Oxidized lipoproteins in hypertensive patients on different modalities of treatment.

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Abstract

Background: In addition to hypercholesterolaemia and smoking, hypertension, a chronic disease with many cardiovascular complications, is one of the major risk factors for cardiac ischemia, and the main risk factor for cerebrovascular disease.

Recent studies have demonstrated some unwanted effects on lipid profile and trace element metabolism caused by antihypertensive drugs.

Aim: To elucidate the role of oxidative stress and lipid peroxidation in hypertension and the effect of different medical therapies on these parameters.

Subjects and methods: The present study included measurement of serum lipid profile, total lipid peroxidation, oxidized HDL (Ox-HDL), and urinary protein in 69 patients aged 30-70 years . They were three groups according to the type of therapy: (atenelol, captopril & no pharmacological antihypertensives, NPAHT).

The results were compared with those of 45 apparently healthy controls (age range = 30-66 years).

Results: showed a significant elevation in serum malondialdehyde (MDA) and the percentage of oxidized non high-density lipoprotein (OX.non-HDL %) with a significant reduction in the percentage of oxidized high-density lipoprotein (OX.HDL %) as compared to the controls. The disturbance in the oxidant / antioxidant balance happened despite the treatment and blood pressure control showing different pictures with different modalities of treatment, being the best with the captopril..

Conclusion: Hypertensives on different modalities of therapy experienced dyslipidemia, (High triglycerides and small dense LDL particles) and oxidative stress presented by changes in the percents of oxidized lipoproteins especially in those on NPAHT.

The suggested mechanisms underlying these events are discussed.

Keywords: hypertension, lipid peroxidation, ox-HDL. captopril , atenelol

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Introduction

Hypertension (HTN) is considered a major health problem that emerges from the wide occurrence of its cardiovascular complications[1] .

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Recent studies indicate that oxidative stress mediated by increased level of reactive oxygen species has a causative role in the pathogenesis of cardiovascular disease (CVDs) such as atherosclerosis and HTN[2], diabetes (particularly in patients with angiopathy), hyperlipidaemia and acutely after myocardial infarction and stroke[3]

The antioxidant and lipid peroxidation products are being extensively studied because of their potential importance

and pathgenetic role in several non-communicable diseases like CVDs and cancer, but the data on HTN is scanty [4]. The present study was undertaken to elucidate the role of oxidative stress, a lipid peroxidation on essential hypertension with the emphasis on the effect of different treatment modalities on these parameters.

Subjects & Methods:

A- Subjects:

The study was conducted on 69 patients aged 30-70 years (mean age = 50.52 ± 8.71 years) with essential hypertension (HTN) attending the medical consultant-clinic at Al-Kadhumia Teaching Hospital, for evaluation of newly diagnosed HTN or re-evaluation of long-standing HTN .

The treated hypertensives were either using pharmacological antihypertensive therapy (PAHT) for a period ranging from two months to nineteen years, or not using PAHT for a period ranging from six months to five years. They were attending the clinic for assessment of control and, if necessary, modulation of therapy. The newly diagnosed patients were first discovered to have HTN during their preliminary attendance to the private clinics few weeks (1-4) before their current visit. During this interval, they had not taken any antihypertensive, with their latest attendance to the consultation clinic was for confirmation of diagnosis and, if HTN persisted, commencement of therapy.

Any patient with other medical illnesses that may have an effect on the measured parameters was excluded from the study, as cardiac, hepatic, endocrine, metabolic diseases, smoking and alcoholism. None of the female patients were pregnant nor on contraceptive pills. Details of clinical state were taken from each subject. Depending on the modality of AHT, the patients were divided into three groups

1-hypertensives on atenelol: They were 27 hypertensive patients on β-blocker (atenolol 50-100 mg/day), age range 34-70 years (mean age = 51.33 ± 8.81 year).

2- hypertensives on captopril: They were twenty-seven hypertensive patients on ACE inhibitor (captopril 25-150 mg/day) , age range 30-65 year (mean age = 50.11 ± 8.53 year)..

3- antihypertensives on no pharmacological antihypertensive therapy (NPHT):

Fifteen hypertensive patients had no pharmacological interference, with age range of 32-64 years (mean age = 49.8 ± 9.33).

