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A STUDY OF LDL SUSCEPTIBILITY TO OXIDATIVE DAMAGE IN PREECLAMPSIA PATIENTS ATTENDING A TERTIARY CARE HOSPITAL, KOLKATA

Biochemistry

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ABSTRACT

Background: Pregnancy Induced Hypertension (PIH) is characterised by elevated systolic and diastolic BP associated with proteinuria. Endothelial dysfunction and inadequate placentation are considered as major contributors. Free radical induced endothelial injury and dysfunction may trigger the pathophysiological events.

Materials and Methods: Fifty (50) preeclampsia mothers (case) and 50 age matched normotensive, non-proteinuric mothers were taken as control group. Maternal serum LDL concentrations and the susceptibility of LDL particles to oxidation were seen.

Results : Preeclampsia mothers were having higher serum LDL concentrations (132.03±11.52 mg/dL) compared to the control group (120.22±8.57 mg/dL). The case group had higher ($p<0.001$) susceptibility of LDL particles to oxidation (0.662±0.112 pmol of MDA/ µg of protein/hr) compared to the control group (0.273±0.026 pmol of MDA/ µg of protein/hr).

Conclusion: Significant increase of maternal serum LDL concentration in preeclampsia patients. LDL particles are more susceptible to oxidative damage in preeclampsia patients.

KEYWORDS

Preeclampsia , Malondialdehyde(MDA) , Lipid peroxidation , LDL particles .

INTRODUCTION:

Hypertensive disorders of pregnancy are an important cause of foeto-maternal mortality and morbidity, involving about 5-8% of all pregnancies in India[1]. The term 'Gestational Hypertension' is used to describe any form of new-onset pregnancy-related hypertension[2].

Although the definition focuses on simply measurable clinical parameters, preeclampsia must be recognized as a multisystem disorder. The risk of preeclampsia markedly increases in women with previous preeclampsia and in those with either pre-existing vascular disease or conditions associated with increased cardiovascular risk, including renal diseases, hypertension, diabetes, thrombophilia, and obesity (Body Mass Index >29)[3]. Pregnancy is considered a 'stress test' that unmasks latent cardiovascular risks[4]. The exact cause of preeclampsia remains largely elusive, but poor placentation is the central predisposing factor.

Role of enhanced placental superoxide generation leading to oxidative stress is increasingly recognized. Endothelial dysfunction due to free radical induced oxidative damage is one of the predisposing factors of preeclampsia and eclampsia[5]. However, adequate antioxidant reserve and free radical disposal system may be most important defence mechanism against preeclampsia. In this way, it can be mentioned that women with relatively low antioxidant defence status are more susceptible to oxidative stress and preeclampsia. Low density lipoprotein (LDL) particles are most vulnerable for oxidation, free radical generation and resultant endothelial membrane injury[6]. In this study, the susceptibility to oxidation of LDL particles is measured to detect any relationship between the preeclampsia and increased LDL susceptibility to oxidation in pregnant women.

MATERIALS & METHODS:

The study was done in collaboration of the Dept. of Biochemistry and the Dept. of Obstetrics & Gynaecology, IPGME&R & SSKM Hospital, Kolkata during January, 2014- June 2015. Altogether, 50 clinically diagnosed preeclampsia patients with proteinuria >300 mg/day, having Systolic Blood Pressure (SBP) >140 mmHg, or the rise of SBP >30 mmHg during gestational age 28-40 weeks, or Diastolic Blood Pressure (DBP)> 90 mmHg, or the rise of DBP >15 mmHg during gestational age 28-40 weeks, were taken in the study group. Whereas 50 age-matched, normotensive, non proteinuric pregnant women were taken as the control group. In both groups, the age limit was 18- 30 years. Pregnant women with pre-existing hypertension, Diabetes or any chronic disease ware excluded from the study.

As a marker of oxidatively modified LDL particles, Malondialdehyde (MDA) was specrophotometrically measured by using Thiobarbituric acid (TBA)-MDA adduct formation and production of pink colour having absorption maximum at 532 nm. For the purpose, initially LDL particles were separated by heparin citrate treatment and subsequent centrifugation of collected sera from both case and control groups. Then the supernatant was decanted and precipitate from 0.5 mL of the serum was resuspended in 1 mL of 1% SDS solution. 1 part was preserved for protein quantification by Lowry method. In the other part, suspended LDL particles were treated with 200 µL of 10 mmol $CuSO_4$ solution along with 800 µL of 64 mmol sodium citrate buffer at pH 5±0.04 and incubated for 3 hours at 37^0 C to induce oxidation of LDL and MDA production by Cupric ions. Finally, the product was mixed with 1 mL of 20% trichloroacetic acid (TCA) and vortexed for 2 min and centrifuged at 4500 rpm for 10 min. The supernatant was treated with 1 mL of 0.67% (w/v) TBA solution and incubated at 95^{0}C for 45 min. Thus produced pink coloured solution was immediately cooled down under running tap water and re-centrifuged at 3000 rpm for 10 min. Absorbance was taken at 532 nm and the concentration of MDA adduct was derived mathematically in respect to its molar

