Original Research Article

Culprit Effect of Oxidative Stress and Dyslipidemia on North Indian Stage I Essential Hypertension Patients

Dr. Archana Agarwal[1], Dr. Rahul Saxena[2]
[1]Assistant Professor, Dept of Physiology, School of Allied Health Sciences, Sharda University, Greater Noida India.
[2]Assistant Professor, Dept of Biochemistry, School of Allied Health Sciences, Sharda University, Greater Noida India.

*Corresponding author
Dr. Rahul Saxena
Email: rahul.saxena@sharda.ac.in

Abstract: Systemic deterioration of biomolecules and increased incidence of oxidative stress play a crucial role in the development of hypertension (HT). In addition, occurrence of dyslipidemia in hypertension significantly contributes the development of cardiovascular disease. In this context, the present study focused on culprit effect of oxidative stress and dyslipidemia on North Indian stage I HT patients in enhancing the risk of cardiovascular disease (CVD). The aim is to estimate the plasma lipid profile, marker of inflammation and oxidative stress in stage 1 hypertension and to determine their role in predicting CVD risk. The study population consists of 40 stage I HT patients (40-55 years) of either sex, recruited as per JNC 7th report and 40 age and sex matched healthy controls. Plasma lipid profile, c-reactive protein (CRP) and ascorbic acid levels were measured along with erythrocyte malondialdehyde (MDA) levels in study group subjects and data was statistically analyzed using standard methods. Plasma total cholesterol, triglycerides, LDL-cholesterol, CRP and MDA levels were significantly high ($p<0.05$) in patient group as compared to healthy controls whereas plasma ascorbic acid and HDL levels were decreased significantly ($p<0.05$) in stage I HT patients. Thus, enhanced oxidative stress and inflammation along with dyslipidemia play a crucial role in HT development and its related complications. Therefore, these markers should not only regularly monitored with increase in blood pressure but also regulated by effective dietary and therapeutic treatment strategy in early stage of hypertension.
Keywords: Malondialdehyde, free radical, inflammation, ascorbic acid, LDL-cholesterol.

INTRODUCTION

Hypertension (HT), often called as silent killer, remains the most common risk factor for cardiovascular morbidity and mortality worldwide [1,2]. It has been well documented that abnormal lipid profile or dyslipidemia in vascular disorders is an alarming condition of future health complications predominantly cardiovascular diseases (CVD) such as myocardial infarction, atherosclerosis etc.[3] Amusingly, the presence of inflammation further enhances the frequency to develop CVD. [4] C-reactive protein, an acute phase reactants, synthesized in liver and raised by many folds following acute inflammation, is a marker of systemic inflammation in several conditions including hypertension, rheumatoid arthritis, psoriasis, cancer and pre-eclampsia [5-7].

Oxidative stress ensues when large amount of reactive oxygen species are produced in the cells that can evade or overwhelm the antioxidant protective mechanisms of cells and tissues, and produce major interrelated impaired cell metabolism including DNA strand breakage, rises in intracellular free Ca^{2+}, damage to membrane ion transporters and other specific proteins leading to cell death. Prime target to free radicals attack is the polyunsaturated fatty acids in the membrane lipids, causing lipid peroxidation, has been found to be a major event in the development of various diseases [8, 9]. Lipid peroxide (malonaldialdehyde) is the most abundant among the reactive aldehydes derived from lipid peroxidation. It has been suggested that binding to these aldehydes to membrane protein may alter their function, tonicity, permeability, rigidity and integrity, and thereby may induce culprit effect [10].

In order to provide protection, antioxidants reduce or eliminate these free radicals. Among non

enzymic antioxidant, vitamin C is an exogenous water soluble antioxidant, functions as primary defense against free radicals in plasma and disappeared more quickly. It plays a key role in protecting plasma lipids against peroxidation, collagen synthesis; wound healing and improving vascular endothelium dependent vasodilatation [11]. Kumar and Das also observed that free radicals are produced in increased amount in HT and altered levels of vitamin C are significantly related with both systolic and diastolic blood pressure [12].

Moreover, it is believable that systemic inflammation, oxidative stress and dyslipidemia are associated with enhanced risk of CVD in hypertensive patients. Inspire of improvement in our knowledge on hypertension from pathologic point of view, the intimate mechanisms involving systemic inflammation, oxidative stress and dyslipidemia in stage I hypertension patients making them more susceptible to develop future CVD are yet not fully understood. In addition, as best of our knowledge, previous studies on hypertension patients have not included systemic inflammation, oxidative stress and dyslipidemia in a single setting [3, 13, 14] Therefore, the present work aims to evaluate the levels of c-reactive protein (CRP), MDA, ascorbic acid and lipid profile in stage I hypertension patients and to determine their role in prediction of CVD risk in same population.

