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RESEARCH ARTICLE

ASSESSMENT OF SERUM NITRIC OXIDE LEVEL IN ESSENTIAL HYPERTENSION

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ABSTRACT

Aim: Nitric oxide, the second messenger is found to be the main factor involved in endothelial dysfunction and its level is found to be altered in hypertensive states. The aim is to elucidate the association between serum NO levels and essential hypertension and to compare with normal individuals.

Materials and Methods: The study sample is comprised of 150 unrelated essential hypertensive patients and130 apparently healthy normotensive controls. Plasma glucose, serum urea, serum creatinine, total cholesterol (TC), high density lipoprotein cholesterol (HDL-c) and triglyceride concentration (TGL) were determined enzymatically and serum NO index (NOx) estimated by Griess method.

Results: Statistically significant low NOx levels, with p value of 0.001 was observed in cases (14.69 + SD 4.45) when compared to controls (18.16 + SD 7.23). Serum NOx level was not influenced by biochemical parameters like plasma glucose and lipid profile.

Conclusion: It was found that the low serum NO index is an independent risk indicator in essential hypertension, based on this study.

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INTRODUCTION

Hypertension is an increase in the systemic arterial blood pressure without an apparent cause and is one of the world's great public health problems (Campanini, 2002) remaining as the leading cause of death, worldwide. More than 90% of hypertensive individuals suffer from essential hypertension. It shows an earlier onset in men, than in women. The factors linked to essential hypertension are age, obesity, smoking and stress. The endothelial lining of blood vessels is critical to vascular health and constitutes a major defense against hypertension. It helps in the regulation of vascular tone and blood flow, by the secretion and capture of paracrine vasoactive substances, which includes vasodilator substances (NO, prostacyclin & endothelium-derived hyperpolarizing factor) and vasoconstrictor substances (Endothelin-1, thrombaxane A2 & platelet-activating factor). Endothelial dysfunction appears to play a pathogenic role in the initial development of atherosclerosis (Ross, 1993; Choen, 1995; Schwartz *et al*., 1981) and of unstable coronary syndromes (Okumura *et al*., 1992), and their diverse risk factors viz hypercholesterolemia (Sorensen *et al*., 1994), smoking (Celermajer *et al*., 1993), hypertension (Panza *et al*., 1990), diabetes mellitus (McVeigh *et al*., 1992), family history of premature coronary disease (Clarkson *et al*., 1997), hyperhomocysteinemia (Woo *et al*., 1997) and aging (Egashira *et al*., 1993). Recent clinical studies have demonstrated that, some drugs well known to reduce the incidence of cardiovascular events, improve endothelial function (Mancini *et al*., 1996; Anderson *et al*., 1995; Treasure *et al*., 1995; Husain *et al*., 1998). NO is the main mediator of vasomotor tone regulation in physiological situations, small amounts being continuously secreted by the eNOS (endothelial nitric oxide synthase) (Palmer *et al*., 1987; Vanhoutte *et al*., 1986) to maintain a reduced arterial tone in the systemic and pulmonary circulation (Stamler *et al*., 1994). The vasodilator activity of NO is due to its interaction with the iron atom of the heme prosthetic group of guanylyl cyclase, causing its activation and increasing the intracellular levels of cyclic guanosine monophosphate (cGMP) (Arnold *et al*., 1977). In smooth muscle cells, this decreases intracellular calcium concentration, causing vascular relaxation (Loscalzo *et al*., 1995). NO prevents binding of leucocytes to the endothelium and decreases inflammation, thereby preventing hypertension.NO is removed from circulation mostly by reaction with free radicals, such as superoxide. Various studies suggest that factors influencing level of NO may have important role in

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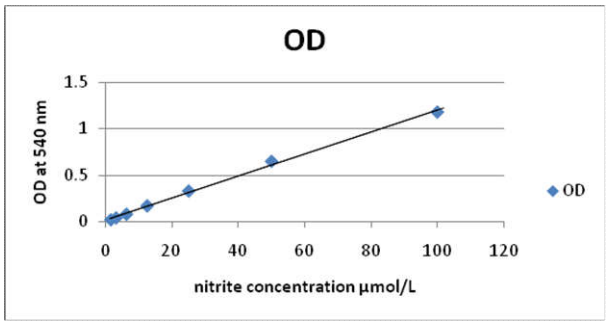
pathophysiology of essential hypertension. Nitrite and nitrates are the oxidative breakdown product of NO [22 and 304] it represents a major storage form of NO in blood and tissues (Bryan, 2006). The most commonly used nitriteassay is based on the Griess diazotization reaction, which isspecific for nitrite and does not detect nitrate. Therefore, nitrate in samples is first be reduced to nitrite; subsequent nitrite determination thus yields nitric oxide index (NOx) that is the total nitrite + nitrate concentration of the sample. Hence, it is proposed to study the level of serum Nitric oxide index in essential hypertension.

