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Research Article

COMPARATIVE STATUS OF OXIDANT /ANTIOXIDANT IN PREGNANCY INDUCED HYPERTENSION AND CERVICAL DYSPLASIA

Shivani Pandey[1], Babita Singh[1], Mita Agnihotari[2], A.A Mahdi[1]

1. Department of Biochemistry, King George's Medical University, Lucknow, India.
2. Department of Obstetrics and Gynecology, King George's Medical University, Lucknow, India.

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ABSTRACT

Present study is aimed to delineate a co-relation between status of anti-oxidant enzymes, lipid peroxidation products with severity of Pregnancy Induced Hypertension (PIH) and cervical dysplasia. The study has been done on 66 patients out of whom 14 were normal pregnancy and had no disease, 31 patients of PIH and 21 patients having cervical dysplasia gr. I-II. Enzymatic activities of Superoxide dismutase (SOD), Catalase (CAT), vitamin C and vitamin E and malonaldehyde levels (MDA, a marker of lipid peroxides) were assessed spectrophotometrically. Mean Malonaldehyde level in PIH patients (8.48 ± 0.96) & Cervical patients (8.31 ± 1.25) in comparison to normal patients (4.88 ± 1.035), the rise was found highly significant ($p < 0.001$). The enzymatic activity of antioxidants such as Superoxide dismutase, Catalase, vitamin C and vitamin E was decreased in PIH patients & cervical dysplasia patients as compared to normal patients ($p < 0.001$). The difference was statistically significant ($p < 0.001$) whereas the same was observed in cervical dysplasia patients. No statistically difference was observed between the levels of antioxidants of PIH patients & cervical dysplasia patients ($p < 0.01$). Co-relation of Hb with MDA level showed that aneamic patients have raised level of MDA as compared to non aneamic patients. There is positive oxidant/antioxidant balance which may aggravate free radical damage during pre eclampsia and eclampsia with cervical dysplasia**.**

Keywords: Pregnancy Induced Hypertension, Cervical dysplasia, Free radical, Malonaldehyde, Antioxidants.

INTRODUCTION

Hypertensive disorders are most common complications in pregnancy & leading causes of maternal mortality. Cervical cancer is the most prevalent genital tract cancer in the world, including India[1, 2]. Each year about 50,000 women's die due to Pregnancy-induced hypertension (PIH). PIH along with cervical cancer, the major cause of death are the salient free radical disease.

PIH includes a group of hypertensive disorders, Gestational hypertension which is without edema and proteinuria, pre-eclampsia and eclampsia with edema and proteinuria. In Pregnancy Induced Hypertension many complex homeostatic modifications occur, some are harmful to the mother and fetus, while others are beneficial. In health, oxidation by free radicals and neutralization by antioxidants remains in balance. When the reactive oxygen species (ROS) are in abundance, oxidative stress occurs which is thought to be the causative factor in PIH[3]. It is earlier known that oxidative stress is exaggerated in PIH. Some recent work has focused on the role of oxidative disturbances in the pregnancy induced hypertension, pre-eclampsia and eclampsia.

It has also been hypothesized that reduction in the antioxidant activity may enhance endothelial cell oxidative damage but studies of various systems have produced conflicting results[4-10]. Lipid peroxidation is a complex process in which unsaturated lipids undergo reaction with molecular oxygen and other reactive oxygen species, which damages cell membrane and other molecules to yield lipid hydroperoxides[11]. Uncontrolled lipid peroxidation is a key

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contributing factor to pathophysiologic condition of preeclampsia[12, 13, 4, & 5]. There is now ample proof for the existence of oxygen derived free radicals in human placenta[12].

In Cervical Dysplasia changes occurs in the histology of their cervices. These abnormalities are precancerous changes in the cells and create a condition called Cervical Dysplasia (CD). It is a multifactorial disease process and several risk factors include, early age intercourse, multiple sex partners, low socioeconomic status, and Human papillomavirus (HPV) infection[1, 2]. Recent data also revealed role of oxidative stress in cervical cancer[14]. Chronic inflammation and infection over a prolonged period of time have been recognized as major risk factor for disease initiation[15].

Invasive cervical cancer develops from precursor lesions of the cervix called cervical intraepithelial neoplasia (CIN). Progression from normal tissue to invasive cervical cancer occurs through a series of increasing grades of cervical dysplasia. CIN I represent mild dysplasia and have a high rate of spontaneous remission (60%) and a low rate of progression to carcinoma. In contrast, approximately 38% of CIN II and III, moderate to severe dysplasia and 16-36% progress to invasive cervical cancer[16]. Because reporting for CIN is not mandatory, the exact incidence is unknown. However, it is estimated that 2.5 million women are diagnosed with low-grade cervical abnormalities annually[17].

Free radicals can acts as initiator or promoter, cause DNA damage, activate procarcinogens and alter cellular antioxidant defense system. This may be due to the damage caused to the tumor suppressor genes or immunological defenses in our body[18]. Superoxide and hydroxyl radicals are oxygen-free radicals, involved in producing oxidative stress. This oxidative stress can be associated with other factors which may lead to various neoplastic transformations[19].

