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A study on serum nitric oxide and hydrogen sulfide in essential hypertension cases in a tertiary care hospital in West Bengal

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ABSTRACT

Background: Essential hypertension is a multifactorial disease of unknown etiology characterized by a chronic elevation of blood pressure without any secondary causes. Nitric oxide and hydrogen sulfide are vasodilatory gaseous transmitters whose deficiency results in endothelial dysfunction and vasoconstriction and hence is one of the etiological factors in the development of hypertension. **Aims and Objectives:** The study aimed to estimate the serum levels of NO and H_2S and to find out their correlation in essential hypertension cases and normotensive controls. **Materials and Methods:** Serum levels of NO and H_2S were measured using chemical methods, in fifty patients with essential hypertension and compared with fifty age-matched controls. **Results:** The mean serum NOx level in essential hypertension cases is 45.45 ± 11.09 µmol/L which is significantly lower (p-value <0.001) than that of controls where it is 166.40 ± 16.63 µmol/L. The mean serum H_2S level in essential hypertension cases is 45.61 ± 11.01 µmol/L which is again significantly lower (p-value <0.001) than controls in which it is 111.54 ± 9.60 µmol/L. A statistically significant positive correlation exists between serum NOx and H_2S levels in overall study subjects. **Conclusion:** This study demonstrates that both the serum levels of NO and H_2S decrease with an increase in BP in essential hypertension cases. There exists a positive correlation between NO and H_2S in overall study subjects. Thus, the simultaneous decline in bioavailability of both NO and H_2S is a significant underlying cause in the development of essential hypertension.

Key words: Nitric oxide; Hydrogen sulfide; Essential hypertension; Kolkata; West Bengal

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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 behavioral 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

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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 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.[13-15] Nitric oxide also demonstrates vasoprotective and anti-atherosclerotic properties, including protection from thrombosis, reduction of adhesion molecule expression and leukocyte adhesion.[16]

Hydrogen sulfide 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 H_2S occurs through glucose, glutathione, inorganic and organic polysulfides (present in garlic) and elemental sulfur.[17] H_2S 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 H_2S in the development of hypertension in mice deficient in Cystathionine ϒ- lyase (CSE).[18] Some previous studies demonstrated that polysulfides present in garlic cause vasorelaxation of rat aortic rings through a H_2S dependent mechanism.[20] One experimental study proved that low doses of the H_2S donor sodium hydrogen sulfide (NaHS) produced short-lived relaxation to the mesenteric artery and intestinal contractility.[21] In conclusion, hydrogen sulfide acts as an effective vasodilator and helps in the reduction of blood pressure, but more studies are required to understand the specific cellular and signaling mechanisms regulating these responses.

The physiological and biochemical interactions of the two endogenously present gaseous transmitters, NO and H_2S, are dubious. A previous study illustrated both the gases act synergistically for their production and physiological action.[22] Whereas other studies have illustrated that NO and H_2S inhibit each other's synthesis and action.[23] There exists a common signaling pathway through which these two molecules are involved in a cascade of chemical reactions to generate reactive intermediates that mediate vasodilatation, vascular remodeling and angiogenesis.[24] Another study showed that NaHS, a H_2S donor, increases nitric oxide production in cultured endothelial cells by inducing endothelial nitric oxide synthase.[25] 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 sulfide in patients with essential hypertension and normal healthy subjects. The study also aims to find whether any correlation exists between serum NO and H_2S levels in overall study population.

MATERIALS AND METHODS

This is a non-interventional, observational, cross-sectional hospital-based study, conducted in the Department of Biochemistry and Medicine, Institute of Post Graduate Medical Education and Research, Kolkata, India for a period of 18 months from January 2018 to June 2019.

The inclusion criteria of the subjects included newly diagnosed cases with BP ≥ 140/90 mm Hg and not on any antihypertensive medication. The exclusion criteria included 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 sulfide modulating drugs.

A total of fifty cases and fifty age-matched controls were included in the study.

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 (NO_3) and nitrites (NO_2). This assay relies on a diazotization reaction that was originally described by Griess in 1879. In the Griess reaction, dinitrogen trioxide generated from the acid-catalyzed formation of nitrous acid from nitrite, reacts with sulfanilamide 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 H_2S were measured by the reaction of sulfide with N, N-dimethyl-p-phenylenediamine sulfate 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.

