted into the plasma as an α2 –glycoprotein.[26] The molecular weight of human ceruloplasmin is described to be 151 KDa.[27]

Albumin(Alb) is the main protein of human plasma.[28] It consists of a single polypeptide chain of 585 amino acids with a molecular weight of 66,500 Da[29], It accounts for about 60% of the total plasma proteins, the liver is the primary site of albumin synthesis, and small amounts of albumin may be synthesized in the mammary glands and skeletal muscle.[30,31]

Patients and Method

Design of Study

This study conducted at AL-Hussein Teaching Hospital in Thi-Qar, Biochemistry Laboratory in College of Science, at the period between 10/9/2014 to 20/2/2015. It included (200) cases, (40) control and (160) patients.

Table (1): Data of patients and controls groups

Groups	NO.
Patients	160
Controls	40

There were (200) male and female subjects, control and hypertension patients with Antihypertensive treatment aged (40-70) years were included in this study. they divided into five groups as the following.

Contrl group:- included forty (40) healthy subjects aged (40-70).

ACEIs group:- include forty (40) patients treated with Angiotensin-Converting Enzyme Inhibitors drugs aged (40-70).

ARBs group:- included forty (40) patients treated with Angiotensin-II Receptor Blockers drugs aged (40-70).

ß.blockers group:- included forty (40) patients treated with Beta.blockers drugs aged (40-70).

CGBs group:- included forty (40) patients treated with Calcium channel blockers drugs aged (40-70).

Collection of Blood Samples

About (5mL) of blood samples from out treated hypertension patients and controls were taken and allowed to clot at room temperature in empty disposable tubes centrifuge to separate it in the centrifuge at 3000 rotor per minute (rpm)for 10 min,the serum samples were separated and stored at (-20ºC) for later measurement of biochemical parameters, unless used immediately.

Biochemical Parameters

Lipid peroxidation Marker (Serum MDA)

Determination of serum MDA level that consider as a lipid peroxidation marker were performed according to the method of **Muslih.**[32] MDA concentrations were calculated, using the molar extinction coefficient of MDA ($\mathcal{E}_{MDA}$) equal to 1.56 x105 mol-1. Cm-1.[33] MDA formed from breakdown of polyunsaturated fatty acid, serves as a convenient index of peroxidation reaction.

Serum Antioxidants

Serum Cp concentration was measured by the method of Menden et al., 1977[34] which using the extinction coefficient of Cp (ε Cp) equal to (0.68) to calculate it concentration. The bromocresol green (BCG) method, colorimetric method, is the simplest technique which have been developed to determine Alb concentration.[35]

Statistical Analysis

Statistical analysis was done using the software SPSS version 17.0; the results were expressed as mean ± standard deviation (mean ± SD). One way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant.

RESULT AND DISCUSSION

Lipid Peroxidation Status (Malondialdehyde)

Table (2) showed non-significant differences in serum MDA between each of ACEIs and ARBs drugs groups, in comparison with control group ($P \leq 0.05$). While a significant increase in the levels of serum MDA can be observed in two patients groups with β.blockers and CCBs drugs in comparison with control group also compared with groups of ACEIs and ARBs drugs. This may be due to that ACEIs and ARBs drugs act indirectly by inhibiting the

renin angiotensin-aldosterone system (RAAS), an important source of ROS in the endothelium.[36,37,38,39]

In the same table, there were no significant differences between CCBs group and ACEIs group but was still significantly higher than the control group ($P \leq 0.05$). In fact calcium channel blockers drugs appear weak antioxidant capacity, by increasing the availability of nitric oxide (NO) in the endothelial cell and increasing the expression of SOD in vascular smooth muscle cells.[40]

Generally hypertension is associated with oxidative stress due to impaired oxidant/antioxidant status. The oxidative stress in hypertension arises when ROS exceeds the level of antioxidant defense system.[41,42] Endothelial dysfunction causes defect in the vasodilator which inactivate nitric oxide (NO) and causes oxidative stress.[43] Rather a constant increase in ROS increases blood pressure and vice versa.[44]

Increased xanthine oxidase activity has also been observed in hypertension.[45] The decreased activity of superoxide dismutase and catalase and reduced levels of ROS scavengers (vitamin E, Glutathione) also may be contributors of oxidative stress in hypertension.[46] Considerable study demonstrated that reactive oxygen species (ROS) play an important pathophysiological role in the development of hypertension, this is due to excessive superoxide formation, decreased nitric oxide bioavailability in the vasculature and kidney, and ROS- mediate cardiovascular remodeling.[47]

Most of the essential hypertensive patients showed as increased activity of rennin angiotensin system (RAS).[48] which can leads to increased production of Ang II from Ang I. Ang-II that activate the membrane-bound NADH and NADPH oxidases.[49] The increased vascular activity of NADH and NADPH oxidase enhances the production of reactive oxygen species (ROS) by several pathways, including the increased activation of xanthine oxidase, the autoxidation of NADH, and the inactivation of superoxide dismutase.

