Section: Biochemistry

Original Research Article

STUDY ON SERUM NITRIC OXIDE AND HYDROGEN SULPHIDE IN ESSENTIAL HYPERTENSION IN A TERTIARY CARE HOSPITAL

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Abstract

Background: This study aims to demonstrate the serum levels of nitric oxide and hydrogen sulphide in patients with essential hypertension and normal healthy subjects. The study also aims to find whether any correlation exists between serum NO and H2 S levels in overall study population. **Material and Methods:** We conducted this hospital based cross-sectional comparative study over a period of 1 year from June 2022 to May 2023 in a tertiary care hospital in the State of Bihar in India. The study commenced after obtaining proper Institutional Ethics Committee approval. The required sample size was calculated as 50 cases and 50 controls rounding to the next nearest number, using the formula suggested for case-control studies. The fasting blood samples collected in a clotted vial were centrifuged at 3500 rpm for 30 minutes, to obtain the serum. The serum samples were stored at -20°C for further analysis. **Results:** The required sample size was calculated as 50 cases and 50 controls rounding to the next nearest number, using the formula suggested for case-control studies. There were 28 (56%) females and 22 (44%) males in the cases, whereas 26(52%) females and 24 (48%) males in controls. The mean serum NOx level in essential hypertension cases is 44.45±10.09 which is significantly lower (p – value <0.001) than that of controls where it is 165.40±15.63. The mean serum H2S level in essential hypertension case 45.60±10.01 which is again significantly lower (p – value <0.001) than that of controls where it is 111.53±8.60. **Conclusion:** The present study demonstrates a reduction in both the levels of serum nitric oxide and hydrogen sulphide in hypertensive patients than in normotensive controls. There also exists a significant positive correlation between the serum levels of NO and H2 S in overall study subjects.

INTRODUCTION

Globally essential hypertension is a major modifiable risk factor for cardiovascular disease, despite a lot of research into its underlying pathophysiology and availability of widespread therapeutic strategies. Clinically, hypertension is defined as the level of blood pressure at which the institution of antihypertensive therapy reduces morbidity and mortality. Hypertension is defined as systolic BP ≥ 140 mm Hg and diastolic BP ≥ 90 mm Hg.[1] Essential, primary or idiopathic hypertension refers to the rise in blood pressure without the presence of any secondary causes like renovascular or endocrinal diseases. Although the exact etiology of essential hypertension is obscure, it is considered to be a multifactorial disease arising due to a combination of genetic, environmental and behavioural factors like obesity, insulin resistance, sedentary lifestyle, stress, high salt or alcohol intake are responsible for its development.[1,2]

Endothelial dysfunction has an important role in pathophysiology in the rise in arterial blood pressure. Many studies in the past have demonstrated that abnormal endothelial function is a main underlying cause in experimental models of chronic hypertension.[3] Endothelium-mediated vasodilatation is markedly impaired in patients with essential hypertension.[4]

Endothelium-derived nitric oxide acts as an important biological mediator in various physiological and pathological processes including cardiovascular diseases like hypertension.[5] Endothelium-derived nitric oxide inhibits the

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synthesis and action of endothelin which is a potent vasoconstrictor.[6]

Nitric oxide is endogenously produced in the human body from L-arginine by endothelial nitric oxide synthase. The NO stimulates guanylyl cyclase to form 3′, 5′-cyclic guanosine monophosphate (cGMP), which causes vasodilatation of the vascular smooth muscles.[7] A previous study showed that an increase in cGMP resulted in reduced calcium influx into cells and vasorelaxation.[8] While a recent study states that NO produces vasodilatation by activation of calcium-dependent K-channels in vascular smooth muscle cells.[9] Mild hypertension develops in transgenic mice deficient in endothelial nitric oxide synthase.[10] Some studies have shown that eNOS gene mutations are more prevalent in patients with essential hypertension than in normotensive persons.[11] Whereas other studies have no significant association between eNOS genotype and hypertension.[12] In hypertension, nitric oxide bioavailability is reduced due to scavenging of NO by reactive oxygen species in circulation, defects in the nitric oxide synthesis pathway, specific eNOS gene mutations, reduction in cofactors required for NO synthesis or due to increased concentration of circulating NO inhibitors.[13,14,15] Nitric oxide also demonstrates vasoprotective and anti-atherosclerotic properties, including protection from thrombosis, reduction of adhesion molecule expression and leukocyte adhesion.[16]

Hydrogen sulphide is another gaseous transmitter that is produced endogenously in the mammalian tissues from L-cysteine mainly by 3 enzymes: cystathionine β-synthetase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptosulfurtransferase. Non-enzymatic production of H2 S occurs through glucose, glutathione, inorganic and organic polysulfides (present in garlic) and elemental Sulphur.[17] H2 S induces vasodilatation through alteration of the K-ATP channel activity and an increase in cGMP concentration in the vascular smooth muscle cells.[18] By acting as a relaxant of vascular smooth muscle or vasodilator, it regulates cardiac function and can be used for cardiovascular therapeutic approaches.[19] A study by Yang et al., illustrated the role of H2 S in the development of hypertension in mice deficient in Cystathionine ϒ-lyase (CSE).[18]

Some previous studies demonstrated that polysulfide's present in garlic cause vasorelaxation of rat aortic rings through a H2 S dependent mechanism.[20] One experimental study proved that low doses of the H2 S donor sodium hydrogen sulphide (NaHS) produced short-lived relaxation to the mesenteric artery and intestinal contractility.[21] In conclusion, hydrogen sulphide acts as an effective vasodilator and helps in the reduction of blood pressure, but more studies are required to understand the specific cellular and signalling mechanisms regulating these responses.