MATERIALS AND METHODS

It is a case control study, single centered, prospective and conducted in a tertiary health center over a period of 10 months. 150 unrelated essential hypertensive patients who were on treatment for 5-10 years were selected as cases, which included 131 males and 19 females, of mean age 50.59 ± 10.52 years. Those with secondary hypertension, diabetes mellitus, renal failure, fever, acute infections, chronic inflammatory states, chronic smokers and those on drugs like oral contraceptive pills, steroids were excluded from study group. 130 apparently healthy normotensives from the out-patient department, during their visit for master health checkup were selected as controls. Confounding factors like age, sex, smoking, alcoholism were matched. Standard anthropometric data (height, weight) and resting blood pressure was recorded in each subject, after a thirty minutes rest on a couch. Blood samples were collected by venipuncture after an overnight fasting. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-c) and triglyceride concentration (TGL) were determined enzymatically. Low density lipoprotein cholesterol (LDL-c) was calculated using Friedwald's formula[304]. Cadmium based reduction of nitrate to nitrite followed by estimation of total nitrite by Griess method is used in this study.



Graph 1. Standardisation graph for nitrite

STATISTICAL ANALYSIS

- Age, sex, smoking, alcoholism, BMI, plasma glucose, serum urea, serum creatinine, serum lipid levels were compared between control subjects and patients by students ' t' test and chi-square test (χ^2).
- Serum NOx distribution between cases and controls were compared by student independent t test. $p< 0.05$ was considered significant. Independent variables included in the analysis were age (quantitative), sex (male/female), smoking (yes/no), alcoholism (yes/no), serum levels of glucose, urea, creatinine, cholesterol, triglycerides, HDL (quantitative).

The analysis was executed by SAS Statistical program Version 6.10 for Macintos.

- The influence of other biochemical parameters on serum NOx level was analysed through Pearson correlation.

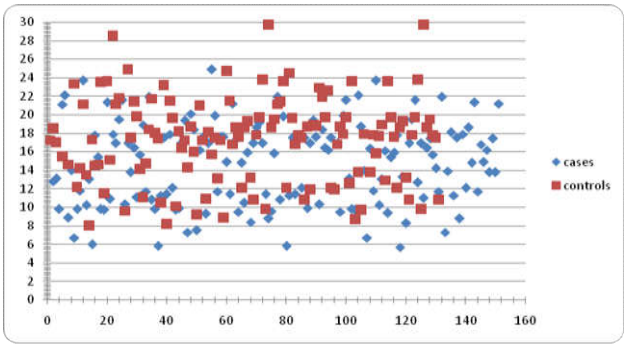
RESULTS

Table 1 & 2: Shows age, sex, BMI and other conventional risk factors distribution in patients and control subjects

	Group				Student independent t-test
	Hypertensives		Control		
	Mean	SD	Mean	SD	
Age	50.59	10.52	51.83	11.60	t=0.94 P=0.36(NS)
Wt	66.23	9.78	64.42	10.39	t=1.51 P=0.13(NS)
Ht	163.78	6.88	162.15	7.75	t=1.86 P=0.06(NS)
BMI	25.13	3.90	24.47	3.41	t=1.47 P=0.14(NS)

		Group				Pearson Chisquare test
		Hypertensives		Control		
		N	%	n	%	
Sex	Male	132	87.4%	118	90.1%	$\chi2$=0.49
	Female	19	12.6%	13	9.9%	P=0.48(NS)
Smoking	No	116	76.8%	96	73.3%	$\chi2$=0.47
	Yes	35	23.2%	35	26.7%	P=0.49(NS)
Alcoholism	No	122	81.5%	104	80.2%	$\chi2$=0.07
	Yes	28	18.5%	26	19.8%	P=0.88(NS)

*Insignificant p value



† Distribution of NO is at lower levels for cases compared to controls

Scatter diagrams 1 represents the distribution of serum NOx level in cases and controls

Table 3. Compares serum NOx levels of cases with controls

	Group				Student independent t-test
	Hypertensives		Control		
	Mean	SD	Mean	SD	
SERUM NOx	14.69	4.45	18.16	7.23	t=4.91 P=0.001***

‡. Statistically significant low NOx levels, with p value of 0.001 was observed in cases (14.69 ± SD 4.45) when compared to controls (18.16 ± SD 7.23)

Table 4 shows the distribution of other biochemical parameters like plasma glucose, serum urea, serum creatinine, lipid profile between hypertensives and controls

	Group				Student independent t-test
	Hypertensives		Control		
	Mean	SD	Mean	SD	
Blood sugar	96.15	6.81	94.69	7.16	t=1.75 P=0.08
S.urea	27.7	5.161	28.1	5.083	t=0.192 p=.848
S.creatinine	.849	.127	.854	0.128	t=.330 p=.742
T.CHOL	162.88	31.13	164.12	42.73	t=0.28 P=0.77
TGL	163.99	48.37	165.09	58.33	t=0.17 P=0.86
HDL	38.04	7.70	44.42	12.00	t=5.38 P=0.001
LDL	96.62	37.44	88.44	37.25	t=1.83 P=0.07

