www.jmscr.igmpublication.org Impact Factor 3.79
ISSN (e)-2347-176x

Journal Of Medical Science And Clinical Research
IGM Publication An Official Publication Of IGM Publication

Reduced Serum Nitrate in Individuals with Primary Hypertension

Authors

Dr Zaheera Sultana S[1], Dr Farquana Qushnood[2], Dr Lakshmi T[3] , Dr Salim A Dhundasi[4], Dr K K Das[5]

[1]Assistant Professor Department of Physiology, Chamrajnagar Institute of Medical Sciences CHAMRAJNAGAR.

[2]Assistant Professor Department of Physiology, Gulbarga Institute of Medical Sciences , GULBARGA.

[3]Assistant Professor Department of Physiology, Chamrajnagar Institute of Medical Sciences , CHAMRAJNAGAR.

[4]Professor & HOD, Department of Physiology, Al Ameen Medical College, Bijapur

[5]Professor, Department of Physiology, Shri B. M. Patil Medical College, Bijapur

E-mail: *dranjumshaik786@gmail.com*

Corresponding Author

Dr Zaheera Sultana S

M.D

Assistant Professor, Department of Physiology,

Chamrajnagar Institute of Medical Sciences, Chamrajnagar. Karnataka, India

ABSTRACT

Background and objectives: *Primary or essential hypertension (PHTN) is the most prevalent form of hypertension accounting 90 % of all cases of hypertension · Evidence suggests that Nitic oxide (NO) plays a major role in regulating blood pressure and that impaired NO bioactivity is an important component of hypertension. Hence this study is taken up to estimate nitrate levels in Primary Hypertension patients compared with healthy individuals.*

Methodology: *The present study was conducted in the Department of Physiology, Al-Ameen Medical College, Bijapur and District Hospital, Bijapur. Thirty five (35) Primary Hypertension Patients (17 male, 18 female) between 35 to 65 yrs age and Thirty nine (39) healthy individuals, controls (20 male, 19 female) between 38 yrs to 65 yrs age visiting Al-Ameen Medical College Hospital, Bijapur and District Hospital Bijapur were selected. Serum Nitrate was estimated by GRIESS METHOD . Statistical analysis was done*

by ANOVA

Results: *Statistically significant variations were found in parameters like age, Ht, Wt, BSA, BMI, PR, SBP, DBP, Serum Nitrate levels in controls and Primary Hypertension patients.*

Interpretation and Conclusion: *In the present study the mean ± SEM of Serum Nitrate in controls was found to be 49.93 ± 1.01 µ mol / lt, and primary hypertension patients was 33.6 ± 0.02 µ mol / lt. . It was found Serum Nitrate levels of PHTN patients were lower when compared with the controls, these difference were found to be statistically significant (t =12.165, p= 0.0000) . Hypertension can produce direct toxic effect on human endothelium; impairment of the release of NO from vascular endothelial cells may thus contribute to the reduced plasma nitrogen oxide concentrations in patients with essential hypertension. . Increased production of superoxide anions, which rapidly deactivate NO, is a characteristic feature of experimental models of hypertension, and plasma indexes of lipid peroxidation are increased in patients with hypertension.*

Keywords: *endothelial nitric oxide synthase, Primary Hypertension, Nitric oxid*

INTRODUCTION

Primary or essential hypertension is the most prevalent form of hypertension accounting 90 % of all cases of hypertension. [1] Reactive nitrogen species comprises of nitric oxide (Nitrate and Nitrite), peroxynitrous acid, S- nitrosothiols. Nitric oxide is a multifunctional effectors molecule that plays a central role in the maintenance of blood pressure, smooth muscle tone, and inhibits platelet and leucocyte adhesion to endothelial cells. iNOS (inducible Nitric oxide synthase), nNOS (neuronal nitric oxide synthase) and eNOS (endothelial nitric oxide synthase) are the three enzymes producing the gas nitric oxide in the human body. eNOS is the main source of NO under physiological conditions .[2]

