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RELATIONSHIP OF NITRIC OXIDE WITH OTHER RISK FACTORS OF HYPERTENSION"

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ABSTRACT

OBJECTIVE: *The aim of this study was to determine the correlation of serum nitric oxide (NO) level with lipids and estradiol that are known components of hypertension. Design and Methods: We had selected 42 hypertensive patients from The National Institute of Cardiovascular Diseases (NICVD), Karachi, Pakistan and 35 randomly selected healthy control subjects. Serum was assessed for cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, nitric oxide and estradiol.*

RESULTS: *Serum cholesterol, LDL-cholesterol and triglycerides were significantly ($p<0.05$) higher in hypertensive patients compared to controls. Serum HDL-cholesterol, nitric oxide and estradiol had decreased levels in hypertensive patients ($p<0.05$) than normal subjects. Serum nitric oxide showed strong correlation with lipid components*

CONCLUSION: *The present study suggests that there is a strong relationship between blood lipids and nitric oxide in hypertensive patients. Decreased nitric oxide and estradiol availability can induce an imbalance between oxidative and non-oxidative mechanisms which may affect the blood pressure.*

KEY WORDS: Hypertension, cholesterol, HDL, LDL, triglycerides, estradiol, nitric oxide.

INTRODUCTION:

Hypertension is known as the "silent killer" as it can lead to heart attack, stroke and renal diseases. Hypertension is highly prevalent among middle aged and elderly persons [1] and one out of every three person over age of 45 is hypertensive in Pakistan. [2]Hypertensive patients have many pathophysiological changes such as atherosclerosis, endothelial dysfunction and inflammations. The most important endothelium-derived vasodilating substance is nitric oxide (NO), an inorganic free radical gas synthesized by the oxidation of l-arginine in a process catalyzed by enzyme nitric oxide synthase (NOS). NO is known to be the principal mediator of several functions, including vasodilation, anticoagulation, leukocyte adhesion, smooth muscle proliferation and the antioxidative capacity of endothelial cells.[3-5] Abnormal activity of endothelial nitric oxide synthase might lead to nitric oxide deficiency and cause clinical hypertension. Clinical studies indicated lower nitric oxide status contributing to endothelial dysfunction in hypertensive patients.[6] Lipid abnormalities are often associated with high blood pressure and might exert a role in the development of hypertension. Hypertensive patients had significantly higher levels of LDL – cholesterol [7] and low HDL – cholesterol level than normal controls. [8] In the PAMELA (Pressioni Arteriose Monitorate E Loro Associazioni) study population, the prevalence of hypercholesterolemia was greater in subjects with hypertension than normal subjects. It was also found that serum HDL – cholesterol is decreased in hypertensive patients than the normal subjects. [9] Elevated levels of cholesterol and LDL cause injurious effect on endothelium. Furthermore, a relation between decrease in cholesterol and improvement in endothelial function was found after lipid lowering therapy. [10] Previous studies have shown that serum triglycerides increased with increasing blood pressure in both genders. [7, 11]Estradiol is the most potent estrogen belongs to a family of endogenous estrogen steroids. Nordby et al. reported lower serum estradiol levels in hypertensive premenopausal women as compared to normotensive women suggesting a role of ovarian hormones in control of blood pressure. [12] It is proposed that protective effects of estradiol in hypertension are mediated through the ability of estradiol to increase nitric oxide synthesis. [13] Konukoglu et al. suggested that the circulating nitric oxide level was lower in obese female subjects with hypertension than in the normal control subjects. [14] Moreover, as nitric oxide is thought to be involved in preventing the oxidation of lipoproteins, Kondo et al. [15] found that a reduction in nitric oxide bioactivity occurs with abdominal