§ HDL levels was found to be significantly lowered in cases

Table 5. Shows the multiple logistic regression analysis

	B	Sig.	Exp(B)	95%CI Lower	Upper
AGE	-.002	.928	.998	.952	1.046
SEX	-.395	.657	.673	.117	3.865
SMOKING	-1.394	.117	.248	.043	1.420
ALCOHOLIC	1.414	.155	4.114	.587	28.846
BMI	.088	.116	1.092	.978	1.220
Bl.SUGAR	.025	.448	1.025	.961	1.093
T.CHOL	-.017	.446	.983	.941	1.027
TGL	.002	.728	1.002	.990	1.015
HDL	.025	.435	1.025	.963	1.090
LDL	.022	.251	1.023	.984	1.062
NOx	.765	.000	2.149	1.759	2.625
Constant	-13.962	.001	.000		

|| It was found that serum NOx level is an independent risk factor for hypertension.

Table 6. Shows pearson correlation analysis

	Type of statistical analysis	Plasma glucose	T.chol	TGL	HDL	LDL	SERUM NOx
Serum NOx level	Pearson Correlation	.070	.028	.157	-.015	-.038	1
Of controls	Sig. (2-tailed)	.429	.751	.073	.865	.669	.
	N	130	130	130	130	130	130
Serum NOx	Pearson Correlation	-.026	.094	-.013	.055	.100	1
Of cases	Sig. (2-tailed)	.749	.251	.872	.503	.223	.
	N	150	150	150	150	150	150

** Correlation is significant at the 0.01 level (2-tailed). It was found that serum NOx level was not influenced by biochemical parameters like plasma glucose and lipid profile.

DISCUSSION

This study was indended to measure serum nitric oxide level (NOx) in essential hypertension and to compare with normal individuals. The hypertensives and controls were perfectly matched with respect to confounding variables like age, sex, BMI, smoking and alcoholism. Those with impaired glucose tolerance, renal failure, acute infections, chronic inflammation and chronic smokers were excluded from the study as these states may present with altered serum NOx level. On comparing serum NOx level of the hypertensives with the controls, it was found to be significantly lowered in hypertensives (14.69 vs 18.16, p= 0.001). It is quite obvious that the serum NO level is an independent risk indicator in essential hypertension, based on this study Previous studies (Afrasyap & Ozturk, 2004; Kumar & Das, 2000: Node, K., Kitakaze, M., Yoshikawa, H., Kosaka, H., & Hori, M. (1997)) also have shown similar supportive results. Node K *et al* (1997), in fact recorded a very similar decline in NO level in hypertensives when compared to controls. NO release from the endothelium, is found to be decreased in patients with established coronary atherosclerosis with hypertension (De Meyer *et al*., 1995).

A reduction in vascular availability of NO determines damage to the endothelium-dependent vasodilation, an increased tendency for platelet aggregation and adhesion of monocytes to the endothelium, thus influencing the proliferation of vascular smooth muscle cells, contributing to the onset and progression of hypertension. Various studies are being conducted to reveal role of various factors influencing nitric oxide level and its activity. Among them S-nitrosothiols, reactive free radicals and ADMA play significant role in modulating nitric oxide activity. S-nitrosothiols are thio-esters of nitrite, and are in steady state of equilibrium with nitrites. *S*-nitrosationhad been implicated in the control of a wide array ofprotein functions and cell activities like regulation of apoptosis, G-protein-coupled receptor based signaling, vascular tone and inflammatory responses (Hess *et al*., 2005).

S-nitrosoglutathione reductase (GSNOR), a member of alcohol dehydrogenase family, has been shown to be the primary pathway through which cells denitrosate intracellular proteins (Liu, 2001). GSNOR has become an important target for developing agents that modulate NO bioactivity inside the cells. The production of superoxide anion and other reactive oxygen species quench NO, thereby reduces its bioavailability (Gryglewski *et al*., 1986). The reduced generation of superoxide and restoration of endothelial-dependent vasodilation appears to be an important mechanism mediating the anti-hypertensive and cardioprotective effects of ACE inhibitors and angiotensin-receptor blockers (Laursen *et al*., 1997). Decreased eNOS expression due to an increase in endogenous inhibitors of nitric oxide synthesis, may be involved in the genesis of endothelial dysfunction (Gail *et al*., 2001). In normotensive animals and humans, administration of methylated arginines, which are competitive NOS inhibitors (Huang *et al*., 1995), caused marked and dose-dependent elevations in blood pressure level. It competes with L-arginine to prevent the synthesis of nitric oxide (Vallance *et al*., 1992) .Studies have shown reduced urinary excretion of NO metabolites and increased plasma levels of ADMA in men with essential hypertension (Vallance *et al*., 1992). Thus, accumulation of this endogenous NOS inhibitor has been hypothesized to contribute to hypertension in patients with chronic renal failure (Wever *et al*., 1999).

Conclusion

The low serum NOx level may be an independent risk factor for essential hypertension. Further studies need to be carried out relating effect of oxidative stress and life style modification in modulating serum NOx level, paving way for newer anti-hypertensive regimens on a long term basis. In future, along with molecular studies, role of epigenetic factors influencing eNOS gene expression can be explored.

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