The results of this study show that ARBs and ACEIs drugs have antioxidant effect can observed by decreasing serum MDA levels in comparison with other drugs which might be due to its effect of inhibition the angiotensin II mediated oxidative stress by blocking the AT-1 receptors, which is similar to the findings of other studies.[50,51] The increase in serum NO levels observed with valsartan, in this study, may be due to blockade of Ang II acting via the

AT1-receptor since Ang II limits NO bioactivity both by reducing NO release and by increasing NO inactivation via an increase in oxidative stress.[52] Oxidative stress affects NO bioactivity by reducing overall availability of locally released NO, both by accelerating NO deactivation and by reducing the endothelial nitric oxide synthase (eNOS) precursors and cofactors such as BH_4 and arginine.

Serum Antioxidants

Serum Ceruloplasmin Concentration

Table (2) showed a significant increase in serum Cp levels in all patient groups in comparison with control group ($P \leq 0.05$). This result matched with the results study of Kedziora *et al.*, 2006.[53] Increased ceruloplasmin levels may be associated with generation of oxidation products i. e. O-2 and H_2O_2 with concomitant production of H2O from H_2O_2 and ceruloplasmin acting as acute phase reactants.[54], Significant increase ($P \leq 0.05$) in Cp levels can be observed in both Beta. blockers and CCBs groups as compared with ACEIs and ARBs groups. Whereas no significant differences in serum Cp levels can be observed between ACEIs and ARBs groups as well as between β.blockers and CCBs groups. High plasma levels of inflammation sensitive proteins such as ceruloplasmin and others, generally, are associated with hypercholesterolemia[55], This explains the high of Cp level in Beta.blockers and CCBs drugs group in comparison with ACEIs and ARBs drugs group. Accordingly ceruloplasmin appears to be correlated with inflammation in hypertension disease. yet the levels of ceruloplasmin, which is also a ferroxidase, were higher in the hypertensive groups as compared to control group.

This finding also represents an important factor of protection, in view of the activity of ceruloplasmin in transporting copper and oxidizing iron for capture by transferrin, i.e., it acts on the most important transition metals with respect to the ability to transfer electrons in their free form in biological systems. Ceruloplasmin functions as ferroxidase by catalyzing the oxidation of (Fe^{2+} to Fe^{3+})[56], and correlates well with its level and antioxidant activity.[57] Ceruloplasmin is an important intravascular antioxidant and it protects tunica intima against free radical injury. Ceruloplasmin is an acute phase protein and is synthesised by the liver in response to tissue damage and inflammation. Ceruloplasmin exhibits a cardioprotective effect and prevents oxygen free radical induced release of noradrenaline, a powerful vasoconstrictor.[58]

Serum Albumin Concentration

Table (4) showed that there were no significant differences in serum albumin levels among all studies groups (P≤0.05), Yet there are a slightly decreases in serum albumin levels in patients groups compared to control groups. In humans, albumin (Alb) is the most abundant serum protein. Its antioxidant activity is essential in maintaining physiological homeostasis because it binds and transports endogenous substances (such as lipids, ascorbate and divalent cations), scavenges oxygen free radicals and preserves microvascular integrity.[59] The albumin molecule has been demonstrated to inhibit copper ion-dependent generation of hydroxyl radicals and lipid peroxidation, thereby preventing the oxidative injury of lipoproteins.[60] Function of Alb as antioxidant subjects to two mechanisms :(1) the Alb sulfhydryl group (=SH-) per molecule, it scavengers several FRs such as (hydrogen peroxide and peroxyl radical) and thus can be considered as one of the primary extracellular defense system[61], and (2) It is known that the free copper either catalyze reaction between (H_2O_2) and ($^{\cdot}O^{2-}$) or react with (H_2O_2), producing the highly reactive hydroxyl radical (Haber Weiss and Fenton reaction).[62] Serum Alb, usually, bind free copper tightly. Consequently although this binding copper is capable to produce (OH•) according to the above mechanism, yet this (OH•) radical is not free, where the reaction of (OH•) radical production take place in the binding sites on albumin molecule surface.[63]

Table 2: Serum malondialdehyde levels in all studied groups.

Groups	*NO.*	*MDA (µmol/L) Mean ± SD*
Cont.	40	$2.76^{c} \pm 0.52$
ACEIs	40	$2.96^{bc} \pm 0.66$
ARBs	40	$2.81^{c} \pm 0.43$
ß.blockers	40	$3.24^{a} \pm 0.69$
CCBs	40	$3.07^{ab} \pm 0.42$
LSD		0.21

Table 3:- Serum ceruloplasmin concentrations in all studied groups.

Groups	*NO.*	*CP (g/l) Mean ± SD*
Cont.	40	$3.73^{d} \pm 0.61$
ACEIs	40	$4.06^{b} \pm 0.75$
ARBs	40	$4.13^{b} \pm 0.59$
ß.blockers	40	$4.46^{a} \pm 0.59$
CCBs	40	$4.41^{a} \pm 0.72$
LSD		0.24

Table 4:- Serum albumin concentrations in all studied groups.

Groups	*NO.*	*Alb (g/dl) Mean ±SD*
Cont.	40	$4.39^{a} \pm 0.32$
ACEIs	40	$4.30^{a} \pm 0.44$
ARBs	40	$4.30^{a} \pm 0.41$
ß.blockers	40	$4.33^{a} \pm 0.36$
CCBs	40	$4.34^{a} \pm 0.34$
LSD		0.14

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