

# Association of serum hydrogen sulphide with hypertension and proteinuria in pre-eclampsia



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Submitted: 12-08-2019

Revised: 19-09-2019

Published: 01-11-2019

## ABSTRACT

**Background:** Preeclampsia is a hypertensive disorder of pregnancy affecting multiple systems and characterized chiefly by hypertension and proteinuria in a previously normotensive and non proteinuric women. The main underlying cause for its pathophysiology is an imbalance between the physiological vasoconstrictor and vasodilator molecules in circulation leading to maternal endothelial dysfunction. Hydrogen sulphide (H<sub>2</sub>S) is a physiological vasodilatory gasotransmitter which plays an important role in the development of hypertension and proteinuria in preeclampsia.

**Aims and Objectives:** The aim of this study was to determine the serum level of hydrogen sulphide and spot urinary protein levels in preeclampsia cases and compare it with age matched controls which were normal pregnant women and to find any correlation, if exists, between these two parameters. **Materials and Methods:** Serum level of H<sub>2</sub>S and spot urinary protein level were measured in one hundred pregnant women with preeclampsia and the values were compared with age matched controls.

**Results:** The mean serum H<sub>2</sub>S level was  $32.31 \pm 12.62 \mu\text{mol/L}$  in patients which was significantly lower ( $p < 0.001$ ) when compared to controls where mean was  $114.50 \pm 20.35 \mu\text{mol/L}$ . The mean spot urinary protein level was found to be  $11.83 \pm 5.06 \text{ mg/dl}$  in preeclampsia cases which was significantly higher ( $p < 0.001$ ) than in controls where it was  $7.18 \pm 2.38 \text{ mg/dl}$ . A negative correlation was found between the serum level of H<sub>2</sub>S and both the systolic BP ( $r = -0.725$ ,  $p < 0.001$ ) and diastolic BP ( $r = -0.639$ ,  $p < 0.001$ ) in preeclampsia patients. A negative correlation was also observed between the serum levels of H<sub>2</sub>S and spot urinary protein in preeclampsia ( $r = -0.541$ ,  $p < 0.001$ ).

**Conclusion:** The present study has elucidated that the serum levels of hydrogen sulphide decreases and the spot urinary protein levels increases in preeclampsia when compared to normal pregnant women and hydrogen sulphide shows a negative correlation with both systolic and diastolic BP in preeclampsia. This study also demonstrates that, there exists a negative correlation between the serum H<sub>2</sub>S level and spot urinary protein level in preeclampsia patients.

**Key words:** Hydrogen Sulphide; Hypertension; Proteinuria; Pre-eclampsia; Kolkata

### Access this article online

**Website:**

<http://nepjol.info/index.php/AJMS>

**DOI:** 10.3126/ajms.v10i6.25962

**E-ISSN:** 2091-0576

**P-ISSN:** 2467-9100

## INTRODUCTION

Preeclampsia is a multisystem disorder of unknown etiology specific to human pregnancy. Preeclampsia is defined as hypertension of at least 140/90 mmHg on two separate occasions for  $\geq 4$  hours apart accompanied by significant

proteinuria of at least 0.3 g in a 24-hour collection of urine (or  $>30$  mg of protein/creatinine ratio), that rises for the first time, after the 20<sup>th</sup> week of gestation in a previously normotensive woman and resolves completely by the 6<sup>th</sup> postpartum week.<sup>1</sup> Proteinuria detected before pregnancy or before 20 weeks of gestation is suggestive

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of some preexisting renal disorder. Preeclampsia is one of the most common causes of maternal and fetal morbidity and mortality worldwide. Preeclampsia is characterized by an antiangiogenic state caused by an imbalance between the elevated synthesis of vasoconstrictors and depressed production of vasodilators, increased oxidative stress and maternal endothelial dysfunction, ultimately leading to uteroplacental hypoperfusion.<sup>2,3</sup> But the underlying pathophysiology for this hypertensive disorder has remained elusive despite a lot of research.

