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Comparison of Serum Arginine, Nitric Oxide Reactive Species in Hypertensive Patients with and Without Microalbuminuria

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ABSTRACT

Aim of the study: Hypertension (HTN) is associated with deranged endothelial dysfunction. The level of serum Nitric oxide affects the endothelial function & is synthesized from Arginine. Hypertension leads to clinical proteinuria & a significant reduction in renal function. Microalbuminuria indicates the renal status, The objective of this study is to find out the difference between serum Arginine, Nitric oxide in patients with hypertension with microalbuminuria & without microalbuminuria and the relation between NO & microalbuminuria.

Material & Method: The study included 62 patients with hypertension without microalbuminuria, 60 patients with hypertension with microalbuminuria and 60 healthy controls were included in our study. The levels of all biochemical parameters, arginine, NO, urinary albumin were estimated and compared .

Result: Serum Arginine, NO levels show significant decrease in both hypertensive patients without & with microalbuminuria when compared with control. There is a significant increase in urinary albumin level in hypertensive patients with microalbuminuria. There exist a negative correlation between NO & urinary albumin in hypertension with microalbuminuria.

Conclusion: As L-Arginine is only source of NO, so therapeutic role of Arginine may be contemplated.

Key words: Hypertension, NO, Microalbuminuria.

INTRODUCTION

As per JNC-9 (Joint National Committee-9) systolic pressure of <120 mm of Hg & diastolic pressure <80 mm of Hg is considered as normal. Blood pressure >140 mm of Hg systolic or > 90 mm of Hg diastolic is considered as hypertensive. [1] Hypertension (HTN) is very common, a very serious condition can lead to development of complications as it is a silent killer as if often remain asymptomatic. [2]

As per WHO report 2016, globally the overall prevalence of hypertension is 24.1% in adult males & 20.1% in adult females. [3] High blood pressure is the third important risk factor for attributable burden of disease in South Asia. The prevalence of hypertension in Indian population may go up to 22.09 & 23.6 percentage wise in India men & woman respectively by 2025. About 25%urban & 12% rural Indians are hypertensive. [4]

Essential or primary hypertension has no known cause. More than 90% of individuals with hypertension have essential hypertension. Genetic factor may play an important role in the development of essential hypertension.[5] Secondary hypertension is due to renal causes resulting from chronic kidney disease or renovascular disease. [6]

Due to complex nature of hypertension it is determined by interaction of multiple genetic, environmental & demographic factors that influence the

hemodynamic variables: cardiac output & total peripheral resistance. [7] Vasoconstriction & vasodilatation influence the normal vascular tone. Vasoconstrictors are angiotensin II & catecholamines & vasodilators are kinins, prostaglandins & nitric oxide. [8]

Nitric oxide (NO) is diatomic free radical, short lived lipid soluble easily passes between cell membrane. [9] NO is an important regulator, mediators & play important role in the nervous, immune, & cardiovascular system. These include vascular smooth muscle relaxation resulting in arterial vasodilatation & increasing blood flow. [10] It is synthesized from amino acid L-Arginine catalyzed by NO synthase (NOS). [11]

It interacts with molecular targets in cell in vascular wall & lumen including membrane receptors. NO activates soluble guanylate cyclase in vascular smooth muscle cells leading to the activation of cGMP causes activation of cGMP-dependent protein kinase & cause vasodilatation. [12]

Arginine a basic amino acid semi essential in nature as it can be synthesized from Glutamine, Glutamate & Proline. [13] NO derived from L-Arginine shifted the focus towards the potential role in cardiovascular control. Many studies revealed that Arginine is a key player in a set of physiological functions that interact to maintain vascular health & homeostasis. [14] Various past studies have suggested that Arginine supplement decreases blood pressure & improve vascular function. There is an increase in incidence in blood pressure & endothelial dysfunction due to deficiency or lack of availability of Arginine. [15]

A frequent complication of HTN is renal damage which is ascertained by estimating urine microalbumin. A urinary excretion of albumin of 30-300 mg/day or 20-200µg / min is called microalbuminuria and an earlier sign of vascular damage. Now-a-days it is considered as a predictor of worse outcome for both kidney & heart patient. [16]