Nitric oxide (NO) as a second messenger is of importance in the maintenance of blood pressure. Being vasodilator it reduces peripheral resistance. It acts via the heme moiety of guanylyl cyclase, which produces cGMP. This reduces the levels of cytosolic Ca++ and also phosphorylates myosin light chain kinase. NO also decreases the activity of platelets and neutralizes free radicals, thus preventing atherosclerosis. Endothelial dysfunction, which is characterized by impairment of nitric oxide bioavailability, is an important risk factor for hypertension. [2] Evidence suggests that NO plays a major role in regulating blood pressure and that impaired NO bioactivity is an important component of hypertension.[3]

The term “endothelial dysfunction” refers to an impairment of the ability of the endothelium to properly maintain vascular homeostasis. Although the term is often used in reference to a loss of bioavailable nitric oxide , endothelial dysfunction also reflects increased production of vasoconstrictors and disturbed regulation of inflammation, thrombosis, and cell growth in the vascular wall. [4]

Nitric oxide has a major influence on basal arteriolar tone and blood pressure. Interestingly, the contribution of NO to resting tone is greater in larger (>200 μm) than in smaller (resistance;< 200 μm) vessel. NO is formed continuously by endothelial NO synthase (eNOS) in the low nanomolar concentration range.[5,6] .Half-life of NO in blood range from 0.05 to 1.8 milliseconds. [7]An estimation of NO could be included as a routine lab investigation to screen people at risk and to device appropriate therapeutic strategy. Estimating total NO is rather cumbersome. Hence this study is taken up to estimate nitrate levels in Primary Hypertension patients compared with healthy individuals.

MATERIAL AND METHODS

The present study was conducted in the Department of Physiology, Al-Ameen Medical College, Bijapur and District Hospital, Bijapur. Thirty five (35) Primary Hypertension Patients (17 male, 18 female) between 35 to 65 yrs age and Thirty nine (39) healthy individuals, controls (20 male, 19 female) between 38 yrs to 65 yrs age visiting Al-Ameen Medical College Hospital, Bijapur and District Hospital Bijapur were selected. All known Primary Hypertension were studied. Patients diagnosed with causes of secondary hypertension viz pheochromocytoma, hyperthyroidism, coarctation of aorta, renovascular diseases, vasculitis , any liver disorder were excluded. The study protocol was explained to the Primary Hypertension and Controls, who volunteered for the study. Informed consent was obtained from each of the participant .A detailed history of subjects was taken.

GRIESS METHOD- A) Materials

I. Griess reagent [Sulphanil amide, N-(1-Naphthyl) ethylene diamine dihydrochloride)]
II. Vanadium (III) Chloride: 8mg dissolved in DDW upto 1ml.
III. Sodium nitrite($NaNO_2$): 1mM $NaNo_2$/ L
IV. Sodium nitrate ($NaNO_3$): 1mM $NaNO_3$ / L
V. Double Distilled Water (DW)
VI. Ethanol

PROCEDURE

Blood (5 ml) for analysis was obtained from the antecubital vein of the primary hypertension and type II Diabetic patients with hypertension as well as from the controls. Blood was allowed to clot and serum was separated by centrifugation at 2500 rpm for 15 minutes.

Serum;

Deproteinization: (Serum : Ethanol, 1:2)

↓

500μl serum + 1000μl Ethanol (1ml)

↓

Vortexed well for 2 to 3 min

↓

Centrifuge (10000rpm for 10min)

↓

Take 0.5mL Supernatant

The supernatant was taken for Nitric oxide determination. 500μl of supernatant was mixed

with 500µl of vanadium chloride. [Vanadium chloride acts as a chemical catalyst, which leads to reduction of sodium nitrate to sodium nitrite], 500µl of Greiss reagent was added into the mixture. Mixed well by vortexing it for 1 to 2 min. This sodium nitrite reacts with Griess reagent. [8]

Sl no	Reagent	Test	Blank	
1	Supernatant	500µl	-	INCUBATION FOR 30 MIN AT 37 ^{0} C
2	DDW	-	500µl	
3	VCl3	500µl	500µl	
4	Greiss Reagent (Sulphanil amide + NED)	500µl (250µl+250µl)	500µl (250µl+250µl)	

Finally the absorbance of the product read spectrometrically by using 540nm filter.