Hydrogen sulphide has traditionally been viewed as a toxic gas, but recent research has established it to be the third member of the gasotransmitter family after nitric oxide and carbon monoxide. Enzymatic synthesis of H<sub>2</sub>S occurs through three enzymes that are cystathionine gamma-lyase (CGL/CSE), cystathionine beta-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3-MST) and involves various intermediate metabolites like cystathionine, L- cysteine, 3-mercaptopyruvate, alpha ketoglutarate, disulphide and persulphides.<sup>4,5</sup> Whereas, non-enzymatic production of H<sub>2</sub>S occurs through glucose, glutathione, inorganic and organic polysulfides (present in garlic) and elemental sulfur.<sup>5</sup>

Hydrogen sulphide is a vasodilatory gasotransmitter that is produced endogenously by human intrauterine tissues and placenta by enzymes namely cystathionine beta synthase (CBS) and cystathionine gamma lyase (CSE).<sup>6</sup> A study by You et al in China, demonstrated that the H<sub>2</sub>S produced by CBS and CSE enzymes in human pregnant myometrium, played a significant role in maintaining uterine contractility during pregnancy and labor.<sup>7</sup> In 2013, Wang et al concluded from their study that endogenous H<sub>2</sub>S is required for healthy placental vasculature and a decrease in CSE/H<sub>2</sub>S activity may contribute to maternal hypertension and placental abnormalities in preeclampsia.<sup>8</sup> In the past few years some research studies have revealed that the downregulation of CBS mRNA expression in placental villous tissue and CSE/H<sub>2</sub>S activity in intrauterine tissues significantly contributed to abnormal placentation and maternal hypertension in preeclampsia.<sup>8,9</sup> H<sub>2</sub>S helps to regulate vascular tone and blood pressure by promoting vasorelaxation via action of ATP-sensitive potassium channels and large-conductance Ca<sup>2+</sup>-activated potassium channels in the vascular smooth muscle cells.<sup>12,13</sup>

Despite quite a few works in this field, there still remains a lacuna in the knowledge about the role of hydrogen sulphide in the pathogenesis of preeclampsia, which requires further research.

In healthy pregnant women, there is about 40-60 % increase in glomerular filtration rate, than in normal,

nongravid women, caused due to the reduction of the plasma oncotic pressure in the glomerular capillaries associated with pregnancy induced hemodilution.<sup>10</sup> In preeclampsia, renal insufficiency occurs due to the spasm of the afferent glomerular arterioles which lead to anoxic damage to the endothelium of the glomerular capillary tuft called glomerular endotheliosis, that finally results in the increased capillary permeability and leakage of proteins in urine.<sup>11</sup> Albumin constitutes 50-60% and alpha-globulin constitutes 10-15 % of the total proteins excreted in the urine.<sup>12</sup> Tubular reabsorption is reduced. Tubular proteins such as beta-2-microglobulin characteristic of tubular damage, occur in severe preeclampsia.<sup>13</sup> In the kidneys, H<sub>2</sub>S increases glomerular filtration rate and inhibits tubular sodium reabsorption and results in significant natriuresis, diuresis and kaliuresis.<sup>14</sup>

In normal pregnancy, endogenous H<sub>2</sub>S also upregulates the synthesis of vascular endothelial growth factor (VEGF) and helps to maintain uterine contractility.<sup>8</sup> VEGF is also an important angiogenic molecule that helps to maintain the integrity of the glomerular filtration barrier. An early event in preeclampsia is placental ischemia that results in the increased production of placental factors like soluble fms-like tyrosine kinase-1 (sflt-1) which is an antagonist of VEGF and placental growth factor (PIGF). The depression in the levels of VEGF and PIGF results in endothelial dysfunction, glomerular endotheliosis and proteinuria.<sup>15</sup>

In a previous study by Wang et al, it was demonstrated that downregulation of CSE activity increased both sflt-1 and soluble endoglin release from human umbilical vein endothelial cells (HUVECs) and vice versa.<sup>8</sup>

So this present study was designed to explore the serum level of hydrogen sulphide and the spot urinary protein level in preeclampsia cases and compare it with those of normal pregnant women. The study also aimed to reveal, if there is any correlation between the serum H<sub>2</sub>S with blood pressure and urinary protein levels in preeclampsia patients.

## MATERIALS AND METHODS

This is a non-interventional, observational, cross-sectional hospital based study, conducted in the Department of Biochemistry and Obstetrics and Gynecology, I.P.G.M.E &R, Kolkata, India.

The inclusion criteria of the subjects included diagnosed preeclampsia that is, those presenting with blood pressure  $\geq 140/90$  mm Hg and proteinuria  $\geq 300$ mg/24 hours or protein creatinine ratio  $\geq 0.3$ , appearing after 20 weeks

of gestation up to 12 weeks postpartum. A total of 100 pregnant women with preeclampsia were included as cases based on these inclusion criteria. One hundred normal healthy pregnant women after 20 weeks of gestation were included as controls.

The exclusion criteria of the study were pregnant women with previously diagnosed hypertension before conception or before 20 weeks of gestation and pregnancy with gestational diabetes mellitus and other complications like cardiovascular, neurological, autoimmune disorders or malignancy were also excluded from the study.