TABLE – 1
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS (MEAN ± S.E.M.) OF CONTROLS AND HYPERTENSIVE PATIENTS

Variables	Controls N = (35)	Hypertensive patients N = (42)
Age (years)	46.03±2.15	48.98±1.70
Gender distribution (M/F)	13/22	17/25
BMI (Kg/m^2)	19.71±0.31	21.38±0.55*
SBP (mmHg)	116.86±0.98	157.14±2.39*
DBP (mmHg)	76.57±0.81	97.86±1.51*
Serum cholesterol(mg/dl)	168.05±2.01	201.14±5.79*
Serum HDL–cholesterol(mg/dl)	55.36±1.19	43.08±1.45*
Serum LDL–cholesterol (mg/dl)	82.52±1.92	124.95±6.57*
Serum triglycerides (mg/dl)	150.82±1.41	165.57±4.01*
Serum estradiol (pg/ml)	39.01±5.38	25.06±2.27*
Serum nitric oxide (um/l)	45.95±1.05	41.54±0.78*

BMI-body mass index, SBP - systolic blood pressure, DBP – diastolic blood pressure

*P <0.05 significant as compared to control subjects.

TABLE – 2
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS (MEAN ± S.E.M.) OF FEMALE CONTROLS AND FEMALE HYPERTENSIVE PATIENTS

Variables	Female Controls subjects N = (22)	Female hypertensive patients N = (25)
Age (years)	46.41±2.56	48.72±1.99
BMI (Kg/m^2)	19.36±0.41	20.71±0.60
SBP (mmHg)	117.27±0.97	160.40±3.29*
DBP (mmHg)	77.73±0.91	97.60±1.45*
Serum cholesterol(mg/dl)	172.90±2.13	196.11±7.22*
Serum HDL–cholesterol(mg/dl)	57.41±1.50	47.12±1.78*
Serum LDL–cholesterol (mg/dl)	85.29±2.49	116.28±7.63*
Serum triglycerides (mg/dl)	151.01±1.81	163.56±4.28*
Serum estradiol (pg/ml)	45.05±8.28	18.46±2.47*
Serum nitric oxide (um/l)	45.63±1.27	41.13±1.14*

* P < 0.05 significant as compared to female control subjects

TABLE – 3
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS (MEAN ± S.E.M.) OF MALE CONTROLS AND MALE HYPERTENSIVE PATIENTS

Variables	Male Controls subjects N = (13) N = (17)	Male hypertensive patients
Age (years)	45.38±3.98 49.35±3.09	
BMI (Kg/m^2)	20.31±0.44 22.36±1.02	
SBP (mmHg)	116.15±2.13	152.35±3.15*
DBP (mmHg)	74.62±1.44	98.24±3.12*
Serum cholesterol(mg/dl)	159.84±2.91	208.53±9.56*
Serum HDL–cholesterol(mg/dl)	51.89±1.61 37.14±1.62*	
Serum LDL–cholesterol (mg/dl)	77.84±2.59 137.69±11.30*	
Serum triglycerides (mg/dl)	150.50±2.32	168.52±7.78*
Serum estradiol (pg/ml)	28.78±1.87 34.77±3.05	
Serum nitric oxide (um/l)	46.49±1.89	42.16±1.01*

* P < 0.05 significant as compared to male control subjects.

TABLE - 4
REGRESSION CORRELATION COEFFICIENT BETWEEN CLINICAL VARIABLES IN HYPERTENSIVE PATIENTS

Variables	Nitric oxide		
	r	r^2	P value
TRIGLYCERIDES	-0.37*	0.14	0.01
cholesterol	-0.35*	0.12	0.02
HDL- cholesterol	0.32*	0.10	0.04
LDL- cholesterol	-0.33*	0.11	0.03

*** P < 0.05 significant as compared to control subjects.**

fat accumulation in women. Previous study demonstrated that both HDL and estrogen stimulate eNOS and the subsequent production of nitric oxide. [16] So, the purpose of the study was to measure the serum levels of nitric oxide, lipids and estradiol among hypertensive and control subjects and also to examine the hypothesis that whether nitric oxide is correlated with serum lipids and estradiol in hypertension

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MATERIAL AND METHOD:

Hypertensive patients were recruited in the study from The National Institute of Cardiovascular Diseases (NICVD), Karachi, Pakistan. Hypertensive patients on hormone replacement therapy (HRT), having diabetes, renal disease, cardiovascular diseases, smoking and pregnancy were excluded from the study. Finally 42 hypertensive patients of ages 25-70 years were included in the study. 35 normal subjects matched for age, sex and racial distribution, having no signs and symptoms of any disease were selected randomly. Three measurements of systolic and diastolic blood pressures were taken on the right arm of each subject with an appropriately sized cuff using mercury sphygmomanometer after 5 minutes rest in a seated position. The average of 2nd and 3rd measurement was used as blood pressure value for each subject. Body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (in meters) i.e. kg/m^2. A questionnaire about demographic, social, medical and family history and written informed consent was obtained from each participant. Venous blood sample was collected after overnight fasting period.

Serum cholesterol, high density lipoprotein cholesterol (HDL) and triglycerides (kits supplied by Randox Laboratories Ltd. United Kingdom) were measured by spectrophotometer (UV mini 1240 –

SHIMADZU). Serum low density lipoprotein cholesterol (LDL) was calculated by the Friedewald formula [17]

LDL cholesterol = (Total cholesterol - HDL cholesterol – triglycerides)

Serum estradiol (kit supplied by Biocheck, Inc. Foster City) was analyzed by Enzyme Linked Immuno Sorbant Assay (ELISA) method (Stat Fax – 2200, Awareness Technology Inc.). Serum nitric oxide was estimated by Griess reagent (kit supplied by Assay Designs, Ann Arbor, MI USA). Data was statistically analyzed by student's t – test. Significance level was P< 0.05. Pearson correlation coefficient was calculated with the Statistica V. 5.0 program (Statsoft, Inc, USA).

RESULTS:

The study population has 17 males and 25 females, whereas in 35 control subjects there were 13 males and 22 females. Demographic and clinical characteristics of controls and study population are shown in table – 1. BMI, systolic and diastolic blood pressure were significantly increased ($p<0.05$) in hypertensive patients as compared to control subjects. Except BMI, the same pattern was observed when male and female patients were analyzed separately (Table – 2 and 3). As shown in Table – 1, there was an increase in serum cholesterol, LDL – cholesterol, triglycerides levels ($p<0.05$) whereas serum HDL – cholesterol, nitric oxide and estradiol levels were significantly ($p<0.05$) decreased in hypertensive patients as compared to normal control subjects. Similar findings were observed in female patients (Table - 2) but in male patients, serum estradiol levels showed insignificant difference (Table - 3). In table – 4, nitric oxide is positively correlated to estradiol, HDL-cholesterol and negatively correlated with cholesterol, LDL-cholesterol and triglycerides.

FIGURE 1.

