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Original Research Article

Dysregulation of H_2S Level in Plasma and Alteration of Biochemical Parameters in Preeclampsia: A Hospital Based Study

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ABSTRACT

Background: Preeclampsia is associated with high risk of maternal morbidity & adverse effect on fetal growth & development in our population. Though exact pathophysiology is unknown but there is compromised renal function causing alteration in various biochemical parameters which may cause detrimental effect on pregnancy outcome.
Aims & Objectives: to find out whether there is any significant difference in the level of H_2S, LDH, and uric acid in patients with preeclampsia in comparison to normal pregnant women.
Materials & methods: 100 preeclampsia patients (cases) were compared with 100 age matched normotensive pregnant women (controls) for biochemical parameters in terms of plasma H_2S, serum uric acid and LDH.
Result: The serum LDH concentrations in patients of preeclampsia is significantly (p =0.001) higher than the value in controls. The serum uric acid concentrations in patients of preeclampsia is also significantly (p =0.011) higher than the value in controls. Plasma H_2S level is also significantly higher (p=0.008) in the patients of pre-eclampsia.
Discussion: The decreased level of H_2S may result into endothelial dysfunction leading to hypoperfusion and placental insufficiency. As a result of reduced renal perfusion and glomerular filtration, Uric acid and LDH may get increased. No correlation of uric acid and LDH with H_2S was observed which suggests the role of some other confounding factors in the pathogenesis of preeclampsia.
Key words: Preeclampsia, Proteinuria, Hypertension, Pregnancy, Eclampsia

INTRODUCTION

Preeclampsia is a maternal hypertensive syndrome that occurs in 4% to 7% of pregnancies worldwide. [1] It is the major cause of maternal mortality which accounts for 15%-20% in developed countries and is a leading cause of preterm and intrauterine growth retardation. [2, 3] Preeclampsia is characterized by persistently elevated blood pressure of greater than 140/90 mm of Hg, proteinuria (Urinary albumin > 300 mg / day) and oedema after 20th week of gestation (ACOG,2002). It is described as a transient but potentially dangerous complication of pregnancy. This usually resolves soon after delivery, but early delivery increases the risk of complications to the baby. This has to be balanced against delay, which increases the risk of eclampsia to develop, with seizures and organ damage threatening the lives of both mother and baby. In later life, both of them are prone to develop chronic diseases including cardiovascular diseases. [4]

The exact etiology and pathogenesis of preeclampsia are unknown, but the growing evidence of an imbalance in angiogenic growth factors [5,6] and abnormal placentation [7,8] at the beginning of pregnancy, followed by generalized inflammation and progressive endothelial damage. [9]

During normal pregnancy, renal blood flow and glomerular filtration rate (GFR) are increased. In preeclampsia, a number of reversible anatomical and pathophysiological changes may occur such as reduced renal perfusion and glomerular filtration. [10]

H_2S is well known as a pungent environmental toxin which has bothered humanity for centuries. H_2S can be generated in mammalian body by three enzymes: Cystathionine γ-lyase (CSE), Cystathionine β-synthase (CBS) and 3-marcaptopyruvate sulphur-transferase (3-MST) from the substrates cystathionine, L-cysteine, homocysteine and marcaptopyruvate. In central nervous system, H_2S acts not only as neuromodulator, but also as a neuroprotectant against oxidative stress. [13] H_2S plays a very important role in maintaining the insulin level. In cardiovascular system, H_2S relaxes vascular smooth muscle by activation of K^+ATP channels and inhibits smooth muscle cell proliferation via mitogen-activated protein kinase signalling pathway. [16] These effects are important for maintaining normal blood pressure. [17] It is also cytoprotective against cellular damage induced by lethal hypoxia or reperfusion injury. [17,18] In regard to angiogenic effect of H_2S, evidence suggests that H_2S promotes angiogenesis via promoting PI3K/Akt or mitogen activated protein kinase / extracellular signal-regulated kinase signalling pathways. In addition, H_2S has antioxidant capacity by direct scavenging of nitrogen or reactive oxygen species. [18]