Control group:

Forty-five apparently healthy subjects were involved as a control group with matching age and sex to the patient group (mean age was 46.04 + 9.43).

None of them were alcoholic, or on any drug that may interfere with the results of the study. Pregnant females and those on contraceptive pills were excluded.

B- Blood Specimens

Ten milliliters of venous blood were drawn from each patient and control after 12 hours fast. The samples were transferred into clean plane tube, left at room temperature for 15 minutes for clotting, centrifuged, and then serum was separated and divided into 2 parts:

1- for measuring the glucose, urea & creatinine (these were measured at the same day of collection in the laborotaries of Al- Kadhumia Teaching Hospital for TG, TC, HDL-C, and LDL-C. the tubes were stored at 4^{o} C for no longer than

seven days[5]. For measuring the total level of oxidized lipids (measured as total malodialdehyde MDA) and specific levels of oxidized HDL (measured as HDL-MDA). The tubes were stored at –20° C until analysis, which was done within one month after collection[6]

C- Methods :

The thiobarbituric acid (TBA) method of *Buege & Aust (1978)*[5] was used to measure **serum MDA**, which reacts with TBA to give a pink color that is read at 535 nm. The MDA concentrations were calculated using the molar extinction coefficient of $1.5*10^5$.

The procedure was also conducted on the fractionated specimens to obtained levels of **oxidized HDL. Oxidized-non-HDL** (oxidized LDL-VLDL) was obtained by subtracting the value of oxidized HDL from the total oxidized lipids i.e. Oxidized-non-HDL = total MDA – oxidized HDL.

Protein in urine was detected by 30% trichloroacetic acid precipitation method [6] to exclude any patient with proteinuria.

Results :

Lipid peroxides profile:

The results of total lipid peroxides, (expressed as s. MDA) and oxidized lipid fractions, which included ox. HDL (expressed as HDL-MDA) and ox.non-HDL are described as absolute values (for s. MDA) and as percentages from the total (for oxidized lipid fractions). These results are shown in Table (3-1).

Serum MDA was highly significantly increased in HTV patients on atenolol (ATE) and captopril (CAP) *($P=10^{-6}$ & 10^{-5} respectively)* when compared to controls and was also significantly increased in HTV on non-pharmacological antihypertensive therapy (NPAHT) *(P=0.03)* as compared to the controls. There was, also a significant variation between hypertensive groups when compared with each other (Table 1).

Hypertensives on atenelol & NPAHT caused a significant reduction of OX.HDL% fraction *($P=0.05$ & 10^{-4} respectively)*; however, HTV on captopril had insignificant reduction in this fraction *(P=0.4)* when all groups were compared with the controls. Also, there was a significant variation between hypertensive groups when compared with each other (Table 1).

In contrast to OX.HDL%, hypertensives on atenelol and on NPAHT had a significant elevation of OX.non-HDL% *($P=0.05$, 10^{-4} respectively)*; however, hypertensives on captopril had insignificant elevation in this fraction *(P=0.45)* when all groups where compared with controls. There was a significant variation between hypertensive groups when compared to each other *($P=10^{-3}$)* (Table 1).

Serum lipid profile:

Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), atherogenic index (expressed as LDL-C/HDL-C) and LDL size index (expressed as TG/HDL-C) are shown in Table 2 .

Table (1): lipid peroxides and oxidized lipoprotein fractions (mean ± SD) in different groups of hypertensive patients and control group.

Groups	n	S.MDA µmol/l	t-test P-value*	OX.HDL %	t-test P-value*	Oxidized nonHDL %	t-test P-value*
HTV on atenalol	27	0.67 ± 0.16	10^{-6}	60.45 ± 11.65	0.05	39.05 ± 11.8	0.05
HTV on captopril	27	0.63 ± 0.16	10^{-5}	70.62 ± 17.1	0.45	29.37 ± 17.1	0.45
HTV on NPAHT	15	0.96 ± 0.36	0.03	55.02 ± 17.09	10^{-4}	44.97 ± 17.09	10^{-4}
ANOVA *P-value*	~	~	10^{-5}	~	10^{-3}	~	10^{-3}
Controls	45	0.46 ± 0.17	~	73.83 ± 17.83	~	26.16 ± 17.83	~

* = Student t-test was done between each HTV group and control group.