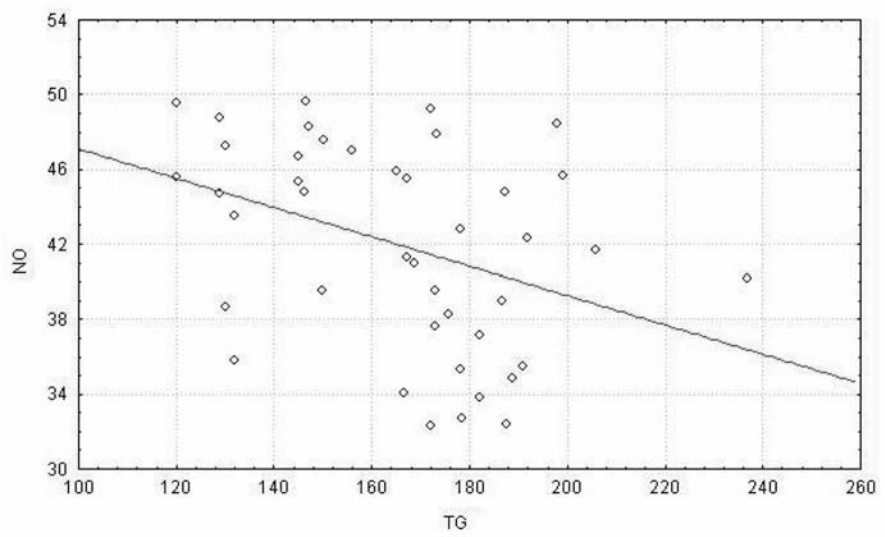
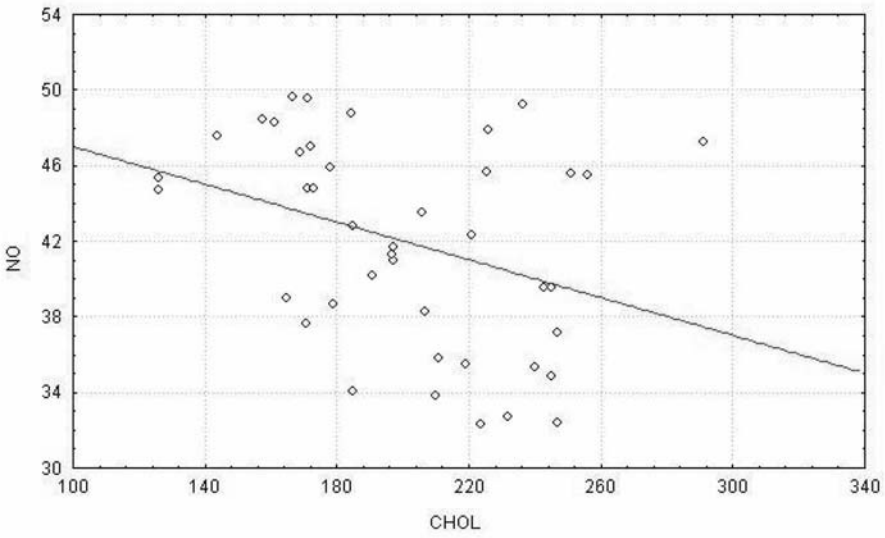


FIGURE 2



DISCUSSION:

Prevalence of hypertension is showing alarming rise due to rapid changes in lifestyle and dietary pattern. Osmani et al. had found that prevalence of hypercholesterolemia was higher in hypertensive subjects compared to non hypertensives. [18] Our results are consistent with the previous studies that serum cholesterol and LDL – cholesterol levels were significantly higher in patients with hypertension compared to controls. [5] Serum triglyceride levels were significantly higher in hypertensive patients when compared with normal subjects as was shown in previous work by Bønna and Thelle. [19] Serum HDL – cholesterol showed a significant decreased level in hypertensive patients than control individuals, which is in agreement with the previous work. [20] It was suggested that lipid abnormalities cause endothelial damage [21, 22] by stimulating the generation of superoxide radicals which directly inactivates NO and may also increase the subsequent oxidation of LDL particles by the formation of peroxynitrite, producing injurious effects on endothelium and eventually elevate blood pressure.

In the present study, hypertensive patients showed a significant decrease in serum estradiol and nitric oxide levels as compared to control individuals (Table-1). Estrogen enhances vascular dilatory mechanisms both in humans and animals. [23] Estradiol also inhibits endothelin synthesis. [24] When estradiol concentration is reduced, endothelin concentration increases that cause oxidative stress and this stress enhance superoxide production. These superoxide anions scavenge nitric oxide [25] contributing to increased vasoconstrictor mechanisms that lead to increased blood pressure. [26] Unexpectedly, we could not found any correlation of nitric oxide and estradiol, the reason for this might be higher levels of estradiol in male patients. Serum nitric oxide has a negative correlation with triglycerides (Fig. 1), cholesterol (Fig. 2) and LDL-cholesterol (Fig. 4) whereas positively correlated with HDL-cholesterol (Fig. 3). This confirms the correlation between lipid abnormalities and endothelial damage which alters nitric oxide concentration in hypertensive patients.

CONCLUSION:

Hypertension is associated with enhanced levels of serum cholesterol, triglycerides, LDL and diminished concentrations of HDL cholesterol, estradiol and nitric oxide. Furthermore, there is a strong relationship found in the present study between blood lipids and nitric oxide among hypertensive patients. Taken together, lower estradiol and nitric oxide levels as well as higher lipids can stimulate super oxide production; causing vasoconstriction which may elevates the blood pressure.

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