Fasting blood samples and spot urine were collected from cases and controls under aseptic conditions after obtaining informed consent.

## ETHICAL CLEARANCE AND APPROVAL

All tests and procedures performed 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.

## LABORATORY ANALYSIS

The collected blood samples were centrifuged at 3500rpm for 30 minutes to obtain the serum. The serum and urine samples were stored at -20 degrees centigrade for further analysis.

Serum levels of H<sub>2</sub>S were estimated by a spectrophotometric method that involved the reaction of sulfide with N,N-dimethyl-p-phenylenediamine sulfate in the presence of oxidizing agent Fe<sup>3+</sup> in hydrochloric acid to form methylene blue whose absorbance was read at 670nm.

The protein content in spot urine was measured by pyrogallol red dye binding method and the absorbance of the blue purple colored complex was measured in spectrophotometer at 600nm.

## STATISTICAL ANALYSIS

The data analysis of this study is done by the statistical software Minitab Version -2018. All the data are expressed in mean  $\pm$  SD. Comparison of data is done by unpaired Student's t- test and Pearson's correlation. The p-value <0.05 was considered as statistically significant.

## RESULTS

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

The mean serum H<sub>2</sub>S level is 32.31  $\pm$  12.62  $\mu$ mol/L in patients while it is significantly lower (p<0.001) when compared with controls where mean was 114.50  $\pm$  20.35  $\mu$ mol/L as shown in Figure 1.

The mean spot urinary protein level was 11.83  $\pm$  5.06 mg/dl in preeclampsia cases which is significantly higher (p<0.001) than in normal pregnant women where the mean was 7.18  $\pm$  2.38 mg/dl as shown in Figure 1.

The scatter diagram plotted between serum H<sub>2</sub>S levels and systolic BP in patients shows negative correlation in Figure 2. The Pearson's correlation coefficient, r is equal to - 0.725. This test is statistically significant with a p-value < 0.001. This implicates that the serum levels of hydrogen sulphide fall with a rise in systolic blood pressure.

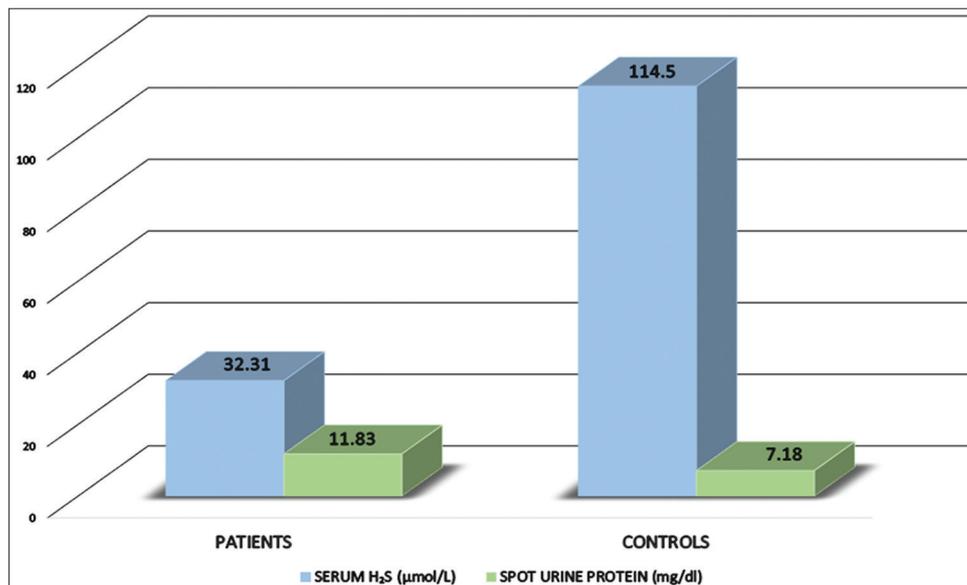
A scatter diagram between serum H<sub>2</sub>S levels and diastolic BP in patients shows a negative correlation in Figure 3. The Pearson's correlation coefficient, r is equal to - 0.639. This test is statistically significant with a p-value < 0.001. This implicates that the serum levels of hydrogen sulphide decrease with an increase in diastolic blood pressure.

A scatter diagram in Figure 4 shows a negative correlation between the serum H<sub>2</sub>S and spot urinary protein levels in preeclampsia cases with the Pearson's correlation coefficient, r equals to -0.541. This test is statistically significant with a p-value <0.001.