The concentration of Nitric oxide in serum sample was determined from standard curve established by 0 to 120µmol/L of sodium nitrate. By taking OD of the serum sample SERUM NITRATE are calculated by using the following formula from the standard curve. [9]

$$\text{NITRATE} = \frac{\text{OD} + 0.0076}{0.0058}$$

RESULTS

Thirty five Primary Hypertension patients between 35 to 65 years were selected for the study. Thirty nine healthy individuals, controls between 38 to 65 yrs age from Bijapur city were the volunteers. All Primary Hypertension patients underwent history taking and a thorough clinical examination. The ANOVA was used to analyse the variation in the parameters of controls, Primary Hypertension patients $P < 0.05$ was considered as a level of significant in all the statistics tests.

Table.No.1: Shows the mean ± SEM of Age, Ht, Wt, BMI, BSA in controls and PHTN patients

PARAMETER	CONTROLS (n = 39) MEAN±SEM	PHTN patients(n = 35) MEAN±SEM	ONE WAY ANOVA
Age (yrs)	53.69 ± 1.69	56.97 ± 0.358	P =0.0757 (NS)
Ht (cms)	154.38± 0.905	157.83 ± 1.79	P = 0.0820 (NS)
Wt (kgs)	51.51 ± 1.01	64.51 ± 1.19	P= 0.0000 (S)
BMI (kg/m^2)	21.61 ± 0.174	25.91 ± 0.111	P= 0.0000 (S)
BSA (m^2)	1.48 ± 0.018	1.65 ± 0.026	P = 0.0000 (S)

Table.No.2: Shows the mean ± SEM of PR, SBP, DBP, Serum Nitrate in controls and PHTN patients

SL No	PARAMETER	CONTROLS (GROUP 1)	PHTN patients (GROUP 2)	ONE WAY ANOVA
		MEAN ±SEM	MEAN ±SEM	
1	PR (bpm)	74.63 ±0.113	80.77 ±0.119	P=0.0000 9 (S)
2	SBP (mmHg)	123.12 ±1.58	157.08 ±1.91	P= 0.0000 (S)
3	DBP (mmHg)	79.64 ±0.451	97.48 ±0.717	P= 0.0000 (S)
4	Serum Nitrate (µmol / lt)	49.93 ±1.01	33.6 ± 0.02	P= 0.0000 (S)

A. Physical Parameters:

Table No. 1 shows the mean ± SEM of age, Ht, Wt, BSA, BMI in controls and Primary Hypertension patients. The height of Primary Hypertension patients was found to be numerically more than the controls and was found to be not significant statistically (t = — 1.764 , p = 0.0820) when compared with controls . The weight PHTN patients was more than the controls and which were statistically significant (t = — 10.0000 , p = 0.0000) . The mean value of BMI and BSA of PHTN patients was more as compared to that of controls. There was a statistically significant difference (t = — 11.5222, p = 0.0000) & (t = — 6.203 , p = 0.0000) respectively.

B. Physiological Parameters:

Table No. 2 Shows the mean ± SEM of PR, SBP, DBP controls and Primary Hypertension patients. The PR of PHTN patients was found to be more compared to that of controls and which was significant statistically (t = — 9.227, p = 0.0000) . The mean value of SBP levels PHTN patients Where higher when compared with the controls, this difference was found to be statistically significant (t = — 15.698, p = 0.0000) . The mean value of DBP levels in PHTN patients where higher when compared with the controls, this difference was found to be statistically significant (t = — 15.431, p= 0.0000).