The physiological and biochemical interactions of the two endogenously present gaseous transmitters, NO and H2 S, are dubious. A previous study illustrated both the gases act synergistically for their production and physiological action. Whereas other studies have illustrated that NO and H2 S inhibit each other's synthesis and action. There exists a common signalling pathway through which these two molecules are involved in a cascade of chemical reactions to generate reactive intermediates that mediate vasodilatation, vascular remodelling and angiogenesis. Another study showed that NaHS, a H2 S donor, increases nitric oxide production in cultured endothelial cells by inducing endothelial nitric oxide synthase.[22] Hence further studies are required to fill this lacuna in understanding the complex interrelationship between the biological actions of these two endogenous gas transmitters which may help to elucidate the significant potential of their interaction in various physiological and pathological processes.

This study aims to demonstrate the serum levels of nitric oxide and hydrogen sulphide in patients with essential hypertension and normal healthy subjects. The study also aims to find whether any correlation exists between serum NO and H2 S levels in overall study population.

MATERIALS AND METHODS

We conducted this hospital based cross-sectional comparative study over a period of 1 year from June 2022 to May 2023 in a tertiary care hospital in the State of Bihar in India. The study commenced after obtaining proper Institutional Ethics Committee approval.

The required sample size was calculated as 50 cases and 50 controls rounding to the next nearest number, using the formula suggested for case-control studies.

Inclusion Criteria

- Newly diagnosed cases with BP ≥ 140/90 mm Hg and not on any antihypertensive medication.

Exclusion Criteria

- Patients suffering from secondary causes of hypertension, renal or endocrinal disorders, pregnancy, preeclampsia, cancer, diabetes mellitus, autoimmune disorders and patients taking antihypertensive medication or any nitric oxide or hydrogen sulphide modulating drugs Fasting blood samples were collected from the cases and controls under aseptic conditions after obtaining informed consent.

Laboratory Analysis

The fasting blood samples collected in a clotted vial were centrifuged at 3500 rpm for 30 minutes, to obtain the serum. The serum samples were stored at -20°C for further analysis.

The method for the indirect determination of NO involves the spectrophotometric measurement of its stable and non-volatile decomposition products, nitrates (NO3) and nitrites (NO2). This assay relies on a diazotization reaction that was originally described by Griess in 1879. In the Griess reaction,

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dinitrogen trioxide generated from the acid catalyzed formation of nitrous acid from nitrite, reacts with sulphanilamide to produce a diazonium ion which is then coupled to N-(1-napthyl) ethylenediamine dihydrochloride (NED) under acidic conditions to produce a red–violet coloured water-soluble azo dye whose absorbance is measured spectrophotometrically at 540nm. The nitrate in the serum is reduced to nitrite with cadmium catalyst and then measured by the Griess reaction.

Serum levels of H2 S were measured by the reaction of sulphide with N, N-dimethyl-p-phenylenediamine sulphate in the presence of oxidizing agent Fe^{3+} in hydrochloric acid to generate methylene blue whose absorbance was read at 670 nm in a spectrophotometer.

RESULTS

The required sample size was calculated as 50 cases and 50 controls rounding to the next nearest number, using the formula suggested for case-control studies. There were 28 (56%) females and 22 (44%) males in the cases, whereas 26(52%) females and 24 (48%) males in controls.

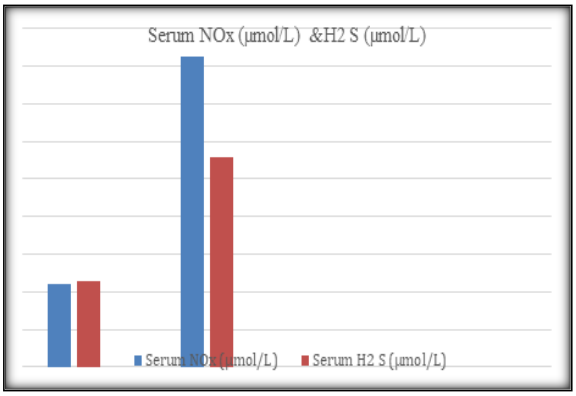

Figure 1: Comparison of serum NOx and H2 S in cases and Controls

Figure 1: The mean serum NOx level in essential hypertension cases is 44.45±10.09 which is significantly lower (p – value <0.001) than that of controls where it is 165.40±15.63.