**Table 1: The clinical and biochemical parameters of the study subjects**

Variables	Patients (n=100) Mean	Controls (n=100) Mean	p- value (with 95% C.I.)
Age (yrs)	25.51 $\pm$ 4.03	24.52 $\pm$ 3.54	0.166 (NS)
Height (cms)	159.45 $\pm$ 7.13	160.2 $\pm$ 6.42	0.683 (NS)
Weight (kgs)	76.43 $\pm$ 8.95	72.07 $\pm$ 8.28	<0.001*
SBP (mm Hg)	148.74 $\pm$ 7.01	123.48 $\pm$ 7.88	<0.001*
DBP (mm Hg)	99.9 $\pm$ 6.6	78.86 $\pm$ 5.32	<0.001*
Serum H <sub>2</sub> S ( $\mu$ mol/L)	32.31 $\pm$ 12.62	114.50 $\pm$ 20.35	<0.001*
Spot urine protein (mg/dl)	11.83 $\pm$ 5.06	7.18 $\pm$ 2.38	<0.001*

\*T-test done, p < 0.05 is considered significant



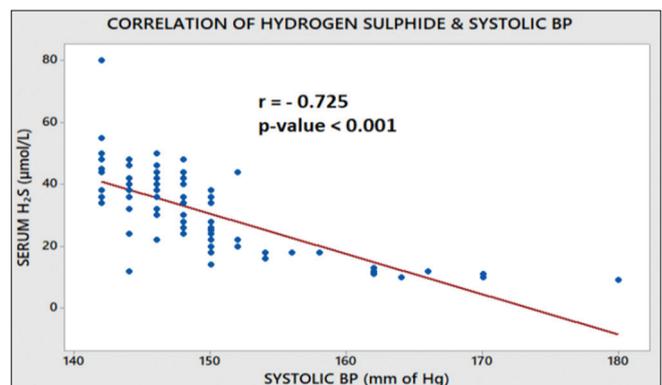
**Figure 1:** Comparison of Serum Hydrogen Sulphide Levels and Spot Urinary protein in Patients and Controls

## DISCUSSION

Hydrogen sulphide has been recognized as a toxicant for nearly 300 years with widespread effects on lungs, brain and kidneys. But now after some research in the past few years, hydrogen sulphide has been known to be an endogenously produced gasotransmitter with some physiological significance.

Although the exact etiology of preeclampsia is not known, but it is established that it is a hypertensive disorder characterized by an anti-angiogenic state. The hypertension is chiefly caused due to a much higher production of vasoconstrictor molecules than vasodilatory molecules by the vascular endothelial cells of intrauterine tissues. Few studies in the past have demonstrated that the H<sub>2</sub>S synthesized endogenously with the help of enzymes in the intrauterine tissues played an important role in maintaining uterine contractility during pregnancy and the downregulation of the H<sub>2</sub>S production was observed in placental tissue obtained from preeclampsia patients.<sup>6,7,9</sup> In 2013, Wang et al, collected human placental tissues from preeclampsia associated pregnancies and normal pregnancies and studied and compared the expression of H<sub>2</sub>S producing enzymes and H<sub>2</sub>S levels in plasma. They concluded from their study that endogenous H<sub>2</sub>S is required for healthy placental vasculature and that a decrease in CSE/H<sub>2</sub>S activity may contribute to the pathogenesis of maternal hypertension and placental abnormalities in preeclampsia.<sup>8</sup>

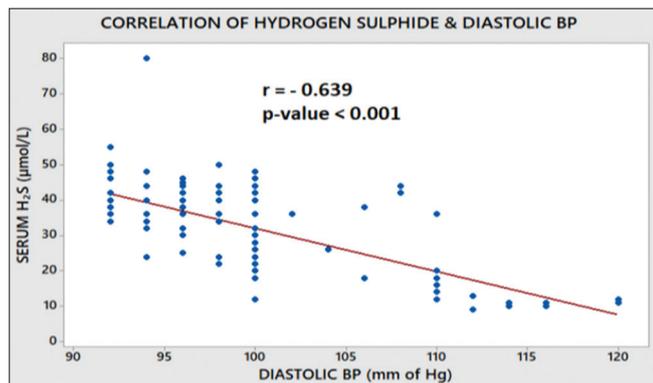
H<sub>2</sub>S induces vasodilation through opening of the ATP sensitive potassium channels in vascular smooth muscle cells of the resistance vessels.<sup>16</sup> H<sub>2</sub>S also upregulates