MATERIAL AND METHOD:
As per "Seventh Report of Joint National Committee on High Blood Pressure (JNC 7th report)", 40 subjects of Stage I essential hypertension [Stage I HT (SBP 140-159 & DBP 90-99 mm Hg)] of either sex (24 males and 16 females) belonged to age group 40-55 years were recruited in the patient group and 40 age matched healthy individuals were recruited in control group (40-55 years) after taking their informed consent form and approval of the study from Ethical committee of the college. Overnight fasting peripheral venous blood sample was collected into plain (5ml) vials from the study group subjects after taking the demographic information, history and limited physical examination such as age, sex, height, weight, blood pressure and confirmation of healthy state. The samples were centrifuged at 2000 rpm for 15 minutes. The separated serum (plain vial) was stored at –80ºC until further analysis. Height and weight were measured with subject barefoot and light dressed. The body mass index (BMI) was calculated as BMI = *weight (Kg) / Height (metre^2).* Obese (B.M.I > 30) and smokers were also excluded from the study.

For the estimation of study group parameters, plasma was separated from the collected blood sample by centrifugation at 1000 g for 15 min at room temperature and stored at -80^{0}C until use. Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate. The heat induced reaction of MDA with thio barbituric acid (TBA) in the acid solution formed a trimethine coloured substance, which was measured spectrophotometrically at 532 nm [15]. Plasma C-reactive protein (CRP) levels were measured using commercially available ELISA kits (R&D Systems, USA), according to manufacturer's instructions.

Plasma ascorbic acid levels were estimated by Mc Cormick and Greene method. Ascorbic acid in plasma is oxidized by Cu (II) to form dehydroascorbic acid which reacts with acidic 2, 4–dinitrophenyl hydrazine to form a red bishydrazone, which is measured at 520 nm [16].

Plasma lipid profile contents (Total Cholesterol, Triglycerides and HDL cholesterol) were analysed enzymatically using kit obtained from (Randox Laboratories Limited, Crumlin, UK). LDL-cholesterol levels were calculated by Friedwald's formula [17].

LDL-C = TC - [(TG/5) +HDL-C]

Statistical analysis:
The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD and compared by using Student's t test. The distribution of 't'- probability was calculated depending on 'n' and significance of test was obtained. P value <0.05 and <0.001 were considered as significant and highly significant respectively.

RESULT
In the present study, mean age and blood pressure of the study group subjects i.e. demographic indices, are depicted in Table 1. Patients with Stage I HT have insignificant variation (p<0.1) with respect to age as compared to healthy controls. Stage I HT subjects were overweight (p<0.05) with respect to healthy controls which reflect the role of increased body weight in HT development as one of the important risk factor. However, incidence of HT in male are more than female HT subjects as 24 males and 16 females were

recruited as Stage I HT patients. Markers of inflammation and oxidative stress status are presented in Table 2. Plasma CRP and erythrocyte MDA levels were found to be significantly high (p<0.001; 39.57% and p<0.05; 30.22% high) in patient group as compared to healthy controls whereas plasma ascorbic acid levels were decreased significantly (p<0.05; 33.33% low) in stage I HT subjects which reflect the role of inflammation and oxidative stress in etiopathology of essential hypertension. Plasma lipid profile, as depicted in Table 2, revealed that plasma total cholesterol, triglycerides and LDL cholesterol levels were significantly high (p<0.05, 32.27%, 29.06% and p<0.001, 34.03% high) in patient group as compared to healthy controls whereas, HDL-cholesterol levels were decreased significantly (p<0.05, 19.4% low) in patient group.

Table-1: Demographic and clinical profile of stage I HT subjects and healthy controls
(Mean ± SD)

S No	Particulars	Control Group (n=40)	Patient Group (n=40)
1)	Age (years)	46 ± 5.0	48 ± 5.4*
2)	Male/Female	20/20	24/16
3)	Height (meter)	1.58 ± 0.31	1.60 ± 0.27
4)	Weight (Kg)	60.2 ± 2.3	69.5 ± 1.9
5)	BMI (Kg/m^2)	24.4 ± 1.2	27.5 ± 1.3**
6)	Systolic blood pressure (mmHg)	112.0 ± 3.4	148.0 ± 4.8***
7)	Diastolic blood pressure (mmHg)	78.6 ± 2.5	94.2 ± 3.40***

where, * p<0.1 : Non-significant, ** p<0.05 : Significant, *** p<0.001 : Highly significant

Table-2: Plasma lipid profile, marker of systemic inflammation and oxidative stress in study Group subjects. (Mean ± SD)