Therefore, it is obvious that, H_2S has potent effects on various physiological responses such as anti-inflammation, vasodilatation, modulation of oxidative and redox state, angiogenesis, neuromodulation, and inhibition of insulin resistance. [19, 20] Moreover, many researchers are trying to explore this gaso-transmitter about its metabolism and its role in different aspects of cell signalling, cell function and protection of cell from oxidative stress. The possible role of hydrogen sulphide in reproduction has not yet been fully investigated. We hypothesized that, because of its anti-inflammatory, pro-angiogenic, vasodilator and anti-oxidative characteristics, dysregulation of H_2S metabolism might play a role in pathogenesis of preeclampsia.

Biochemical parameters like uric acid and LDH are also investigated by different study groups to find out their role in pre eclampsia.

Hyperuricemia has been reported to be associated with adverse pregnancy outcomes in preeclampsia including increased preterm delivery, foetal growth restriction and foetal death. Elevation in circulating uric acid in preeclampsia is due to decreased GFR and changes in renal tubular handling and or uric acid production.

Altered renal handling is an important mechanism underlying hyperuricemia in preeclampsia, by decreased renal clearance of uric acid mainly because of increased tubular reabsorption. There is increase in xanthine oxidase in preeclampsia, which is responsible for endogenous uric acid production.

Uric acid is responsible for initiating inflammatory cascades through increased production of monocyte chemo-attractant protein-1, IL-1β and tumour necrosis factor-α (TNF-α). It interferes with normal spiral artery vascular remodelling by inhibiting trophoblast integration into uterine microvascular endothelial cell monolayer causing shallow trophoblastic invasion which is the characteristic of the disease.

Uric acid is biologically relevant antioxidant and rapidly oxidized in the presence of free radicals. In antioxidant-depleted environment, behaves as pro-oxidant contributing to oxidative endothelial damage.

Elevated level of LDH has also been reported as important finding. In preeclampsia, vascular endothelial dysfunction increases the sensitivity of the vasculature to vasoactive substances, with a subsequent reduction of perfusion and loss of fluid from the intravascular compartment. [11] These hemodynamic changes along with the activation of a coagulation cascade with microthrombi formation as a result of endothelial damage will result in the various clinical complications associated with pre-eclampsia. [11,12] The multi-organ dysfunction in severe preeclampsia caused by vascular endothelial damage, including maternal liver, kidney, lungs, nervous system, blood, and coagulation system, will lead to excessive LDH leakage and elevated levels in serum due to cellular dysfunction. [13]

Severely pre-eclamptic women with LDH levels of 800 IU/l showed a significant increase in complications in terms of eclampsia, abruptio placentae and various other complications like renal failure, HELLP syndrome, cerebrovascular accidents compared to women who had lower serum LDH levels. [14] Maternal and perinatal mortality is increased significantly in patients with levels of LDH >800 IU/l, suggesting chronic hypoxia because of hypoperfusion and placental insufficiency as a result of endothelial dysfunction. [14,15]

With these view, The study was done to measure the plasma H_2S level and that of serum uric acid and lactate dehydrogenase (LDH), in patients of preeclampsia & healthy pregnant women. The correlation of uric acid level and LDH activity with plasma H_2S level was also aimed to find out.

MATERIALS & METHODS

This observational case control study was undertaken in the Department of Biochemistry in collaboration with the department of Obstetrics and Gynaecology, NRS Medical College and Hospital, Kolkata, West Bengal, during the period from July 2016 to June 2017. A total number of 100 patients suffering from preeclampsia were included in the study. They were initially diagnosed by clinical and biochemical parameters in the Department of Obstetrics and Gynaecology of Nilratan Sircar Medical College & Hospital, Kolkata. For control, 100 samples were collected from normal pregnant woman attending for pre-natal check-up to OPD of Obstetrics & Gynaecology of the same institution. The approval of the study was taken from the Institutional Ethics Committee of NRS Medical College & Hospital.

