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Comparative study of nitric oxide and malonyldialdehyde in pregnancy induced hypertension, normal pregnant and non-pregnant women.

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ABSTRACT

Normal pregnancy is associated with progressive anatomical and physiological changes in all the systems of the body. About 7-16% of normal pregnancies are complicated by pregnancy-induced hypertension (PIH). Increased oxidative stress coupled with decreased antioxidant status is an important pathophysiological pathway in PIH. Total 180 women were included in the study which were further divided as PIH (n=60), normal pregnant (n=60) and normal non-pregnant (n=60) .Serum Malonyldialdehyde was estimated using Thiobarbituric acid reactivity method. Nitric oxide levels were estimated using Griess reaction. Statistical analysis was carried out using SPSS for windows 10.0 software (SPSS Inc., Chicago, IL, USA). The results revealed that both Malonyldialdehyde (2.9 $\pm$ 0.5 Vs 1.7 $\pm$ 0.2 Vs 4.6 $\pm$ 0.9; $p<0.05$) and Nitric oxide levels (66.0 $\pm$ 11.0 Vs 29.1 $\pm$ 5.3 Vs 115.7 $\pm$ 17.9; $p<0.05$) were significantly increased in normal pregnant women in comparison to normal non-pregnant women. These levels were found to be further significantly increased in women having PIH. A positive correlation was also found between Malonyldialdehyde and Nitric oxide levels in women having PIH ($r=0.41$). It is hypothesized that this marked increase in nitric oxide levels in women with PIH is an indicator of increased oxidative stress and therefore, has the potential for being used as a marker for PIH.

Keywords-Pregnancy, Malonyldialdehyde, Nitric oxide, Oxidative stress, PIH.

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INTRODUCTION

Pregnancy induced hypertension (PIH) is defined as hypertension which develops as a consequence of pregnancy and regresses postpartum. About 7-16% of pregnancies are complicated by increased blood pressure during gestation. [1] Increased oxidative stress is the widely accepted cause of PIH. Lipid peroxidation is a complex process where unsaturated lipids undergo reaction with molecular oxygen and other reactive oxygen species, which damages cell membrane and other molecules to yield lipid hydroperoxides.[2] Malonyldialdehyde (MDA) is an end product of lipid peroxidation. It is a volatile, low-molecular weight ($C_3H_4O_2$; molecular weight 72.07), short chain, 1, 3 dicarbonyl compound and moderately weak acid, PKa=4.46. [3] In women with PIH, Malonyldialdehyde levels increase beyond normal pregnancy levels by second trimester. [4] Malonyldialdehyde levels in normal pregnancy are also higher than non-pregnant individuals. [5]

Nitric oxide (NO) has a lone electron in outer shell and thus can act as free radical. It is synthesized from arginine by the action of enzyme nitric oxide synthase (NOS). [6] It exists in several isoforms of which Type III NOS (e NOS) synthesized in endothelial cells causes vasodilatation and is responsible for generalized vasodilatation which characteriscally occurs in normal pregnancy. [7] PIH is mediated by vascular endothelial dysfunction. [8] Studies related to involvement of Nitric oxide in PIH are controversial with some studies favoring it and some against it. Varying-[lower [9], similar [10] or even increased [11, 12]] levels of Nitric oxide metabolites have been reported in different studies in women with PIH.

In the present study, Malonyldialdehyde and Nitric oxide levels were estimated in women having pregnancy induced hypertension and results were compared with those obtained in normal pregnant and normal non-pregnant groups.

MATERIAL AND METHODS

The study was carried out on 60 patients of PIH, 60 normal pregnant women and 60 normal non-pregnant women. These were selected from antenatal clinic and maternity ward. The cases were chosen according to standard definition of PIH irrespective of parity and socioeconomic status. Presence of any other preexisting medical disorder served as exclusion criteria. The controls chosen had normal blood pressure, showed no abnormal weight gain and were matched for age, parity and socioeconomic status with PIH cases. The study was approved by institutional ethical committee.