There exists a significant correlation between blood pressure & microalbuminuria. It is also even associated with high normal blood pressure & biomarker of increased cardiovascular risk. Microalbuminuria is generally the first clinical sign of renal dysfunction in diabetic & hypertension. Therapy either prevents or delays the development of microalbuminuria & delay the end organ damage. [16]

There are various postulations regarding the development of microalbuminuria in essential hypertension, like changes in renal hemodynamics, change in selective permeability, insufficient tubular reabsorption & structural damage to glomeruli & arterioles. [17]

This study was under taken to evaluate the Serum Arginine & nitric oxide reactive species & to find out the correlation between serum Arginine, NOS & urinary albumin level.

MATERIAL & METHODS

A case control study was conducted in the Dept. of Biochemistry, SCB Medical College, Cuttack in collaboration with Dept. of Medicine. The study was approved by IEC and written consents were taken.

A total of 182 subjects were selected. They were divided in to three groups. Group-I (60 numbers) were chosen as control having normal blood pressure & no microalbuminuria .Group-II (62 number) were chosen hypertensive without microalbuminuria & Group-III (60 numbers) were hypertensive with microalbuminuria.

Subjects suffering from endocrinal disorder, cerebrovascular disease, CKD, autoimmune disorder, smokers & alcoholics were excluded from the study.

Collection of Sample

In sitting position the blood pressure of all subjects was measured after taking 15 min rest. 5 ml of fasting venous blood was collected taking aseptic measure. Of which

4 ml was kept in clot activator vacutainer for biochemical analysis & 1 ml was transferred to oxofluoride tube for plasma sugar estimation. 5 ml of urine was collected in a sterile urine container for urinary albumin measurement.

A) Fasting plasma sugar, serum lipid profile, urea, creatinine, were estimated by using standard commercial kits adopted to autoanalyzer.

B) Estimation of Serum Arginine

Purified human Arginine antibody used to coat microtiter plate wells which were solid-phase antibody. Samples were added to well antigen (Arginine in serum) antibody – antigen complex was formed. This complex forms antibody-antigen-enzyme antibody complex (sandwich technique) after addition of HRP-conjugate solution. When TMB substrate was added to the wells HRP catalyses the reaction so that TMB-substrate produced blue colour. This reaction was terminated by addition of sulphuric acid solution which changed the colour from blue to yellow. This was measured spectrophotometrically at 400 nm.

C) Estimation of serum Nitrites by Griess method [18]

Griess reaction involves formation of a chromogen during the reaction of nitrite (NO_2) with sulphanilamide & heterocyclic amine of naphylene-ethyleme-diamine,(Griess regent) under condition of low pH. During this reaction acidified nitrite undergo ditozation with sulfanilamide to form diazonium salt. This diazonium salt then complex to N(1-naphthyl) ethylenediamine to form a magenta colored compound which was measured at 540 nm'

D) Measurement of urine albumin (Pyrogallon Red method)

Protein combines with pyrogallon and molybdate in acidic medium to form a blue purple coloured complex. Intensity of colour formed was directly proportional to the amount of protein present in the sample compared with standard at 600 nm.

Statistical Analysis

All data were expressed in mean ± SD. The statistical analysis was carried out by using one way ANOVA {post hoc test) to find out test of significance & Pearson's correlation was done by using SPSS version 20.0. The 'p' value <0.05 was considered to be significant.

RESULTS

Demographic characteristic

All the demographic character of different study groups are listed in table no-1. There was no significant difference in mean age. The table shows the incidence of hypertension is more in males than females.

Clinical Findings

The recording of blood pressure was done in sitting position.

a) SBP: As shown in table-II, the systolic blood pressure was significantly raised in group-II & group-III study populations as compared to control (group-I) population ($p < 0.001$). But there was no significant difference between group-II & group- III study population.($p < 0.93$)

b) DBP: Similarly diastolic blood pressure was significantly raised in group-II & group-III study populations ($p < 0.001$) as compared to control (group-I) population, while there was no significant increase in diastolic pressure between group-II & group-III patients.