Ethical clearance and approval

All tests and procedures conducted in this study involving human subjects were in accordance with the ethical standards of the institutional and national research committee and with the 1975 revised Helsinki declaration and its later amendments and other comparable ethical standards.

Statistical analysis

The data analysis in this study is done by the statistical software Minitab Version-2016. All the data are expressed in mean ± SD. Comparison of data is done by unpaired Student's T-test and Pearson's correlation. The p-value < 0.05 was considered statistically significant.

RESULTS

The clinical and biochemical variables of the study subjects are depicted in Table 1.

The mean serum NOx level in essential hypertension cases is 45.45 ± 11.09 µmol/L which is significantly lower (p-value <0.001) than that of controls where it is 166.40 ± 16.63 µmol/L as shown in Figure 1.

The mean serum H_2S level in essential hypertension cases is 45.61 ± 11.01 µmol/L which is again significantly lower (p-value <0.001) than controls in which it is 111.54 ± 9.60 µmol/L as shown in Figure 1.

A scatter diagram (Figure 2) plotted between serum nitric oxide and systolic BP in patients shows a negative correlation. The Pearson's correlation coefficient,

Table 1: The clinical and biochemical parameters of study subjects

Variables	Patients' Mean (n=50)	Controls' Mean (n=50)	p-value (with 95% C.I)
Age (yrs.)	36.6 ± 5.39	36.6 ± 5.39	NS*
Height (cms)	159.16 ±9.43	160.62 ± 6.09	NS*
Weight (kgs)	61.13 ± 8.61	60.48 ± 11.82	< 0.05**
SBP (mm Hg)	147.96 ± 7.14	121.98 ± 5.21	< 0.05**
DBP (mm Hg)	100.76 ± 6.25	80.52 ± 3.72	< 0.05**
Serum NOx (µmol/L)	45.45 ± 11.09	166.40 ± 16.63	< 0.001**
Serum H_2S (µmol/L)	45.61 ± 11.01	111.54 ± 9.60	< 0.001**

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

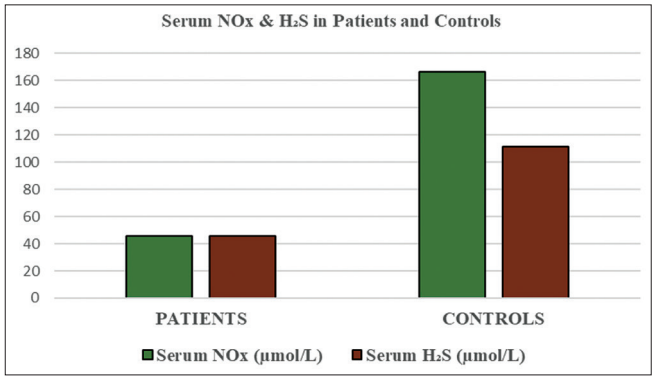

Figure 1: Comparison of serum NOx and H_2S in Patients and Controls

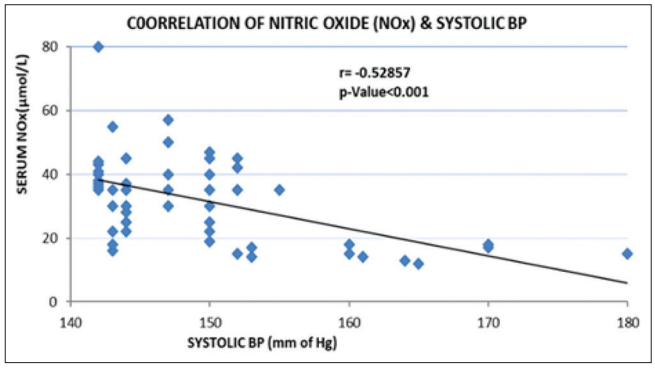

Figure 2: Correlation between serum NOx and systolic BP in Patients

r =-0.52857 and the result is statistically significant with a p-value < 0.001. A scatter diagram (Figure 3) plotted between serum nitric oxide and diastolic BP in patients shows a statistically significant (p-value <0.001) negative correlation with Pearson's correlation coefficient, r =-0.47376. This implicates that serum nitric oxide concentration falls with a rise in systolic and diastolic blood pressure.

