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Original Article

Study Of Serum Nitric Oxide And Hydrogen Sulphide In Essential Hypertension

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ABSTRACT

Background:
Hypertension is one of the leading causes of morbidity and mortality worldwide. Among different contributing factors, Nitric Oxide and Hydrogen Sulphide, has recently come out as important regulatory molecules in this regard. But very few studies have been done till date, especially on Indian population to delineate any possible relation between these factors and blood pressure in the hypertensive patients.

Methods:
In the Present study 50 Hypertensive patients and 50 age and sex matched control subjects were studied in the Dept of Biochemistry, IPGMER, Kolkata. Their Systolic and Diastolic blood pressure were measured. Serum Nitric Oxide was measured by Diazo method and the Serum Hydrogen sulphide was measured by N, N-dimethyl-p-phenylenediamine method.

Results:
Serum Nitric Oxide and Hydrogen Sulphide levels were significantly higher in the Patients in comparison to the controls. Both Nitric Oxide and Hydrogen Sulphide levels were found to have significant negative correlation with both Systolic as well as Diastolic Blood Pressure. Moreover, a significant positive correlation was found between serum Nitric Oxide and Hydrogen Sulphide levels among the Hypertensive Patients.

Conclusion:
The present study indicates an important association of the two novel Gaso transmitters, Nitric Oxide and Hydrogen Sulphide with the hypertension in our population. So there is need to more regularly measure the serum levels of these two molecules in hypertensive patients. Therapeutic modalities targeting to correct the dysregulation of these molecules should also be studied for possible ways to treat hypertension.

INTRODUCTION

Hypertension is one of the major cause of increased premature morbidity and mortality throughout the world. Approximately 7.6million death (13-15% of the total) and 92 million disability-adjusted life-years worldwide were attributable to high blood pressure.

Hypertension is also a burning problem in our community as during the last decades, the prevalence of hypertension is increasing in the younger age groups. Along with the change of the modern lifestyle, this disease caused increased mortality and morbidity in our population.

Previous studies have already established a role of the nitric oxide in hypertension and reduction in bioavailability of nitric oxide is associated with hypertension. Hydrogen sulphide is another gaso-transmitter also involved in vasodilatation like nitric oxide. Few studies have reported the interaction of nitric oxide and hydrogen sulphide in the vasculature and signal transduction pathways.

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Very few studies have been directed to elucidate the status of H2S and NO simultaneously in a situation like hypertension. Hence the current study is conducted to evaluate the role of H2S and NO in this condition in order to elucidate newer pathophysiology in quest of new therapeutic target.

In our body nitric oxide contributes to the maintenances of vascular tone to increase blood flow. Endothelium dependant vasodilatation is mediated in part by nitric oxide. Nitric oxide produced from the endothelial cells is a strong vaso-relaxant and anticoagulant factor. Its effect is mediated by guanosine 3,5 cyclic monophosphate produced by soluble guanyl cyclase. The activation of nitric oxide synthase (NOS) triggers the production of nitric oxide, in the vascular endothelium, diffuses into the vascular smooth muscle cells and causes vasodilatation by relaxing the vascular smooth muscle cells[1].

Hydrogen sulphide is also endogenously generated from L-cysteine & has important biological function within the vasculature, regulate vessel tone and prevent over-growth of new vessels. It acts as a vasorelaxant through K-ATP channel causing smooth muscle relaxation and plays an important role in maintaining blood pressure. It also helps in regulation of angiogenesis. In human body H2S formation occurs via the trans-sulphuration pathway involving cystathionine beta synthase (CBS) and cystathione gamma lyase (CGL) along with cysteine catabolism via 3 mercaptopyruvate sulphur transferase (MST) [2-4].

In this background the aim of our study is to find out the status of both the above vaso-transmitters in serum of patients with essential hypertension.

Methods:

STUDY DESIGN – The present study was undertaken as a cross sectional, hospital based observational study.

STUDY SETTING AND TIMELINE– Department of Biochemistry and Department of Medicine IPGME&R, KOLKATA. Patient with diagnosed essential hypertension were selected from Department of Medicine, IPGME&R for one and half years. The analysis of bio chemical parameters were done in the Department of Biochemistry IPGME&R. The result was compared with age and sex matched healthy persons as controls.

PERIOD OF STUDY– January 2018 to June 2019 (18 months)
STUDY POPULATION- Fifty (50) patients of essential hypertension and fifty (50) age and sex matched controls were included in the study.
Following approval of Ethical Committee, blood samples were collected from study population for initial screening and estimation of desired parameters only after obtaining informed consents.