The mean serum H2S level in essential hypertension case 45.60±10.01 which is again significantly lower (p – value <0.001) than that of controls where it is 111.53±8.60.

Table 1: Distribution of patients according to sex

Sex	Case	Percentage	Control	Percentage	p value
Male	22	44%	24	48%	0.212 --?
Female	28	56%	26	52%	
Total	50	100%	50	100%	

Table 2: Baseline and demographic characteristics of the study group

Parameter	Case(Mean±SD)	Control(Mean±SD)	p value
Age (years)	37.7±6.04	37.7±6.04	NS*
Height (cms)	149.16±9.05	150.62±6.05	NS*
Weight (kgs)	60.13±7.61	59.48±10.82	<0.05**
SBP (mm Hg)	146.92±7.12	120.98±5.19	<0.05**
DBP (mm Hg)	100.66±6.15	80.42±3.62	<0.05**
Serum NOx (µmol/L)	44.45±10.09	165.40±15.63	<0.001**
Serum H2 S (µmol/L)	45.60±10.01	111.53±8.60	<0.001**

NS*= Not Significant. **T-test done, p < 0.05 is considered significant

DISCUSSION

Essential hypertension characterized by the chronic elevation of blood pressure, of unknown etiology, affects about 95% of hypertensive patients all over the world. Hypertension is a complex disorder that poses a significant risk factor for the development of other cardiovascular disorders. It is associated with endothelial dysfunction and impaired vasodilatory response to vasodilators in circulation. Nitric oxide and hydrogen sulphide are the two endogenously produced gaseous signalling molecules in circulation that have a profound effect on human vasculature.

In animal models, it was seen that intravenous infusion of nitric oxide synthase inhibitors resulted in reduced NO bioavailability and a rise in blood pressure.[23] A significant positive association was found between eNOS gene polymorphism and the development of essential hypertension. Inhibition of nitric oxide production by IL-6 contributes to the development of resistant hypertension. Up regulation of neuronal nitric oxide synthase (nNOS) has a protective role in hypertensive cardiomyopathies. In obesity-related hypertension, impaired L-arginine transport can reduce NO bioavailability, increase oxidative stress and trigger the development of hypertension [20] transgenic mice lacking in endothelial nitric oxide synthase developed mild hypertension.[10] Some studies illustrated a higher prevalence of eNOS gene mutation in patients with essential hypertension than in normotensive persons.[11] A randomized controlled trial conducted on high-risk pregnant women showed that dietary supplementation with L-arginine and antioxidants reduced the development of hypertension in pregnancy and incidence of preeclampsia,[16] Serum NO concentration was

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found to be reduced in preeclampsia in several studies.

Traditionally known as a toxic gas, H2 S is now considered to be an important endogenous gaseous transmitter molecule having a wide range of physiological and pathological roles in the human body. It acts as an endothelium-derived relaxing factor (EDRF) and induces vasodilatation via K-ATP channels and the cGMP pathway. Studies have demonstrated a fall in serum hydrogen sulphide level in patients with Grade 2 and grade 3 hypertension and patients with portal hypertension.[23,24] In a previous study, the expression of cystathionine ϒ- lyase (CSE) and serum H2 S level was reduced in patients with pulmonary hypertension. Hyperhomocysteinemia causes homocysteinylation of endogenous enzyme cystathionine ϒ- lyase and hence reduced production of H2 S resulting in the development of hypertension and cardiovascular diseases. A reduced level of H2 S in serum and urine and suppression of CSE gene expression and activity in the thoracic aorta is seen in spontaneous hypertensive rats. Exogenous administration of NaHS has been found to attenuate the elevated BP in spontaneous hypertension in rats. In patients with early-onset preeclampsia, CBS mRNA expression was significantly down regulated in placental villous tissue which resulted in reduced H2 S production.[17] H2 S mediated vasodilatation mainly depends on the activation of the ATP-sensitive K-channels in vascular smooth muscle cells.

Research in the past has separately illustrated the roles of these gas transmitters in the development of hypertension. NO and H2 S act by a different mechanism to mediate vasodilatation. Few studies in the past elucidated that crosstalk exists between these two molecules. H2 S therapy contributed to cardio protection by up regulation of eNOS activity and NO bioavailability. Inhibition of eNOS activity attenuates H2 S induced vasodilatation.[22] Hydrogen sulphide improved the endothelial function by up regulating the peroxisome proliferators-activated receptor delta/ eNOS signalling pathway and helped in the amelioration of hypertension in both humans and rats. While a few reports in the past have demonstrated that both NO and H2 S act via a common signalling pathway to mutually potentiate each other's action, there are also studies elucidating their antagonistic roles.[24, 25]

CONCLUSION

The present study demonstrates a reduction in both the levels of serum nitric oxide and hydrogen sulphide in hypertensive patients than in normotensive controls. There also exists a significant positive correlation between the serum levels of NO and H2 S in overall study subjects.

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