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Original Article

Nitric oxide-cyclic GMP signal transudation pathway in pregnancy induced hypertension

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ABSTRACT

Due to the conflicting nature of the role of nitric oxide in pregnancy induced hypertension, we had undertaken this study to estimate the levels of nitric oxide, cyclic GMP and calcium in order to follow the signal transduction pathway in PIH at different periods of gestation. Venous samples were obtained from 40 pregnant women with diagnosed PIH (study group) and 20 healthy pregnant women (control group) at 28 weeks, 36 weeks and post delivery (within 48 hours). Blood samples were assayed for serum nitric oxide, plasma cyclic GMP and serum calcium. A comparison between the study and control group showed higher levels of nitric oxide, cyclic GMP and calcium in study group at each period of gestation with maximum levels at 36 weeks. There was significant correlation between serum nitric oxide and plasma cyclic GMP (r=o.2869) in study group at 36 weeks of gestation. Serum nitric oxide is increased in pregnancy induced hypertension, and nitric oxide, cyclic GMP- calcium signal transduction pathway is very important for the biological role of NO.

1. Introduction

Pregnancy induced hypertension (PIH) is one of the common complications of pregnancy and contributes significantly to the cause of perinatal morbidity and mortality. The incidence of preeclampsia in hospital statistics varies from 5-15% [1]. Primigravidas and women at either end of reproductive age have been found to be more susceptible to it.

PIH is a multisystemic spectrum of disorder wherein intense vasospasm due to increased sensitivity of the vasculature to pressor agents compromises perfusion to most of the organs that is further aggravated by the activation of coagulation cascade with simultaneous micro thrombi formation. Also plasma volume is decreased by the loss of intravascular fluids thereby reducing organ blood flow. Aberration of the interaction between placental and maternal tissue is probably the primary cause. The acute stage of PIH might be the clinical biological expression of the endothelial injury [2], and perhaps a part of a more extensive and exaggerated inflammatory response mediated by placental oxidative stress [3].

Nitric oxide (NO) produced by vascular endothelial cells is a potent vasodilator [4]. Alteration in the release of NO in fetoplacental and maternal circulation may have a role to play in pathogenesis of PIH with some studies favoring [5,6] and some studies challenging it [7]. Nitric oxide exerts its effects by binding to heme group of guanylate cyclase enzyme resulting in profound (50-200 times) increase in rate of conversion of guanosine 5' triphosphate (GTP) to cyclic GMP. cGMP dependent protein kinase 1 (PKG1) mediates vascular relaxation by nitric oxide (8) resulting in phosphorylation of myosin light chain kinase, which has a reduced affinity for calcium calmodulin complex. This reduces the vascular smooth muscle contraction and hence the vascular tone. Reports on the exact role of NO in PIH are still conflicting. Some studies suggest that increase in NO in PIH is a compensatory response to maintain homeostasis [9,10]. Other studies however have shown same (11) or lower levels in cases of PIH when compared to normal pregnancy [2,12]. This conflicting nature of the reports available at the current time spurred us to devise this study to assess the role of nitric oxide-cGMP signal transduction pathway in hypertensive disorders complicating human pregnancy at different periods of gestation.

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2. Materials and Methods

The prospective study was conducted in the Departments of Biochemistry, and Obstetrics and Gynecology of the Lady Hardinge Medical College and associated Smt. Sucheta Kriplani Hospital at New Delhi, India.
60 subjects were selected from pregnant women attending the antenatal clinic or admitted in the antenatal wards, with informed consent. They were assigned into two groups:

Study group: Consisting of 40 pregnant women with diagnosed PIH i.e. with BP =140/90 mmHg with or without proteinuria (High Blood Pressure in Pregnancy-2000).

Control group: Consisting of 20 healthy pregnant women.

Sample Collection: Venous samples were collected from all patients at 28 weeks and 36 weeks of gestation, and post delivery (within 48 hrs).

Fresh serum was used to measure serum calcium levels by Arsenazo III colorimetric method [13] and for the other routine investigations. Rest of the blood samples were stored at 20^0C till batch analyzed.

NO in serum was determined indirectly by measurement of its stable decomposition product nitrite (N_02^-) employing the Griess reaction according to modified method of Mathew et al (14). Plasma cGMP was measured in heparinized plasma by competitive Enzyme Immuno Assay (EIA) (15).

Results obtained were analyzed on SPSS statistical package and subjected to students t- test and normal distribution for statistical evaluation.

3. Results

Gestational hypertension was seen in 27.5% cases of PIH, preeclampsia in 65% cases and eclampsia in 75% PIH pregnancies (as per the report on High Blood Pressure in Pregnancy-2000).The mean age of PIH patients was 24.2 ± 3.7 years, ranging from 15-35 years. There were 72.5% nulliparous women compared to 53.3% nulliparous patients in the control group (refer table I). This relates well with the fact that the incidence of PIH is higher in nulliparous women. The diagnosis of PIH is made when the blood pressure is 140/90 mm Hg or greater. In our study, at 28 weeks of gestation, the mean systolic BP in PIH was 146.2 ± 6.1 mm Hg and diastolic BP was 95.4 ± 7.6 mm Hg. In the control group the mean systolic BP was 105.1 ± 13.4 mm Hg and diastolic BP was 69.40 ± 9.3 mm Hg (refer table I).

Table 1. Clinical profile of patients in study and control groups.