Deleterious effects of these oxidants are counteracted by antioxidants such as Superoxide dismutase (SOD) & Catalase. In addition to the body's defense mechanism there are few vitamins that provide the body with the much-needed immunity and a mechanism of self-defense to fight against various pathogens. Studies indicate that the level of these antioxidants in the body decrease in cases of carcinogenesis. The levels of vitamin E were found to vary in a study of cervical carcinogenesis[20].

Despite extensive research, the mechanism that causes PIH are unknown Free radical Injury is associated with both cancer & PIH, but how the extent of oxidative stress correlates this, it is not clear. Therefore, the present study attempts have been made to evaluate co-relation if any between status of antioxidant enzymes, (SOD, Catalase, vitamin C and vitamin E) and lipid peroxidation product during PIH and Cervical dysplasia. The study evaluates indirect evidence of free radical injury & antioxidant states to epithelial tissue in cervical cancer, and its status compared to PIH.

MATERIALS AND METHODS:

Pregnant women attending Antenatal OPD and Labor room of the Department of Obstetrics and Gynecology, C.S.M. Medical University, Lucknow, were selected for the study. Study included total 66 patients age group of 20-35 years with 28-38 weeks of gestation out of which 14 had normal pregnancy and had no disease, 31 patients were of PIH & 21 patients were of cervical dysplasia gr. I-II.

Selection cases: Selection cases of PIH were done after assessing for BP > 140/90 mm Hg, proteinurea, edema.

Selection criteria of cervical dysplasia included – local examination (inspection of genetalia for obvious lesion)

- Per speculam (for cervicities vaginitis erosion)
- Atypical coloscopic finding which indicated cervical dysplasia.
- Biopsy of lesion for type of lesion and grading of cervical dysplasia.

Exclusion criteria: In both groups illness like anaemia, diabetes mellitus, essential hypertension, renal insufficiency, cardiovascular disease which by themselves are known to alter free radical status were excluded from study.

Six ml venous blood was collected with the informed consent of all patients. It was taken from both normal as well as study groups for determination of Oxidative damage in terms of Lipid peroxidation product- Malondialdehyde (MDA) and for assessment of antioxidant status by determining activities of Superoxide dismutase, Catalase vitamin C and vitamin E.

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Biochemical Analysis:

The following parameters were estimated in the controls and cases.

☐ Plasma Malondialdehyde (MDA) level by Bernheim *et* al[21]. Lipid peroxidation, an index of Oxidative stress was estimated & expressed as n mol MDA/ml.

☐ RBC haemolysate SOD activity by Misra and Fridovich[22]. One unit of enzyme was defined to cause 50% inhibition of auto oxidation of epinephrine in the assay by 1ml enzyme preparation. Specific activity of enzymes was defined as units/mg protein.

☐ RBC haemolysate Catalase activity by Beers and Sizer[23]. One enzyme may catalyze thousands of reactions every second. In the UV range H_2O_2 shows increase in absorption. The decrease in absorbance is used to calculate catalase activity in sample. Specific activity of enzymes was defined as units/mg protein.

☐ Plasma vitamin C level by Harris and Ray[24]. As a reducing agent and antioxidant agent it directly reacts with $O2^{\bullet}$ and $OH^{\bullet}$ and various lipid hydroperoxides. Specific activity of enzymes was defined as mg/dl of plasma.

☐ Serum vitamin E level by Baker and Frank[25]. Specific activity of enzymes was defined as µmole/ml.

☐ Urinary protein estimation by Harold varley[26]. This test was carried out in PIH patients for proteinurea determination.

Statistical Analysis: The results are expressed as mean ± S.E. One way analysis of variance (ANOVA) followed by Newman Keuls multiple comparison tests has been applied to test the significance of the data. A p-value < 0.05 was considered to be statistically significant.

Results: There was an increase in Plasma MDA level in PIH patients (8.48 ± 0.96 nmoles/ml) & Cervical dysplasia (CD) patients (8.31 ± 1.25 nmoles/ml) as compared to normal patients (4.88 ± 1.035 nmoles/ml), the rise was highly significant ($p < 0.001$) for PIH & $p < 0.001$ for Cervical dysplasia (Table- II). Although when the level of Plasma MDA compared within PIH & Cervical dysplasia patients, found to be non significant PIH vs CD ($p < 0.01$).

SOD activity was decreased in PIH patients (0.347 ± 0.069) & in cervical dysplasia patients (0.319 ± 0.78) as compared to normal patients (0.704 ± 0.109). The difference was statistically significant $p< 0.001$ in PIH & $p < 0.001$ in cervical dysplasia. But again there was no statistically difference when the activity of SOD was compared between PIH & Cervical dysplasia ($p < 0.01$) (Table- II).

Catalase activity was also decreased in PIH patients (0.112 ± 0.02) & cervical dysplasia patients (0.098 ± 0.02) as compared to normal patients (0.304 ± 0.089). The difference was statistically significant ($p < 0.001$) in PIH & in cervical dysplasia $p < 0.001$. No statistically difference was observed between the activity of Catalase of PIH patients & Cervical dysplasia patients ($p < 0.01$) (Table- II).