Table (2): Lipid profile (mean ± SD) in different hypertensive and control groups.

Group	HTV ON ATE	HTV ON CAP	HTV ON NPAHT	ANOVA *P*-value	CONTROLS
N	27	27	15	~	45
TG(mmol/l)	1.76± 0.67	1.64 ± 0.53	2.21± 0.42	~	1.18 ± 0.54
*t-test P-value**	0.0001	0.0007	0.04	0.009	~
TC(mmol/l)	4.92 ± 1.12	5.1 ± 0.79	5.1 ± 1.2	~	4.34 ± 0.96
t-test P-value*	0.02	0.001	0.01	0.7	~
HDL-C (mmol/L)	1.04 ± 0.23	1.14 ± 0.24	1.09 ± 0.33	~	1.18 ± 0.3
*t-test P-value**	0.04	0.5	0.3	0.3	~
LDL-C(mmol/l)	3.07 ± 1.06	3.03 ± 0.66	2.99 ± 1.31	~	2.66 ± 0.93

*t-test P-value**	0.08	0.15	0.2	0.9	~
Atherogenic index (LDL-C/HDLC)	3.01 ± 0.11	2.87 ± 0.75	2.9 ± 1.33	~	2.45 ± 1.29
*t-test P-value**	0.09	0.19	0.24	0.8	~
LDL size index (TG/HDL-C)	1.79 ± 0.89	1.55 ± 0.78	2.4 ± 0.75	~	1.13 ± 0.79
*t-test P-value**	0.001	0.03	0.000003	0.007	~

*Student t- test was done between each HTV group and control groups.
.ATE : Atenelol , CAP : captopril

Discussion:

The results showed that, oxidative stress increases in hypertensive (HTV) patients regardless the modality of treatment, this is clear from the highly significant elevation of serum MDA level and is in agreement with the results of previous reports [7,8]

This elevation in serum MDA may be due to the association of high blood pressure (BP) with a loss of balance between pro-oxidation and anti-oxidation, energy depletion, and accelerated aging in the target organs, such as heart, kidney and brain [9]

Essential HTV patients are more prone than the normotensive subjects to oxidative stress; because of an increased production of reactive oxygen metabolites and of lipid peroxidation products[10], something that was reported to be frequent among such patients. This may results in a poor control of BP and hence progression of target tissue damage[11]. The latter will be reflected by an elevation in serum MDA as an index of oxidative stress; however, the presence of undesirable side effects of these drugs might contribute to some rise in serum MDA, this contribution varies from one type of therapy to another. Beta-adrenergic blockers were reported to increase cholesterol side effects besides the central nervous system effects. However, ACE inhibitors have fewer side effects in general [12].

It remains to be determined whether these events are simply consequences of tissue damage or strictly involved in primary pathogenic mechanism of HTN. Therefore, a study of oxidant and antioxidant factors seems to be useful in HTV patients in order to evaluate oxidative stress and to correct, if possible, the observed abnormalities with dietetic or pharmacological therapy[13].

The term " ***oxidized non-HDL"*** refers to the oxidized form of VLDL and LDL. The oxidation of LDL appears to be involved in the development of atherosclerosis. The oxidation of LDL leads to alteration of the LDL apolipoprotein B (apoB) recognition site and in the unregulated uptake of LDL by the macrophages via the scavenger receptor. The subsequent accumulation of cholesterol-loaded macrophages (foam cells) in the sub-endothelium

leads to the accumulation of fatty streaks and atherosclerotic plaques [14]*;*

In the in-vitro studies, OX-.LDL was shown to be taken up by the macrophages three to ten times than native LDL[15].