Patients having seizure disorders, eclampsia, disseminated intravascular coagulation (DIC), pregnancy induced hypertension (PIH) and pre-existing hypertension , patients with history of repeated abortion &patients with history of renal failure, diabetes mellitus, alcoholism, hepatic dysfunction were excluded from the study.

5 to 6 ml of blood sample was collected from both cases & controls after proper explanation & informed consents, aseptically by disposable syringe. 3ml of blood was transferred in a EDTA vial and gently shaken & the remaining blood was transferred into a clotted vial. The clotted blood sample was centrifuged at 2500 rpm for 5 minutes. The serum was separated, analysed and was kept in aliquots and stored in minus forty degree Centigrade (-40°C) in deep freezer. The EDTA blood sample was kept in 4-8^{0}C.

Measurement of H2S concentration in plasma

The estimation of plasma H2S levels was done following the methods reported earlier which is further modified and standardized in our laboratory. [21]

Principle: $Zn^{2+}$was added to serum sample to deposit H_2S, HS^- and S^{2-}, as well as serum protein. Then NaOH is used to re-dissolve serum protein. ZnS deposit was re-dissolved by the addition of N, N-dimethyl-p-phenylenediamine, and the remnant protein was deposited by tri-chloroacetic acid. After centrifugation, ferric chloride was added to the supernatant fluid to generate methylene blue, which was analysed by spectrophotometer at 670 nm.

For measurement of plasma H2S level, sodium hydrogen sulphide (NaHS) has been used to construct the calibration curve. When NaHS is dissolved in water, HS- is released and forms H2S, with H+ ions present in water. Different dilutions of NaHS from a stock solution of 250 µ mol/ml were made in different test tubes, and under the same conditions of the method of estimation of H2S in plasma and the same technique was followed to take the absorbance (obtained at 670nm) and plotted on a graph.

Serum LDH was estimated by UV kinetic method & Uric acid was estimated by Uriase method. All the parameters were measured using standardized kits in semi auto analyzer.

Statistical Methods: The data was tabulated and analysed using standardized statistical methods (SPSS20).

RESULTS

In this study, 100 patients of preeclampsia and 100 healthy, age and gestational age matched control subjects were studied. Biochemical parameters, namely serum LDH and uric acid were measured from the samples of the whole study population. The biochemical parameters of both patients and controls subjects are shown in table 1.The serum LDH concentrations in patients of preeclampsia (235.6 ± 104.14 IU/L) is significantly (p =0.001) higher than the value in controls (122.4 ± 18.63 IU/L). The serum uric acid concentrations in patients of preeclampsia (5.95 ± 1.03 mg/dl) is also significantly (p =0.011) higher than the value in controls (4.66 ± 0.46 mg/dl). The plasma H2S level in patients of preeclampsia (11.18 ± 3.12 µmol/ml) is significantly (p =0.008) lower than the value in controls (36.79 ± 5.41 µ mol/ml)

The biochemical parameters of both patients and controls subjects are shown in table 1.

Parameters	Cases(100) Mean ± SD	controls(100) mean ± SD	t-value	p-value
Serum LDH (IU/L)	235.6±104.14	122.4±18.63	4.79	0.001
Serum Uric acid (mg/dl)	5.95±1.03	4.66±0.46	5.20	0.011
Plasma H_2S conc.(µmol/L)	11.18±3.12	36.79±5.41	-21.2	0.008

Independent 't' test done. p value < 0.05 considered significant at a confidence interval of 95%.