The plasma vitamin C values were also decreased in PIH patients (08.66 ± 2.32) & cervical dysplasia patients (7.54 ± 2.21) as compared to normal patients (`14.70 ± 3.52). The difference was statistically significant ($p < 0.001$) in PIH & in cervical dysplasia $p < 0.001$. No statistically difference was observed between the levels of Vitamin C of PIH patients & Cervical dysplasia patients ($p < 0.01$) (Table- II).

The serum vitamin E level was also decreased in PIH patients (11.65 ± 1.03) & cervical dysplasia patients (10.76 ± 1.98) as compared to normal patients (13.43 ± 3.45). The difference was also statistically significant ($p < 0.001$) in PIH & in cervical dysplasia $p < 0.001$. No statistically difference was observed between the level if Vitamin E of PIH patients & Cervical dysplasia patients ($p < 0.01$) (Table- II).

Co-relation of hemoglobin (Hb) with MDA level showed that aneamic patients have raised level of MDA as compared to non aneamic patients (Table- III).

Discussion: ROS functions as signal transducers in normal physiology, however, their overproduction may result in numerous human health problems. Although the body's own defense mechanism plays a crucial role to control the levels of these free radicals, the levels of antioxidants that counterbalance these oxidative radicals get impaired themselves. The present study was planned to detect co-relation between status of anti-oxidant enzymes, lipid peroxidation products with severity of Pregnancy Induced Hypertension (PIH) and cervical dysplasia.

Estimation of Malonyldialdehyde shows the extent of lipid peroxidation, i.e. free radical injury or oxidative stress. In the present study, Plasma MDA level in PIH patients & Cervical dysplasia patients was found higher when compared to normal patients. There was no significant result within PIH & Cervical dysplasia patients. The results are in accordance

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Table-I: Clinical Parameters in Control & Study groups

Characteristic	Normal n= 14	PIH n=31	Cervical dysplasia n=21
Age in years (mean)	26.50	30.50	32.44
Mean gestational age in weeks	35	34	35
Mean Systolic blood pressure in mm Hg	112.54	148	145.54
Mean Diastolic blood pressure in mm Hg	70.72	98.45	91.50
Protienuria (Mean) in gm/day	Nil	2.95	Nil
Oedema	Nil	++	Nil

Table –II:

Study group	MDA (nmoles/ml of plasma)	Significance (p)	SOD activity (units/mg protein)	Significance (p)	Catalase activity (units/mg protein)	Significance (p)	Vitamin C (mg/dl)	Significance (p)	Vitamin E (µmole/ml)	Significance (p)
Control	4.88±1.035		0.704±0.109		0.304±0.089		14.70±3.52		13.43±3.45	
PIH	8.48±0.96	p<0.001#	0.347±0.069	p<0.001#	0.112±0.02	p<0.001#	08.66±2.32	p<0.001#	11.65±1.03	p<0.001#
CD	8.31±1.25	p<0.001@ p<0.01$	0.319±0.78	p<0.001@ p<0.01$	0.098±0.02	p<0.001@ p<0.01$	7.54±2.21	p<0.001@ p<0.01$	10.76±1.98	p<0.001@ p<0.01$

Table-III: Correlation of Hb% to Free radical damage in terms of MDA level

S.No.	Hb gm%	MDA nmol/ml of plasma
1	< 8	9.39
2	8.1--9	7.51
3	9.1—10	6.43
4	>10	5.42

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with values obtained in other studies[27, 28]. In hypertension, increased blood pressure responsiveness to vasoconstrictors[29, 30], and reports of retinal arteriolar vasospasm/ischemia[31, 32] in preeclampsia are also consistent with endothelial cell dysfunction.

Total SOD and Catalase activity in placental homogenates reportedly increases with gestational age[33, 34]. Our study agrees with other workers[35-39] though the fall in vitamin E level in both the subgroups of cases as compared to the control was significant. There was statistically significant decrease in levels of vitamin C & vitamin E in PIH and cervical dysplasia when compared with control.

Manju *et al*[40]and Kim *et al* [41] have similarly demonstrated the involvement of rising LPO and compromised antioxidant levels. According to study by Roes in uncomplicated pregnancy, concentration of vitamin C decreased during pregnancy. Hence in normal pregnancy there seems a balance between antioxidant and oxidant concentration despite modest oxidative stress[42].

A significant higher level of serum lipid peroxide in form of MDA is observed whereas significantly lowered activity of SOD and Catalase is observed when compared to healthy subjects[43]. There was a concomitant decrease in hemoglobin as a response to oppose the oxidative stress. Thus increased levels of lipid peroxidation product MDA and decreased levels of Hb and antioxidants indicate oxidative stress in PIH & Cervical dysplasia. There was rise in oxidative stress with progression of gestation of pregnancy & Cervical dysplasia.

From the above observations we conclude that with increase in severity of the disease the oxidative stress increases & is dependent on the nutritional status and body status with nutrition. Hence there is a definite correlation between oxidative stress and initiation of pathogenesis of PIH and Cervical dysplasia.

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