ETHICAL CONSIDERATIONS: The study followed the Helsinki declaration guidelines and ICMR guidelines for human studies. It was undertaken after obtaining the written approval from the Institutional Ethics committee.

Inclusion Criteria

I. Patient with diagnosed essential hypertension.
II. Newly diagnosed cases, no H/O antihypertensive medication.
III. Diagnosis of essential hypertension established by clinical evaluation and different biochemical, radiological and cardiological investigations.

Exclusion Criteria

I. Patients suffering from Secondary causes of hypertension.
II. Patient suffering from Carcinogenesis, metabolic disease complicated with different organ failure (with renal failure, myocardial infarction etc.) and other endocrinal disorder.
III. Patient with pregnancy, eclampsia and diabetes mellitus.
IV. Patients on antihypertensive medication.
V. Patient with any hydrogen sulphide and nitric oxide modulating drugs.

Method of assay of NOx (nitrate plus nitrite) in serum

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 process is based on a diazotization reaction originally mentioned and described by Griess in 1879. In this method of nitrite assay as described first by Johann Peter Griess in 1879, nitrites are made to react with sulfanilic acid under acidic conditions. The resulting diazonium cation reacts with the aromatic amine 1-naphthylamine in the subsequent coupling reaction and generates a red–violet coloured with with maximum absorbance at 540 nm.

Measurement of H_2S concentration in serum:

Zinc sulphate was added to serum sample for precipitating the H_2S, HS^-, S^{2-} and serum protein with Zn^{2+}. NaOH was used thereafter to re-dissolve the serum protein. The precipitated ZnS was re-dissolved using the N, N-dimethyl-p-phenylenediamine. Other proteins were deposited using trichloroacetic acid. Finally, after the centrifugation process, ferric chloride was added to the supernatant to produce the chromogenic methylene blue that was analysed using spectrophotometer at 670 nm.

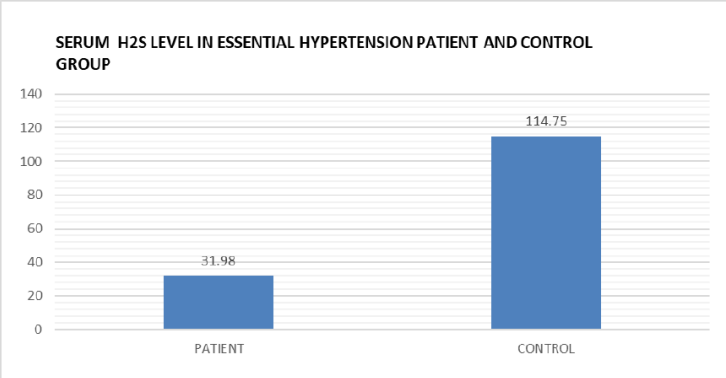
Statistical analysis:

All data collected are tabulated on Pre-Designed Excel data sheet and statistical analysis done by SPSS 17.

Results:

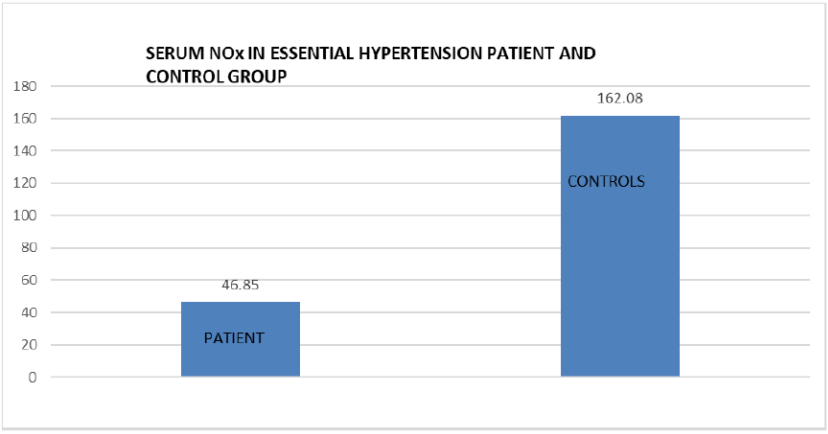
The mean serum H_2S level is 45.07 ± 7.32µmol/L in patients with essential hypertension , while it is higher in control subjects, where mean is 111.36 ± 9.76 µmol/L. These levels show significantly different values when compared by Independent T test ($p < 0.001$).

Figure 1: Bar diagram showing comparison of means of Serum H2S level between patients and controls



The mean serum NO_x level of essential hypertensive patients is 45.16± 11.23µmol/L and that of control subjects is 166.03±16.88 µmol/L. The levels were found to be significantly different when compared by Independent T test ($p < 0.001$).