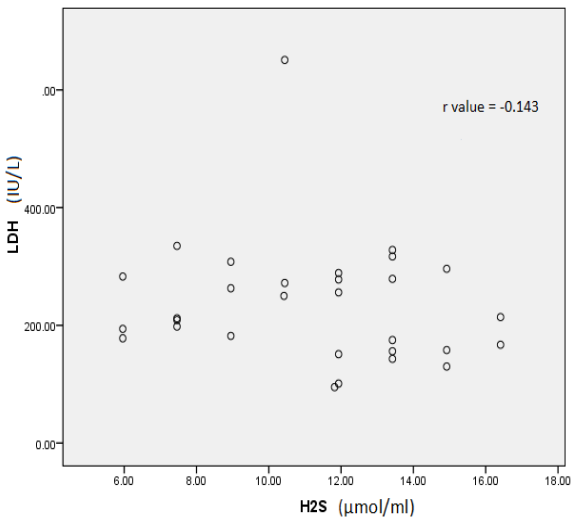

Fig 1: Correlation between H_2S & LDH in study population

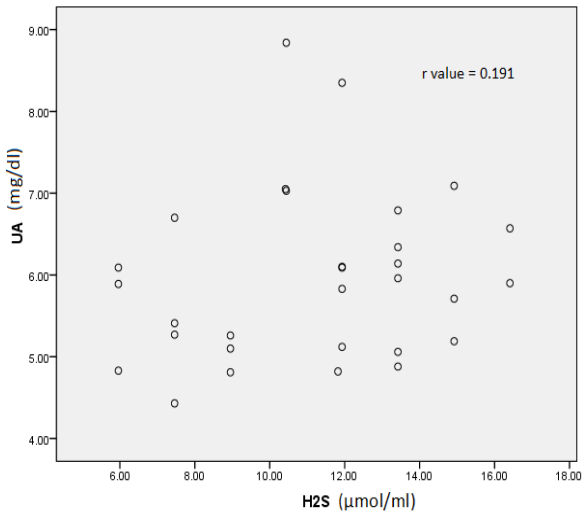

Fig 2: Correlation of H_2S & Uric acid in study population

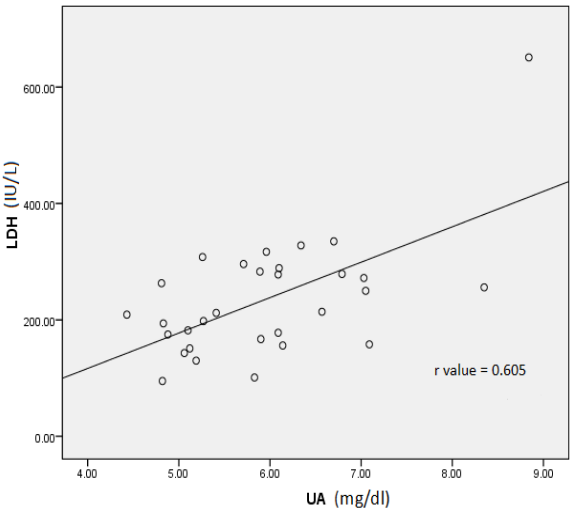

Fig 3: Correlation of uric acid & LDH in study group

Figure (1). shows there is a negative correlation (r = - 0.143) between plasma H2S and serum LDH. It means when plasma H_2S is decreased LDH get increased. However, this correlation was not found to be statistically significant.

Figure (2) shows there is no significant correlation (r = 0.191) found between plasma H_2S and serum uric acid.

In the figure 3, it is obvious that there is a statistically significant (p<0.001) positive correlation (r = 0.605) between uric acid and LDH.

DISCUSSION

Preeclampsia is one of the pregnancy specific hypertensive disorders with multisystem involvement and widespread vascular endothelial malfunction and vasospasm that occurs after 20th week of gestation or as late as 4 to 6 weeks of postpartum. In India, it is the major burden of maternal and perinatal morbidity and mortality with 8 - 10% incidence rate among the pregnant women. [14]

Alteration in biochemical parameters are seen in various studies in preeclampsia. Though exact pathogenesis is unknown, defective placental angiogenesis may be the probable cause. Elevated serum uric acid level is often an important finding associated with preeclampsia, which usually subsides after delivery but may give rise to long standing hyperuricemia.