Biochemical Parameters

Table -III shows the comparison between biochemical parameters among the groups

There was no significant difference in fasting plasma sugar level between different groups.

Serum urea level showed no significant difference between group-I & group-II study population, and also between group-II and group-III population, but there was significant difference between group -I and group -III study population.

No significant difference in value of serum creatinine was observed between different groups There was a significant difference in serum LDL level in group-II & III(increase} when compared with group-I population and also between group-II & group-III(increase} study population.

Serum VLDL level was significantly increase in both group-II & group-III when compared with group-I study population. But there was no significant difference between group-Ii & group-III study population. Table-IV shows the comparison of special parameters between different groups, which showed a significant decrease in serum arginine level in group-II & group-III when compared to group-I study population. But there was no significant difference between group-II & group-III study population.

There was significant decrease in serum NO level among group-II & group-III population compare to group-I population as well as group-III when compared with Group-II study population.

There was no significant deference in urinary albumin level among group-I and group-II study population. But group-III population showed a significant decrease as compared to group-I and group-II study population. Pearson correlation analysis was done to evaluate the relation between serum NO & urinary albumin. We observed a negative correlation which was significant.

Table-1 : Demographic characteristics of different study group.

Characteristics	Control (n=60) (Group-1)	HTN without microalbuminuria (n=62)(Group-II)	HTN with microalbuminuria (n=60)(Group-II)
Age (in Year)	48.98±8.06	48.30±5.30	47.83±5.79
Sex			
Male	35	43	40
Female	25	19	20

Table-II: Compression of Systolic blood pressure (SBP) diastolic blood pressure (DBP) between different group.

Parameter	Control (n=60) (Group-1)	HTN without microalbuminuria (n=62)(Group-II)	HTN with microalbuminuria (n=60)(Group-II)	'p' value
SBP (mm of Hg)	130.23 ± 5023	157.82 ± 7.20	158.23 ± 7.3	* p<0.001 ** p<0.001 *** p<0.93
DBP (mm of Hg)	81.61 ± 4.27	± 7.74	95.03± 4.30	P<0.001 ** p<0.001 *** p<0.93

*Significance between group-I & group-II, ** significance between group-I & group-III
*** significance between group-II & group-III

Table-III: Comparison of different biochemical parameters between the study groups

Parameter	Control (n=60) (Group-1)	HTN without microalbuminuria (n=62)(Group-II)	HTN with microalbuminuria (n=60)(Group-II)	**'p' value**
FPG (mg/dl)	97.56 ± 10.76	95.72 ± 11.61	93.75 ± 11.11	*p<0.63 **p<0.15 ***p<0.59
Urea (mg/dl)	21.28 ± 5.48	20.50 ± 4.56	23.6.26	*p<0.70 **p<0.09 ***p<0.01
Creatinine(mg/dl)	0.93 ± 0.26	0.96±025	1.00±0.27	NS
Cholesterol (mg/dl)	180.8±21.52	224.74±22.56	235.66±26.54	*p<0.001 **p<0.001 ***p<0.03
Tg (mg/dl)	119.85±46.21	210.04±29.25	206.58±50.66	*p<0.001 **p<0.001 ***p<0.88
HDL (mg/dl)	49.46±7.98	50.53±7.06	5006±6.65	NS
LDL (mg/dl)	107.51±18.94	132.64±17.85	145.31±23.6	*p<0.001 **p<0.001 ***p<0.02
VLDL (mg/dl)	23.73±9.12	41.08±4.80	40.50±6.59	*p<0.001 **p<0001 ***p<0.92

*Significance between group-I & group-II,
** significance between group-I & group-III,
*** significance between group-II & group-III

Table-IV Comparison between special parameters among different groups

Parameter	Control (n=60) (Group-1)	HTN without microalbuminuria (n=62)(Group-II)	HTN with microalbuminuria (n=60)(Group-II)	'p' value
Arginine(ng/ml)	100.41±74.75	27.47±2.4	15.85±2.09	*p<0001 **p<0001 ***p<0.338
NO(µmol/lt)	25.49±3.03	7.93±3.41	4.93±077	*p<0.001 **p<0.001 ***p<0.001
Urinary albumin(mg/dl)	10.22±6.87	11.28±3.46	172.4±48.65	*p<0976 **p<0001 ***p<0.001