A scatter diagram shown in Figure 4 illustrates a negative correlation between serum H_2S and systolic BP in cases of essential hypertension. The Pearson's correlation coefficient, r = -0.56115. It is statistically significant with a p-value <0.001. In Figure 5, the scatter diagram plotted between serum H_2S and diastolic BP in patients, shows a negative correlation with Pearson's correlation coefficient, r = -0.6126 and p-value <0.001.

A scatter diagram plotted between serum NOx and H_2S levels in overall study subjects shows a positive correlation as shown in Figure 6. The Pearson's correlation coefficient, r = 0.948 and the result is statistically significant with a p-value <0.001.

DISCUSSION

Essential hypertension characterized by the chronic elevation of blood pressure, of unknown etiology, affects about 95% of hypertensive patients all over the world.[2] 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 sulfide are the two endogenously produced gaseous signaling 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.[26] A significant positive association was found between eNOS gene polymorphism and the development of essential hypertension.[27] Inhibition of nitric oxide production by IL-6 contributes to the development of resistant hypertension.[28] Upregulation of neuronal nitric oxide synthase (nNOS) has a protective role in hypertensive cardiomyopathy.[29] In obesity-related hypertension, impaired L-arginine transport can reduce NO bioavailability, increase oxidative stress and trigger the development of hypertension.[30] Transgenic mice lacking in endothelial nitric oxide synthase developed mild hypertension.[10] Some studies illustrated a higher prevalence of eNOS gene