Elevated serum LDH level has also been found in various studies is associated with preeclampsia, which may be due to the result of increased cell damage in hypoxia and oxidative stress.

The biochemical parameters in our study have been given in Table-1. The serum LDH levels of the patients (235.6 ± 104.14 mg/dl) are significantly (P<0.001) higher than the controls (122.4 ± 18.63mg/dl). In a study, Qublan et al. found the similar result. They identified LDH as a biochemical marker of adverse pregnancy outcome in preeclampsia and eclampsia. [15]

The values of serum uric acid in the patients (5.95 ± 1.03) are also found significantly increased (P<0.011) than the healthy controls (4.66 ± 0.46). These are also in accordance with the previous study (Qublan et al).

Although this study has limited sample size but the observations are similar with the previous studies. There is significantly increased level of serum uric acid and LDH level which are suggestive of endothelial damage and decreased placental oxygenation in preeclampsia.

In our study, the plasma H_2S levels in the patients with preeclampsia are 11.18 ± 3.12 µmol/ml with the values ranging from 5.96 to 16.41 µmol/ml. This is significantly lower (P<0.008) than the plasma H_2S concentration found in the controls (36.79 ± 5.41µ mol/ml) with a range from 27.32 to 46.21 µmol/ml in healthy controls.

Wang et al. have reported that genetic deletion of the H_2S producing enzyme cystathionine γ-lyase (CSE) leads to the constriction of blood vessels, thus developing hypertension4. Li et al. have reported that H_2S performs reversible and rapid vasorelaxation through opening of K^+ATP channels and elevated cGMP levels in vascular smooth muscle cells (SMCs). [22]

H_2S enhances the efficiency of the antioxidant enzyme superoxide dismutase to scavenge superoxide and increase the level

of reduced glutathione (GSH) biosynthetic enzyme c-glutamyl cysteine synthase. It also modulates mitochondrial function, as it is a potent and reversible inhibitor of cytochrome c oxidase. Thus it obtuse cellular respiration, which in turn reduces mitochondrial reactive oxygen species (ROS) production and decreases mitochondrial uncoupling.

Bir et al. demonstrated that H_2S plays a more dominant role in angiogenesis under hypoxic conditions. It causes microvascular growth which is mediated through upregulation of HIF-1/VEGF/Akt mediated pathway in vascular endothelial cells. [23] H_2S can reverse the down-regulating VEGFR2 by specific phosphorylation at Tyr 996 of the receptor153 and that VEGFR2 acts as a receptor for H_2S during angiogenesis. It has been reported that K^+ATP channel plays a mediator in the angiogenic effects of H_2S. [23]

However, the Pearson correlation analysis demonstrates that the plasma H_2S concentration in pre-eclamptic patients are negatively correlated (r = - 0.143) with the levels of LDH (Figure 5.8), but this correlation was not statistically significant. We have further observed that the H_2S levels are not significantly correlated (r = 0.191) with the serum uric acid levels. Probably, because of small number of sample these correlations were found to be insignificant, and further study with larger number of samples may be needed to get more conclusive evidences.

The changes in the biochemical parameters according to severity are important for consideration as a biomarker, which is not done in this study.

CONCLUSION

Plasma H_2S, serum LDH and uric acid concentrations are positively correlated with preeclampsia & are significantly higher in pre-eclamptic patients in comparison to normal pregnant subjects. Levels of Serum LDH and uric acid are found to be positively correlated in patients of preeclampsia.

Due to time constrains, cost of reagents and feasibility in our department, sample size of the study is restricted. A multi centred study would have been a better choice.

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