Furthermore, OX.LDL is chemotactic for circulating monocytes, while decreasing the motility of tissue macrophages[15,16], and also stimulates the release of growth factors and cytokines by endothelial cells to further stimulates monocyte migration. The oxidized form of LDL is known to be cytotoxic to endothelial cells and may cause an autoimmune type of response against endothelial cells. Also OX.LDL inhibits endothelial-derived relaxing factor (EDRF)-mediated vasorelaxation. EDRF appears to be crucial in maintaining coronary vasodilation and its activity is impaired in hypercholesterolaemia and atherosclerosis [15,16]

According to the results of the current study, there was a significant elevation of OX.non-HDL in HTV groups on atenolol (ATE) and those on no pharmacological antihypertensive therapy (NPAHT) as compared with the controls, the NPAHT being the highest. This suggests that those patients are more prone to have atherosclerotic changes than those receiving medications with the presence of smaller forms of LDL particles (as evident from higher TG / HDLc molar ratio). On the other hand hypertensives on atenelol have significantly higher level of OX.non-HDL than patients on captopril , who showed insignificant increase in this fraction as compared to the controls; this is due to the fact that ACE inhibitors inhibit LDL oxidation and attenuate atherosclerosis independent of lowering BP and this may due to direct inhibition of angiotensin II-dependent effects[17].

As concerning ***oxidized HDL,*** it has been postulated that HDL has a protective effect against the formation of OX.LDL, and one of the mechanisms is mass transfer of lipid peroxidation products between lipoproteins[18] (i.e. from LDL to HDL) forming OX.HDL which was markedly reduced in the present HTV, mostly those on no medications on the expense of the increase in the atherogenic form of LDL (OX.LDL).

Percentages of OX.HDL were also reduced in HTV people receiving medications but to a lesser extent and with a variable degree depending on the antioxidant properties of the antihypertensive drug being only slightly reduced in those on captopril. This indicates that untreated HTV were more prone to have atherosclerosis than those receiving medications. Also, HTV on atenelol had a higher reduction in percentages of OX.HDL than those on captopril this reveals the antiatherogenic protective effect of captopril against vasculopathy.

The findings of this study, suggest that the studied drugs (atenolol & captopril), in addition to their antihypertensive effect that control Blood pressure, possess antioxidant properties that make them an important means to retard the progression of atherosclerosis in hypertensive peoples.

The antioxidant property of capotril was well evident through the results of MDA, OX.non-HDL and OX.HDL (when results of the above parameters were compared with the healthy controls and other hypertensive groups), and it exceeds that of atenelol and probably overcome its antihypertensive effect[19].

Reported increased nitric oxide (NO)

production, decreased production of thiobarbiturate reactive substances and enhancement of endogenous antioxidant defense during the treatment with capotril. Therefore, the goal of appropriate antihypertensives should not be restricted to achieving normotensive BP values, but should also aim to reduce other risk factors of cardiovascular diseases[20] .

Further improvement in cardiovascular risk can be obtained only with a comprehensive approach to hypertensive patients, including dietary and lifestyle modifications and the use of antihypertensive drugs with beneficial effects on lipid metabolism. It has to be noted that, some of these drugs have divergent effects on lipid metabolism. While β-blockers decrease HDL-C and increase triglyceride (TG), ACE inhibitors do not affect lipid metabolism [20 , 21] .

In the present study, There was also a significant increase in s.TG and s.TC level in the untreated hypertensives. While there was no significant changes in s.HDL-C and s.LDL-C levels, these results are consistent with the fact that untreated HTN has been shown to be associated with a significant elevation in s.TG and s.TC levels[22] . The results of this study and others support the hypothesis that hyperlipoproteinaemia is aften observed in untreated hypertensives patients[23], and when HTN is associated with high plasma lipid levels, it is an atherosclerogenic factor of greator clinical significance causing CVDs [24] .

However, the role of TG in CVD is a controversial subject. Many epidemiological trials do not identify hypertriglyceridaemia as an independent risk factor when the cholesterol and, in particular the HDL-C level, are taken into consideration, Nevertheless, these results must be interpreted with caution as hypertriglyceridaemia represent a very heterogenous entity which is closely related to many factors that may affect coronary risk (hypertension, insulin resistance, sedantarity, and even tobacco consumption). Therefore, hypertriglyceridaemia and hypo-HDL-aemia may be the results of the same primary abnormality, as the HDL-C level is more stable, it is the parameter, which will be identified as a protective factor in epidemiological trials [25].

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