Mean of	Study Group (n=40)	Control Group (n=20)
Age (in years)	24.2±3.7	23.3±2.3
Parity (gravida)	1.3±0.6	1.6±0.7
Systolic BP (mmHg)	146.2±6.1	105.1±13.4
Diastolic BP (mmHg)	95.4±7.6	69.4±9.3

Proteinuria is an important indicator of the severity of PIH often developing late in the course of the disease. In the study group, 52.5 % patients at 28 weeks, 80 % at 36 weeks and 55 %patient during post delivery had proteinuria (1+ or more on urinary dipstick).

The mean serum uric acid levels in PIH cases were insignificantly higher than the cut off value (4.5 mg/dl) at all periods of gestation as compared to the control group.

There was a significant increase in serum NO levels (p= 0.005) in the study groups from 28 weeks to 36 weeks, which decreased significantly after delivery (p= 0.001)(refer table Π, figure 1).

In the present study, there was a significant increase in the plasma cGMP levels in the study group from 28 weeks to 36 week of gestation (p=0.01), which decreased significantly in post delivery (p= 0.005) (refer table Π, figure 1). A comparison between the study and the control group showed significantly (p= 0.001) higher levels of plasma cGMP in the study group at each period of gestation studied.

Serum calcium levels showed an insignificant variation during the course of pregnancy both in the study and the control groups. This may be due to the fact that in India pregnant women are prescribed calcium tablets empirically in antenatal period.

4. Discussion

Hypertension in PIH patients as compared to control group can be explained by generalized vascular endothelial cell dysfunction, which leads to an imbalance between vasodilatory prostacyclin (PGI_2) and vasoconstrictor products such as endothelin and thromboxane (TXA_2) [16].

Although the finding was not significant but higher concentration of uric acid was found in PIH patients as compared to control group. H.Pasaoglu et al [17] also reported higher uric acid concentration in preeclamptic group compared with control group.

As shown in results, comparison between the study and the control groups depicted a higher level of serum NO in the study group at each period of gestation that was significant at 28 weeks (p= 0.025) and 36 weeks (p=0.01). Similar findings were reported by Lucy et al [18] showing production of NO in established preeclampsia was higher in the uteroplacental, fetoplacental and peripheral circulation than in normotensive pregnancies. Nobunaga T et al [19] showed higher levels of NO metabolites in PIH patients compared to those with long term hypertension suggesting that increased NO was specific to PIH. Also it has been suggested that endothelial damage occurs in PIH resulting in a compensatory increase in production of NO from healthy endothelium [11,20]. Shaamash et al [21] concluded that serum NO production is increased in normal pregnancy and more so in preeclampsia and eclampsia.

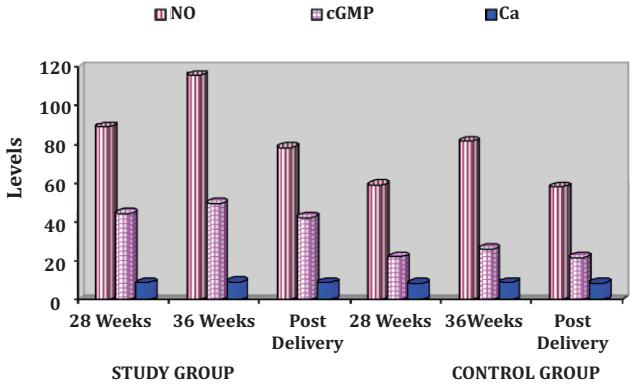
It is hypothesized that NO generates cGMP in endothelial cells after directly activating soluble guanylate cyclase by binding to its ferroheme group [22] and the relaxation response to NO is directly proportional to the concentration of cGMP [23]. cGMP acts as second messenger, to activate the cascade of protein kinase that maintains myosin light chain in dephosphorylated state hence leading to smooth muscle relaxation. Schneider et al [24] have reported a higher cGMP in preeclampsia relative to measurements in normal pregnancy. Several studies have conclusively shown that NO with cGMP as its second messenger plays definite role in maternal vasodilatation [25,26] and is determinant in control of vascular reactivity.

Serum NO and plasma cGMP correlated significantly (r= 0.2869)in the study group at 36 weeks. This may suggest an

Table 2. Levels of NO, cGMP and Ca in the study and control groups at different periods of gestation.

	Study Group (MEAN± SD)			Control Group (MEAN± SD)		
Age (in years)	28 Weeks	36 weeks	Post Delivery	28 Weeks	36 Weeks	Post Delivery
NO (/L)	89.14±38.97	115.58±41.23	78.41±31.32	59.39 ± 41.54	81.56 ±41.58	58.10±37.42
cGMP (pmol/ml)	44.34±9.42	49.75±9.68	49.75±9.68	22.08±3.39	26.27±4.97	21.86±6.20
Ca (mg/dl)	8.87±0.86	9.20±0.94	8.82±0.93	8.50±0.90	9.05±1.08	8.80±0.8

Figure1. Bar diagram for the levels of NO, cGMP and Ca in the study and control groups at different periods of gestation.



enhanced NO-cGMP-Ca system as a result of the increased NO release in response to endothelial dysfunction in PIH. Increased NO release in PIH in our study supports the hypothesis that an increase in NO production may be a compensatory response to maintain homeostasis thus improving blood flow in fetoplacental circulation and decreasing platelet adhesion in the uteroplacental circulation The increase in plasma cGMP and calcium levels in the study group paralleled the rise in serum NO levels in these patients, thus following the NO-cGMP-Ca signal transduction pathway which is highly conducive to the biological role of nitric oxide in transcellular communication.

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