S.No	Particulars	Control Group (n=40)	Patient Group (n=40)	% increase	% decrease
1.	Ascorbic acid (mg/dl)	1.38 ± 0.25	0.92 ± 0.27**	-	33.33%
2.	Malondialdehyde (µmolMDA/ml)	2.68 ± 0.16	3.49 ± 0.21**	30.22%	-
3.	Total Cholesterol (mg/dl)	158.38 ± 16.2	209.50 ± 19.0**	32.27%,	-
4.	Triglycerides (mg/dl)	104.74 ± 14.5	133.8 ± 18.3**	29.06%	-
5.	HDL cholesterol (mg/dl)	44.2 ± 3.24	35.64 ± 3.26**	-	19.4%
6.	LDL cholesterol (mg/dl)	93.5 ± 12.63	125.32 ± 14.4***	34.03%	-
7.	CRP (mg/L)	3.31 ± 0.14	4.62 ± 0.15***	39.57 %	-

where, *p<0.1 : Non-significant, **p<0.05 : Significant, *** p<0.001 : Highly significant

DISCUSSION

Dyslipidemia has been known to be associated with several clinical conditions such as cardiovascular disease, hypertension, diabetes and vascular diseases [3, 18, 19]. In this context, association of dyslipidemia with inflammation and oxidative stress in stage I HT in North Indian population has yet not be clarified and documented. It is speculated that occurrence of dyslipidemia in hypertension is associated with inflammation and oxidative stress with unsolved conundrum and the mechanism behind its complex interplay is responsible for the development of future CVD risk. In the present study, plasma CRP levels were increased significantly (p<0.05) in stage I HT patients which reflect the significant role of inflammation in etiopathophysiology of HT. Similarly, plasma ascorbate levels were found to be significantly low in stage I HT patients along perturbs lipid profile as compared to healthy controls. Reduction in vitamin C levels could not be only due to its free radical scavenging action but also in maintaining the body antioxidant reserve and in normalization of vascular superoxide formation which prevent endothelial dysfunction [20]. Salonen *et al.* in their study also observed that low ascorbate level is significantly related with both diastolic and systolic blood pressure [21]. Our findings are in concordance

with that of several previous studies conducted separately and involving relatively small patient populations [13, 22, 23]. According to them, enhanced oxidative stress induces the development of atherogenic dyslipidemic profile in HT patients by modulating the lipoprotein lipase activity via antilipoprotein lipase antibodies. In addition, atherogenic complexes of autoantibodies to oxidized LDL are generated in response to an oxidative inflammatory effect which enhances the accumulation of LDL in the endothelial wall and thereby enhances the CVD risk in HT patients [24].

Perturbation of systemic oxidative balance, i.e. uncontrolled ROS production plays a crucial role in enhancing the disease complexity in HT [25]. Endothelial cells and vascular smooth cells produce ROS which oxidize low density lipoprotein and thereby initiate atherosclerosis. In addition, involvement of ROS in cell membrane damage via lipid peroxidation and its resultant products such as lipid radicals (L∘), lipid peroxides (LOO∘), lipid hydroperoxides and highly reactive aldehydes which play a crucial role in the development and progression of vascular complications [26]. In this context, marked increase in erythrocyte MDA levels (i.e. marker of lipid peroxidation) were observed in stage I HT subjects ($p<0.001$) as compared to healthy controls which clarify the etio-pathogenic role of ROS via lipid peroxidation, in shaping HT patients more susceptible to develop future incidence of CVD and its related complications. Our findings were in concordance with that of previous study carried out on HT patients having rheumatoid arthritis and were at high risk to develop CVD.[22] According to them, lipid peroxides are toxic to the cellular components, and responsible for initiation of complex cascade that promotes atherosclerotic plaque formation, prostacyclin synthesis, enhancement of cytosolic free calcium and peripheral vascular resistance and thereby leading to development of CVD complications in HT patients with abnormal lipid profile [27].

CONCLUSION

On the basis of findings of present study, we can conclude that dyslipidemia along with enhanced inflammation and reduced plasma ascorbate level are associated with increase in blood pressure and play an etiopathological role in the development of HT. Furthermore, as the blood pressure rises, plasma vitamin C level decreases due to its role in reducing oxidative stress by limiting lipid peroxidation as it

scavenges free radicals and by maintaining body's antioxidant reserve. Thus, it is suggested that apart from conventional CVD risk factors assessment, estimation of CRP along with markers of oxidative stress should be encouraged in stage 1 hypertension patients. Moreover, dietary counseling may help in the management of blood pressure and enable them to reduce the cardiovascular risks. In addition, present study also suggested that the consumption of diet rich in antioxidants along with regular monitoring of lipid profile could be recommended to the patients suffering with stage 1 hypertension in order to reduce the risk of CVD and its related complications.

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