C. Biochemical Parameters

Table No. 2 Shows the mean ± SEM of Serum Nitrate in controls was found to be 49.93 ± 1.01 µ mol / lt, PHTN patients was 33.6 ± 0.02 µ mol / lt. It was found Serum Nitrate levels of PHTN patients were lower when compared with the controls, these difference were found to be statistically significant (t =12.165, p= 0.0000) .

DISCUSSION

In the present study the mean ± SEM of Serum Nitrate in controls was found to be 49.93 ± 1.01 µ mol / lt, and primary hypertension patients was 33.6 ± 0.02 µ mol / lt. . It was found Serum Nitrate levels of PHTN patients were lower when compared with the controls, these difference were found to be statistically significant (t =12.165, p= 0.0000) .

In the study entitled "Reduced Plasma Concentrations of Nitrogen Oxide in Individuals with Essential Hypertension" by Koichi Node et al., in 1997 conducted on 108 Hypertension patients (78 men and 30 women with a mean± SEM age of 49±3 years). A total of 127 normal subjects (81 men and 46 women) aged 50±3 years were matched with the patients for sex and approximate age and served as the control group. The mean ± SEM of plasma nitrogen oxide (nitrate plus nitrite) in Essential Hypertension patients was 15.7 ± 1.1 mmol / L and that of controls was 22.8 ± 1.4 mmol / L . The plasma concentration of NO was reduced in individuals with essential hypertension relative to that in control subjects and which was significant statistically ($p < 0.001$). [10]

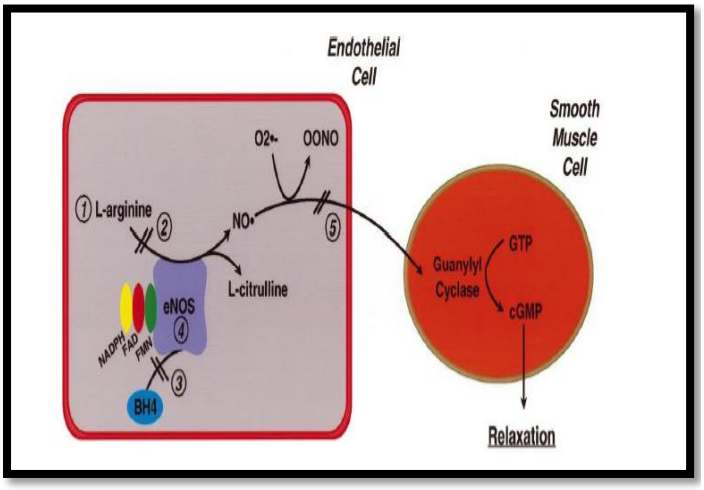
Shubhangi Arora, et al., conducted a study entitled "Nitric Oxide and eNOS Gene in Essential Hypertension " in 2009 on 45 patients (selected from the department of Cardiology All India Institute of Medical Sciences, ages between 25 to 55 yrs and not on any antihypertensive medications) and 45 controls (healthy volunteers with normal blood pressure, ages between 25 to 55 yrs. The mean ± SEM of NO in the patients with Essential Hypertension was 4.0 +/-1.7 µM and that of controls was 6.7 +/-3.2 µM .The NO in patients with Essential Hypertension was 42% less than that of the controls. The difference is highly significant ($p<0.001$). [2]

It has been seen that SBP and DBP inversely correlated with plasma nitrate owing to the decline of antioxidative activity (lipid peroxidation enhanced by the lack of antioxidant activities) which was associated with decreased NO production and the severity of hypertension.

In the present study it was found Serum Nitrate levels of Primary Hypertension patients were lower when compared with the controls, these difference were found to be statistically significant ($t =12.165$, $p= 0.0000$) .