Figure 2: Bar diagram showing comparison of means of Serum NOx level between patients and controls:



When analysed by Pearson correlation, significant negative correlation was found between Serum NOx levels with Systolic (Coefficient r=-0.52857 & $p < 0.001$), as well as diastolic blood pressure ($r = -0.47376$ $p < 0,001$).

Figure 3: Scatter diagram showing a negative correlation between serum nitric oxide levels and systolic blood pressure in patients

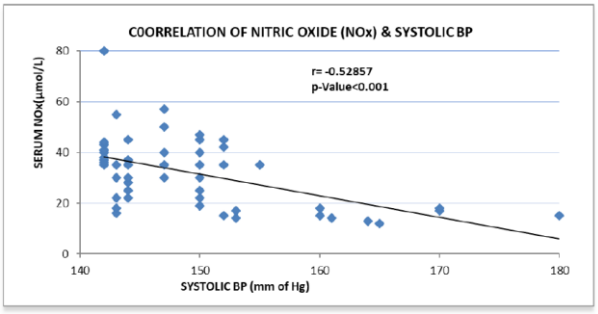
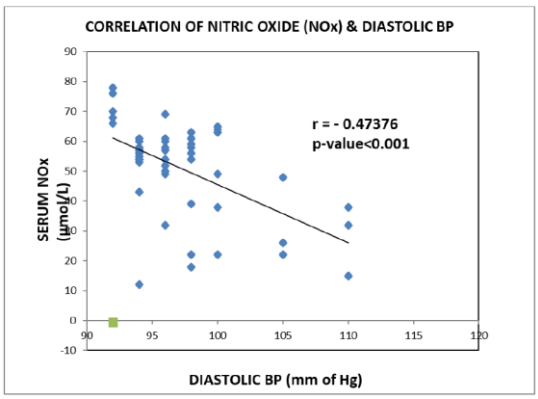


Figure 4: Scatter diagram showing a negative correlation between serum nitric oxide levels and diastolic blood pressure in patients



When analysed by Pearson correlation, significant negative correlation was found between SerumH2S levels with Systolic (Coefficient r-0.56 & p <0.001), as well as diastolic blood pressure (r = -0.6126, p <0,001).

Figure 5: Scatter diagram showing a negative correlation between serum H2S levels and systolic blood pressure in patients

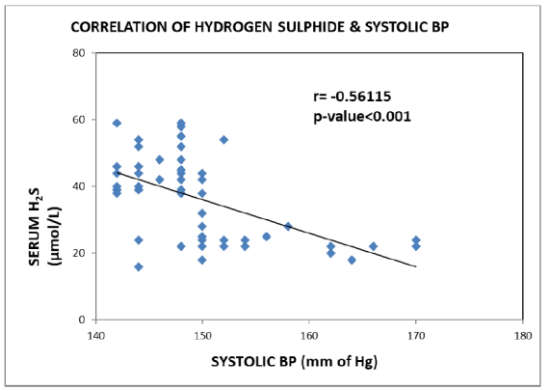
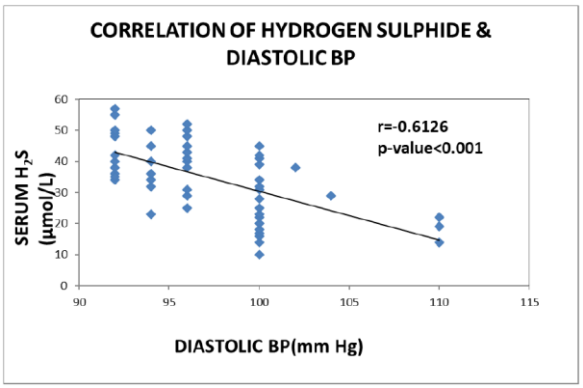
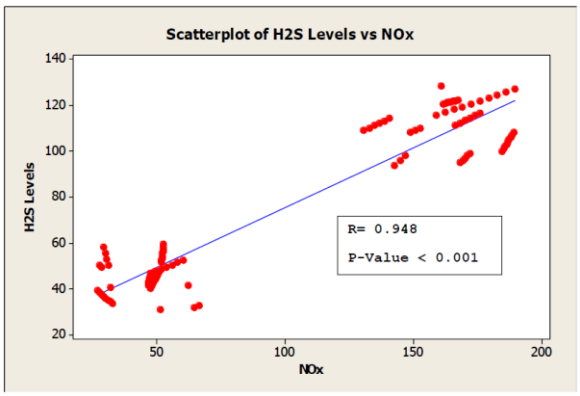


Figure 6: Scatter diagram showing a negative correlation between serum H2S levels and systolic blood pressure in patients



Serum NOx and H2S levels show significant positive correlation when analyzed by Pearson Correlation (r=0.948, p <0.001)

Figure 7: Scatter diagram showing Correlation between NOx and H2S Levels in Overall Study Subjects



Discussion:

Essential hypertension is the commonest variety of hypertension. H_2S and NO are recognized as the predominant members of a new family of signalling molecules, gaso-transmitter in human beings, both of which have profound effect on vasculature [5,6,7,8]. Thus the serum levels of nitric oxide decrease in hypertensive patients as compared to normotensive persons. This result supports the vasodilatory role of nitric oxide in the vasculature.