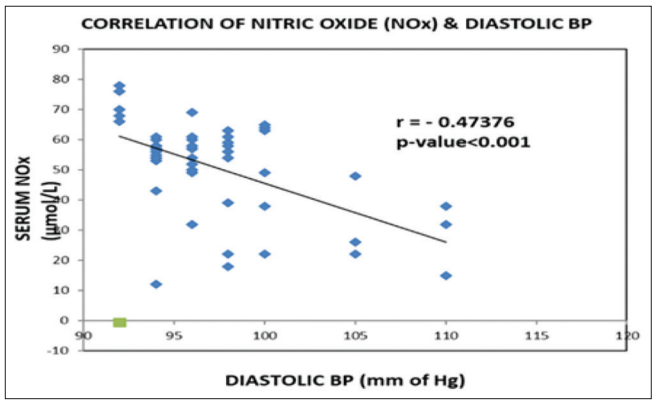

Figure 3: Correlation between serum NOx and diastolic BP in Patients

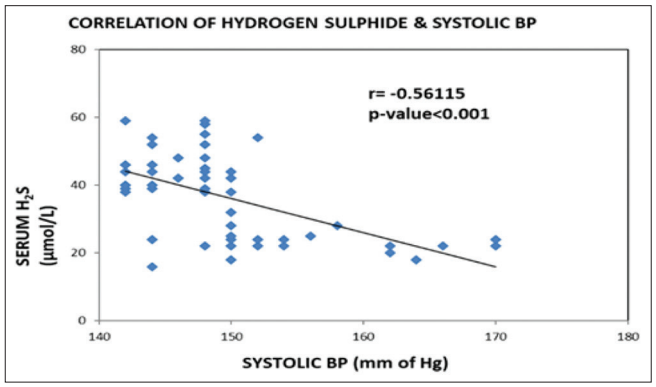

Figure 4: Correlation between serum H_2S and systolic BP in Patients

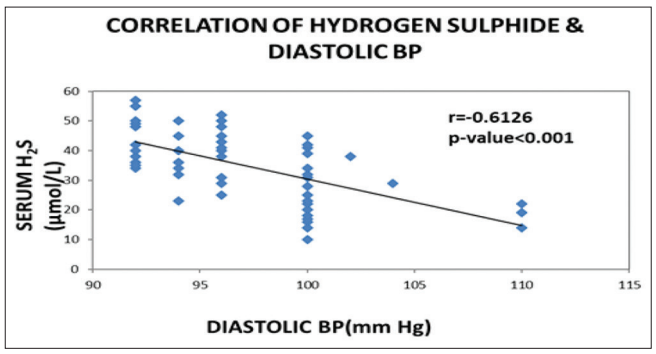

Figure 5: Correlation between serum H_2S and diastolic BP in Patients

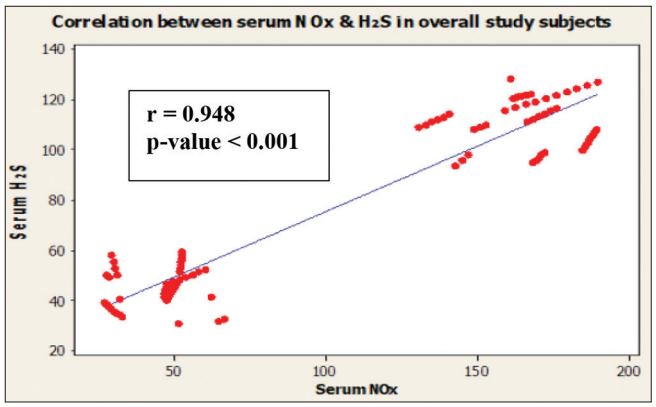

Figure 6: Correlation between serum NOx & H_2S levels in overall study subjects

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 found to be reduced in preeclampsia in several studies.[31]

Traditionally known as a toxic gas, H_2S 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.[32] Studies have demonstrated a fall in serum hydrogen sulfide level in patients with Grade 2 and grade 3 hypertension and patients with portal hypertension.[33,34] In a previous study, the expression of cystathionine ϒ- lyase (CSE) and serum H_2S level was reduced in patients with pulmonary hypertension.[35] Hyperhomocysteinemia causes homocysteinylation of endogenous enzyme cystathionine ϒ- lyase and hence reduced production of H_2S resulting in the development of hypertension and cardiovascular diseases.[36] A reduced level of H_2S 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.[37] In patients with early-onset preeclampsia, CBS mRNA expression was significantly downregulated in placental villous tissue which resulted in reduced H_2S production.[17] H_2S mediated vasodilatation mainly depends on the activation of the ATP-sensitive K-channels in vascular smooth muscle cells.[38]

Research in the past has separately illustrated the roles of these gasotransmitters in the development of hypertension. NO and H_2S act by a different mechanism to mediate vasodilatation. Few studies in the past elucidated that crosstalk exists between these two molecules. H_2S therapy contributed to cardio protection by upregulation of eNOS activity and NO bioavailability.[39] Inhibition of eNOS activity attenuates H_2S induced vasodilatation.[22] Hydrogen sulfide improved the endothelial function by upregulating the peroxisome proliferator-activated receptor delta/eNOS signaling pathway and helped in the amelioration of hypertension in both humans and rats.[40] While a few reports in the past have demonstrated that both NO and H_2S act via a common signaling pathway to mutually potentiate each other's action, there are also studies elucidating their antagonistic roles.[22,23]

The present study demonstrates a reduction in both the levels of serum nitric oxide and hydrogen sulfide in hypertensive patients than in normotensive controls. There also exists a significant positive correlation between the serum levels of NO and H_2S in overall study subjects.

Recently novel H_2S based therapeutic agents are being investigated for their use in cardiovascular diseases.[41] Research work has already established the therapeutic potential of various nitric oxide donors in the maintenance of normal blood pressure in cardiovascular disorders.[42] NO and H_2S acting through a common intermediate pathway can be further utilized to delve into the therapeutic potential of using combination therapy for the maintenance of normal BP and early prevention and treatment of essential hypertension. However, a large-scale study is required in this direction to further evaluate the pathophysiological interaction and the therapeutic potential of NO and H_2S in the management of essential hypertension.

Limitations of the study

The sample size for this study could have been larger. Newer and sophisticated techniques for the measurement of nitric oxide and hydrogen sulfide could not be undertaken. The levels of an enzyme involved in NO and H_2S production in the serum like NOS, CBS and CSE, could not be estimated especially at the tissue levels. Most of our study subjects included the patients attending our hospitals, so the study may not reflect the overall scenario in the community.

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MN-Concept and design of the study, statistically analyzed and interpreted the results, prepared the first draft of the manuscript; **SC**-Concept and coordination, review of literature, final preparation and critical revision of manuscript; **ACR**-Concept and design of the study, data analysis and interpretation; **AK**-Manuscript preparation and critical revision of manuscript; **UKB**- Concept and design of the study, data analysis and interpretation, Final approval.

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