The plasma concentration of nitrogen oxide in systemic venous blood is determined by synthesis, degradation, and clearance of NO. Daily activity and the consumption of food or water may also affect nitrogen oxide concentration. As for the synthesis of NO, NO is continuously synthesized from L-arginine in a reaction catalyzed by NO synthase, with most NO present in the circulation originating from endothelial and smooth muscle cells. Hypertension can produce direct toxic effect on human endothelium; impairment of the release of NO from vascular endothelial cells may thus contribute to the reduced plasma nitrogen oxide concentrations in patients with essential hypertension. Decreased synthesis of NO might also result from abnormal handling of intracellular calcium and a consequent reduction in the activity of NO synthase. Hypertension impairs endothelium-dependent dilation of rat coronary arteries as a result of superoxide anion–mediated degradation of NO. Indeed, increased production of superoxide anions, which rapidly deactivate NO, is a characteristic feature of experimental models of hypertension, and plasma indexes of lipid peroxidation are increased in patients with hypertension .[10]

Potential mechanisms of decreased NO activity in essential hypertension:



I. Decreased availability of the substrate L arginine;
II. Antagonism of NO biosynthesis (e.g. by increased levels of asymmetric dimethylarginine [ADMA], reduced dimethylarginine dimethylaminohydrolase [DDAH]);
III. Decreased availability of cofactors (e.g. reduced generation of tetrahydrobiopterin [BH4]);
IV. Decreased activity of eNOS (e.g. reduced expression; decreased stimulation; phosphorylation,myristoylation, palmitoylation); and
V. Increased degradation of NO. [11]

ACKNOWLEDGEMENT

Authors would like to thank all the participants of the study. Authors also acknowledge the great help received from the scholars whose articles cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES

1. K.Park. Park's text book of preventive and social medicine. 20th Edn. India: M/s Banarsidas Bhanot Publishers; 2009. 325-343.
2. Arora S, Das N, Srivastava K. Nitric Oxide and eNOS Gene in Essential Hypertension. International Journal of Collaborative Research on Internal Medicine and Public Health (IJCRIMPH). 2009;1(2):56-71.
3. Hermann M, Flammer A, Lüscher TF. Nitric oxide in Hypertension. The Journal of Clinical Hypertension. 2006;8:17-29.
4. Tabit CE, Chung WB, Hamburg NM, Vita JA. Endothelial dysfunction in diabetes mellitus: molecular mechanisms and clinical implications. Reviews in endocrine & metabolic disorders. 2010;11(1):61-74.
5. Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolio AP, Cockcroft JR. Nitric oxide regulates local arterial distensibility in vivo. Circulation. 2002;105(2):213-7.
6. Kelm M, Preik-Steinhoff H, Preik M, Strauer BE. Serum nitrite sensitively reflects endothelial NO formation in human forearm vasculature: evidence for biochemical assessment of the endothelial l-arginine–NO pathway. Cardiovascular research. 1999;41(3):765-72.

7. Rassaf T, Preik M, Kleinbongard P, Lauer T, Heiß C, Strauer BE, et al. Evidence for in vivo transport of bioactive nitric oxide in human plasma. Journal of Clinical Investigation. 2002;109(9):1241-8
8. Han Moshage, Bart KoK, Johannes R, Huizenga, and Peter L.M.Jansen. Nitrite and Nitrate determinations in plasma: a critical evaluation. Clin chem. 1995: 892 - 896.
9. Ghasemi A, Hedayati M, Biabani H. Protein precipitation methods evaluated for determination of serum nitric oxide end products by the Griess assay. Journal of Medical Sciences Research. 2007;2(0):29-32 .
10. Node K, Kitakaze M, Yoshikawa H, Kosaka H, Hori M. Reduced plasma concentrations of nitrogen oxide in individuals with essential hypertension. Hypertension. 1997;30(3):405-8.
11. Cardillo C, Panza JA. Impaired endothelial regulation of vascular tone in patients with systemic arterial hypertension. Vascular Medicine. 1998;3(2):138-44.