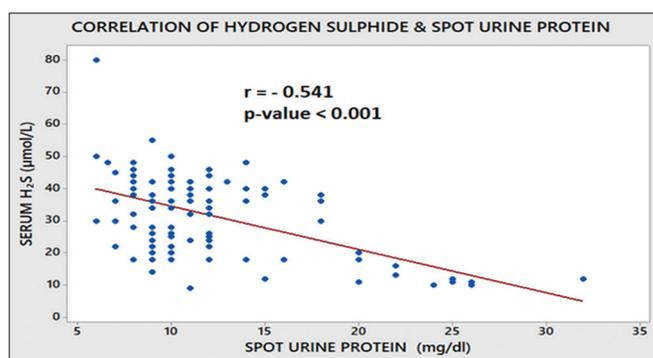
**Figure 2:** Correlation between serum H<sub>2</sub>S levels and systolic BP Pearson's Correlation done. p-value <0.05 considered significant

synthesis of vascular endothelial growth factor (VEGF) which is a potent angiogenic factor regulating maternal endothelial cell integrity.<sup>8,17</sup> In a study in the past it was seen that in preeclampsia, a potent antiangiogenic molecule known as soluble fms like tyrosine kinase 1 (sflt1) was produced from the placenta in the initial stages of hypoxia.<sup>18</sup> sFlt-1 antagonizes the proangiogenic effect of VEGF and placental growth factor (PlGF) and results in the maternal syndrome of preeclampsia.<sup>19</sup> VEGF also helps to maintain the integrity of the glomerular filtration barrier.<sup>20</sup> H<sub>2</sub>S is also endogenously produced in the kidneys by CBS and CSE enzymes and affects renal vasculature.<sup>21</sup>

In preeclampsia, the increased level of vasoconstrictors in circulation cause spasm of afferent glomerular arterioles, glomerular endotheliosis, increased capillary permeability and leakage of proteins in urine.<sup>11</sup>



**Figure 3:** Correlation between serum H<sub>2</sub>S levels and diastolic BP Pearson's Correlation done. p-value <0.05 considered significant



**Figure 4:** Correlation between serum H<sub>2</sub>S levels and spot urinary protein levels Pearson's Correlation done. p-value <0.05 considered significant

Despite a lot of research in the past, there still remains a lacuna regarding the role of H<sub>2</sub>S in the pathogenesis for the causation of hypertension and proteinuria in preeclampsia.

The present study elucidates a significant reduction in the levels of hydrogen sulphide and a significant rise in the level of spot urinary protein in patients with preeclampsia compared with normal pregnant women. This study shows a negative correlation between the serum levels of H<sub>2</sub>S and the systolic and diastolic blood pressure in preeclampsia. It also elucidates a negative correlation between serum H<sub>2</sub>S and urine protein levels in preeclampsia.

Since preeclampsia is a hypertensive disorder and hydrogen sulphide functions as a vasodilator, so it may be a possibility that hydrogen sulphide could be playing a role in the pathogenesis of preeclampsia. The rise in urinary protein with a fall in serum H<sub>2</sub>S implies that H<sub>2</sub>S may be indirectly responsible for the destruction of the glomerular filtration barrier and proteinuria. From review of literature, it may be considered that the downregulation of endogenous H<sub>2</sub>S synthesis in preeclampsia may have caused the increased synthesis of sft-1 followed by reduction of VEGF or over activity of vasoconstrictor molecules in circulation that led to proteinuria.

There is no known treatment for preeclampsia till today and the only therapy is delivery of the fetus and placenta. So it is considered that the placenta is the root cause for preeclampsia. Hence, this study can be used as a base to further research into the potential use of various H<sub>2</sub>S based therapies for early prevention and treatment of maternal complications like proteinuria and hypertension in preeclampsia.

But a further large scale study with a larger sample size is required in this direction to establish the therapeutic role of H<sub>2</sub>S and its association with the pathophysiology of hypertension and proteinuria in preeclampsia.

## LIMITATIONS OF THE STUDY

The sample size of the study could have been larger and newer sophisticated techniques for measurement of plasma H<sub>2</sub>S could not be undertaken. The H<sub>2</sub>S producing enzymes in placental tissues could not be measured.

## ACKNOWLEDGEMENTS

I would like to express my heartfelt thanks and gratitude to my guide, Dr. Utpal Kumar Biswas, The then Professor of the Department of Biochemistry, IPGME&R, Kolkata, for his immense support and patience in guiding me through my research work.

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**Authors Contribution:**

**SC-** Concept and design of the study, reviewed the literature, manuscript preparation, statistically analyzed and interpreted and critical revision of the manuscript; **UKB-** Concept and design of the study, data analysis and interpretation, drafting of manuscript, Final Approval; **AK-** Manuscript preparation and Critical revision of the manuscript.

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**Source of Support:** None, **Conflict of Interest:** None.