Written consent was taken from all the patients prior to sample collection. Under sterile conditions, single venous sample was collected. Patients in the study group had sample collected at the time of diagnosis of PIH prior to institution of any treatment and from normal pregnant females after 20 weeks of gestation. Simultaneously, samples from non-pregnant women were also collected.

Fresh sample was used to perform routine investigations. Serum was stored at -70^{o} C till Malonyldialdehyde and Nitric oxide levels were analyzed.

Malonyldialdehyde levels were estimated using thiobarbituric acid reactivity method. [13] Thiobarbituric acid reacts with lipid peroxides to give a red colored pigment. The intensity of the color obtained was proportional to the concentration of lipid peroxides, which was measured using Spectrofluorometer.

Nitric oxide is very unstable compound therefore; its level in serum was determined indirectly by the measurement of its stable decomposition product nitrite and nitrate, employing the Griess reaction. [14] Griess reaction involves formation of chromophore during reaction of nitrite with Sulphanilamide and N-(-naphthyl) ethylenediamine (Griess reagent) to produce a purple azo compound, which was measured photometerically.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS for windows 10.0 software (SPSS Inc., Chicago, IL, USA). Values were reported as mean and standard deviation. The difference between groups was compared by Kruskal-Wallis or Mann- Whitney test for continuous variables and by Wilcoxon test for paired comparison. Spearman's rank correlation was applied to test for association between continuous variables. A two-tailed p value<0.05 was considered statistically significant.

RESULTS

The present study was conducted on 60 cases of PIH and results were compared with equal number of age matched normal pregnant and normal non-pregnant women. The results obtained are summarized in the tables.

There is physiological hyperlipidemia in normal pregnancy, as shown in Table I. Serum cholesterol levels were significantly elevated in normal pregnant women in comparison to normal non-pregnant women (258.3 $\pm$ 28.1 Vs 187.5 $\pm$ 15.9;$p<0.05$). In PIH, there is altered lipid metabolism and there is significant increase in serum cholesterol levels in these women in comparison to normal pregnant women (319.5 $\pm$ 35.3; $p<0.05$). High availability of unsaturated lipids contributes to increased lipid peroxidation which is shown as increased Malonyldialdehyde levels in normal pregnant women increased in comparison to normal non-pregnant women and further increased in women having PIH in comparison to normal pregnant women (2.9 $\pm$ 0.5 Vs 1.7 $\pm$ 0.2 Vs 4.6 $\pm$ 0.9 ;$p<0.05$) as shown in Table II. Similarly, levels of Nitric oxide were increased in normal pregnant women compared to normal non-pregnant controls. These levels were further significantly increased in women with PIH in comparison to normal pregnant women (66.0 $\pm$ 11.0 Vs 29.1 $\pm$ 5.3 Vs 115.7 $\pm$ 17.9; $p<0.05$ Table II).

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TABLE I
BASELINE DATA IN NORMAL NON PREGNANT, NORMAL PREGNANT, PREGNANCY INDUCED HYPERTENSION (PIH) GROUPS

PARAMETER	NORMAL NON PREGNANT MEAN±S.D.(RANGE) N=60	NORMAL PREGNANT MEAN±S.D.(RANGE) N=60	PIH MEAN±S.D.(RANGE) N=60
Age (years)	26.0±4.6(20-35)	26.2±4.4(20-35)	26.1±4.2(20-35)
Syst.BP(mmHg)	119±5(106-126)	118±6(106-130)	144+13(130-190)
DiastBP(mmHg)	77±5(68-84)	75±5(64-84)	98±6 * (90-120)
Hb (g/dl)	11.0±0.8(9.8-12.5)	10.9±0.9(8.7-12.5)	10.1±1.5(6.0-12.5)
Urea (mg/dl)	24.7±4.7(15-30)	20.6±3.5(15-30)	26.2±14.8(18-100)
Uric acid (mg/dl)	2.8±0.3(2.5-3.5)	3.0±0.4(2.5-4.0)	5.0±0.8 †‡ (3.0-6.5)
Calcium (mg/dl)	8.7±0.07(7.5- 11.0)	8.5±0.9(7.5-11.0)	8.3±1.0(7.5-11.0)
Total Cholesterol (mg/dl)	187.5±15.9(163-218)	258.3±28.1* (221-310)	319.5±35.3†‡(280- 370)
Proteinuria	Absent	Absent	Present