In the present study a significant negative correlation is found to exist between the serum Nitric oxide levels and systolic BP & diastolic BP as shown in figure 3 & 4. Several previous studies have also demonstrated similar decreased levels of NO_x in hypertension as compared to age matched normotensive persons. Several earlier reports have established that there is a deficient synthesis of vasodilatory molecule –nitric oxide and prostacyclin (PGI_2) in this condition.[9,10,11] On the other hand, the vascular endothelial cells and activated leucocytes produce an increased amount of vasoconstrictor molecules like endothelin-1, thromboxane A_2, angiotensin II, TNF α and other cytokines. As a result the endothelial dysfunction and vasospasm occur and arterial blood pressure are elevated [9,10,11].

In our body, nitric oxide contributes to the maintenance of vascular tone to increase blood flow. Endothelial dependent vasodilatation is mediated by guanisine 3,5-cyclic monophosphate produced by soluble guanylnylcyclase. The activation of nitric oxide synthase (NOS) triggers the production of nitric oxide in vascular endothelium, diffuses into vascular smooth muscle cells and causes vasodilatation by relaxing vascular smooth muscle cells. The endothelial derived NO produced by eNOS is supposed to play a major role in maintaining the inherent vascular tone. Its decrease leads to an increased vascular tone that heralds a significant rise in blood pressure. In previous studies the eNOS were seem to maintain the circulatory haemostasis through vascular smooth muscle relaxation. Some mutations of eNOS has been reported to be associated with the decreased plasma level of nitric oxide in hypertensive patients [12].

In contrast to nitric oxide, which has a clear vasorelaxant action, hydrogen sulphide has both vasorelaxing and vasoconstricting effect on the cardiovascular system depending on the concentration. Hydrogen sulphide has already been reported as an important antihypertensive agent. The reduced production of hydrogen sulphide and alteration in its function are involved in the initiation of spontaneous hypertension [5,6,7].

Thus the results of this study also demonstrated that the level of the vasodilatory hydrogen sulphide molecule is decreased in patients with hypertension. Previous studies have reported that hydrogen sulphide plays crucial role in regulating the blood vessel tone as well as outgrowing new vasculature. It acts as a vasorelaxant through K^+-ATP channels causing smooth muscle relaxation and plays a role in maintaining vascular tone. It also helps in regulation of angiogenesis[13].
A negative correlation has been found to exist between serum H_2S levels and systolic as well as diastolic BP in the present study

(Figure 5 & 6). Previous studies has shown that H_2S producing enzyme, cystathionine-γ-lyase, has depressed activity in hypertensive patients, this in turn resulted in decreased H_2S levels in peripheral circulation[14].

Some recent studies suggest that H_2S and NO signalling pathways closely interact in some areas that play important roles in several processes involving mutual attenuation or potentiation of biological response in body. They demonstrate a common signalling pathway exist where H_2S and -NO crosstalk, mediates their effect on vascular function such as in vasodilatation, vascular remodelling and angiogenesis[15**].** This is reflected in the current study as evidenced by a significant positive correlation beween H2S and NO levels in the overall study subjects (Figure 7).

Recently, several H_2S based therapeutic strategies are being utilized for the treatment of various cardiovascular diseases. The H_2S releasing compounds that have been developed for clinical use include sulphide salts like sodium hydrosulphide, sodium sulphide and synthetic H_2S donar like S-allylcysteine and S-propyl-cysteine. These drugs increases the H_2S production and bio-availability in circulation. These drugs are currently in various phases of clinical trial and may prove to be very beneficial in hypertensive patients[13].

In conclusion, hydrogen sulphide and nitric oxide, two most important gaseous signalling molecule play a critical role in hypertensive patients. The present study contains a very small sample size and have several limitations mentioned later. Further large scale study in this
direction in future may help in ther understanding of complex pathophysiological role of H2S and NO in hypertension and may help develop new treatment modality.

Conclusion:

1. Nitric Oxide and Hydrogen Sulphide needs to be routinely measured and monitored in Hypertensive patients.
2. More Information about the two gaso-transmitters may be used to develop newer therapy for Hypertension.

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Conflict of Interest: None
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