*significance between group-I & group-II, ** significance between group-I & group-III
*** significance between group-II & group-III

Table-5: Correlation of Serum NO with Urine albumin in hypertensive with microalbuminuria

Parameter	'r' value	'p' value
NO(µmol/dl) vs Urine albumin(mg/dl0	-0.557	0.001

DISCUSSION

Hypertension (HTN) is very common, a very serious condition can lead to development of complications as it is a silent killer as if often remain asymptomatic. [2] Essential hypertension that produces clinical proteinuria & significant reduction in renal function. Urinary microalbuminuria is a marker of renal function status & independent predictor of cardiovascular morbidity and mortality. Risk of of developing renal failure, ischemic & hemorrhagic stroke & peripheral arterial disease is doubled. [16]

In normal condition, NO pathways, RAAS & insulin are major functions involved in a complex biochemical network, any alteration can lead to hypertension. [15]

Arginine, only source of NO is a key player in physiological functions that maintain vascular health & homeostasis inhibit ACE activity. Lack of Arginine has the potential effect to cause rise in blood pressure. [13]

We observed that blood pressure of group-II population (urine albumin excretion within normal limit) & in group-III (urine albumin excretion within 30-300 mg/dl) were in stage -1 & stage-2 as JNC-9 criteria (table-II). Hence severity of hypertension leads to increase excretion of albumin in urine. Jose L et.al. also documented the similar finding. [17]

Serum urea level showed no significant increase in group=II & group-III population with respect to group-I p<0.709 &p<0.09 respectively. But there was a significant rise in group-III study population when compared with group=II population, (p<0.01). There was no significant alteration in creatinine level between groups. Macroalbuminuric hypertensive cases have higher mean urea value than normo - albuminuric hypertensive patients. (table-III).

The study on lipid profile (Table-III) there was significant rise in value of cholesterol in group-II & group-III (p<0.001) in compared to group-I (control). There also observed significant rise in group-III (p<0.001) when compared with group-II. Our finding of hypercholesterolemia corroborates the study by Alan Daugherty et,al, [19] They demonstrated that hypercholesterolemia exerts stimulatory effect on angiotensinogen that activates RASS & increase in vascular tone. Olivier Fenon et.al. proposed the inhibitory effect of hypercholesterlemia in synthesis of nitric oxide. They have described elevated level of LDL – cholesterol stimulates production of caveolin & promotes its inhibitory interaction with endothelial nitric oxide synthase. [20]

The value of LDL showed a significant rise in group-II & group-III population (p<0.001) compared to group-I population and also between group-III (p<0.02) within group II.

There was significant rise in Tg level in group-II & group-III populations (p<0.001) as compared to group-I population, while there was no significant difference between group-II & group-III

population. (table -III) There was no significant alteration in HDL level.

The concentration of Arginine values were significantly lower in group-II & group-III population ($p<0001$) as compared to group-I population. The mean value is lower in group-III than group-II population. Hence lower in arginine level may be a cause of hypertension & endothelial dysfunction. This is supported by the study conducted by Perticone et.al. [21]

In our study mean serum concentration of nitric oxide values were found to be significantly reduced in group-II & group-III ($p<0.001$) as compared to control, Similar finding was found group-III ($p<0.001$) when compared to group-II population. Same observation was reported by Raymond et.al.. [22] So reduced value may be cause of hypertension.

Comparison of mean urine albumin level showed a rise in hypertensive group with microalbuminuria compared to control group, similar results were also obtained by Mattei P et.al. [23] Microalbuminuria is generally the first clinical sign of renal dysfunction. Albumin excretion rate has been the mainstay for early detection of nephropathy. [24]

There was a significant negative correlation between serum NO & urine albumin with 'r' value of -0.55 & p vale 0.001. Our finding is in accordance with Denver et.al. [25]

CONCLUSION

The study shows a positive correlation between arginine & NO as well as a negative correlation between NO & microalbuminuria. So consideration of therapeutic role of Arginine in hypertensive patients can be contemplated with large & multicentric studies.

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