*p<0.05 in comparison with normal non- pregnant women
†p<0.05 in comparison with normal pregnant women
‡p<0.05 in comparison with normal non-pregnant

TABLE II
DISTRIBUTION OF WOMEN VIS-a-VIS SERUM MDA AND NITRIC OXIDE LEVELS IN NORMAL NON-PREGNANT, NORMAL PREGNANT AND PREGNANCY INDUCED HYPERTENSION (PIH) GROUPS.

WOMEN IN DIFF.GROUPS	MDA LEVELS (nmol/ml) (MEAN±S.D.)	NO LEVELS (μM/L) (MEAN±S.D.)
NORMAL NON-PREGNANT	1.7±0.2	29.1±5.3
NORMAL PREGNANT	2.9±0.5 *	66.0±11.0 *
PIH	4.6±0.9 †	115.7±17.9†

*p<0.05 in comparison with normal non-pregnant women
†p<0.05 in comparison with normal pregnant women

DISCUSSION

Pregnancy induced hypertension is considered to be one of the most significant health problems complicating 7-16% of all pregnancies. [1] Estimation of Malonyldialdehyde shows the extent of lipid peroxidation, i.e. free radical injury or oxidative stress. In the present study, 80% of normal non-pregnant women had values between 1-2nmol/ml, which increased marginally for normal pregnant women to between 2-3nmol/ml whereas most of the PIH patients had values exceeding 4.1nmol/ml (Table II). The results are in accordance with values obtained in other studies. [4,5] The data depicts increased lipid peroxidation i.e. increased oxidative stress in women with PIH.

Nitric oxide when secreted at physiological level activates soluble guanylate cyclase system, resulting in increased production of cyclic guanosine monophosphate (cGMP) and thus causes relaxation of smooth muscles and which provides constant vasodilator tone. [7] In the present study, Nitric oxide levels are significantly increased in normal pregnant women compared to normal a non-pregnant woman which is also in accordance with other studies [15,16] (Table-II,). This is a consequence of Nitric oxide reacting with lipid-derived radicals to terminate lipid peroxidation propagation reactions. [15,16]

Nitric oxide is a double-edged sword as at optimum levels it acts as vasodilator, whereas when generated at high levels it is converted to pro-oxidant species as peroxynitrite and nitrogen dioxide. [17] Peroxynitrite mediates peroxidation of unsaturated fatty acids. [18] Invitro, peroxynitrite oxidizes diverse classes of lipids forming conjugated diene, Malonyldialdehyde, lipid peroxide and lipid hydroxide etc.. [18] In the present study Nitric oxide levels in PIH group were found to be significantly higher than those in normal pregnant women. In several other studies also Nitric oxide levels were found to be significantly higher in PIH group in comparison to normal pregnant group. [14, 19]

In the present study, in PIH group both serum Malonyldialdehyde and Nitric oxide levels were found to be significantly increased compared to those observed in other two groups. A significant positive correlation was found between serum Nitric oxide levels and Malonyldialdehyde levels in PIH group ($r=0.41$; $p<0.05$). Both Nitric oxide signaling pathways and lipid peroxidation reactions involve free radical species that react at extremely fast rates, therefore, convergent interactions between these pathways are expected.

Therefore, in the light of above discussion it can be hypothesized that this marked increase in serum Nitric oxide levels in women having pregnancy induced hypertension is contributing towards pathphysiology of PIH i.e. increased oxidative stress leading to vasospasm. Nitric oxide therefore, has the potential for being used as a marker for PIH.

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