extinction coefficient (1.56X105 $mol^{-1}cm^{-1}$). The entire procedure was repeated once again, without adding $CuSO_4$ to it. The difference between Copper induced and non-induced MDA levels was used to obtain susceptibility of LDL particles to oxidative stress and results were expressed as picomol of MDA/ µg of protein/hr.

RESULT:

50 diagnosed cases of preeclampsia and 50 age-matched controls in the age group of 18-30 years were evaluated using simple statistical methods. The susceptibility of LDL particles to oxidative stress and results were expressed as pmol of MDA/ µg of protein/hr. All the results were expressed with Mean±SD in table 1.

Table 1:

	Case group	Control group
No of Pregnant women	50	50
Age(Yrs)	22.75±3.3	24.31±3.61
LDL concentration(mg/dL)	132.03±11.52	120.22±8.57
LDL oxidisability (pmol of MDA/ µg of protein/hr)	0.662±0.112	0.273±0.026
Gestational age(wks)	25.62±3.48	25.92±3.61
Systolic Blood Pressure(mmHg)	155.7±12.8	113.5±8.5
Diastolic Blood Pressure(mmHg)	95.56±4.35	74.9±9.6

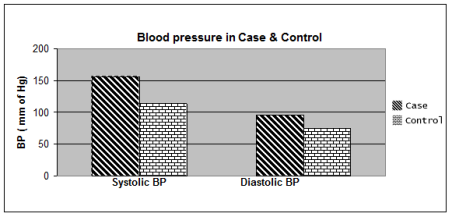

Figure 1: Systolic and Diastolic blood pressure in case and control groups(n=50) .

There is no significant difference (p>0.05) between the age of case group(22.75±3.3 yrs) compared to control group (24.31 ± 3.61 yrs). There is also no significant difference (p>0.05) in gestational age between the case group(25.62±3.48 wks) and the control group (25.92±3.61 wks).

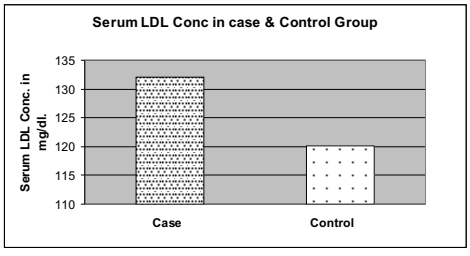

Figure 2 : Distribution of serum LDL cholesterol concentration in case and control groups(n=50)

Women with preeclampsia have significantly (p<0.05) higher serum LDL cholesterol concentration. There was no significant (p>0.05) difference in LDL protein content between the two groups.

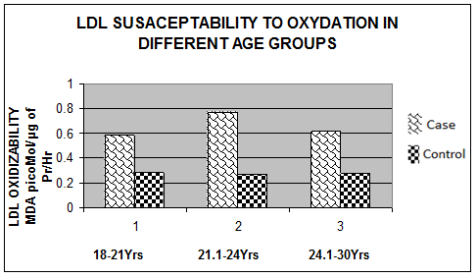

Figure 3 : LDL susceptibility to oxidation in different age groups in case and control subjects.

LDL susceptibility to oxidative stress in preeclampsia group was 0.662±0.112 pmol of MDA/ µg of protein/hr, whereas the same in control group was 0.273±0.026 pmol of MDA/ µg of protein/hr. These two values of susceptibility to oxidative stress were statistically significant (p<0.001), calculated by unpaired t test.

DISCUSSION:

Preeclampsia remains one of the most serious complications of pregnancy[7]. Although the exact pathophysiological events of the disease remains poorly understood, placental ischemia, immune maladaptation, genetic factors are probably all involved to some extent. Recent investigations suggest endothelial cell injury as the initiator of the pathophysiological events. Uncontrolled lipid peroxidation may play an important role.

The present study was conducted to assess the role of oxidative stress in preeclampsia by comparing generation of one of the important markers of lipid peroxidation, namely Malondialdehyde in LDL particles. Isolated LDL particles from maternal sera were subjected to an in vitro challenge by cupric ion as oxidising substance to detect susceptibility of those LDL particles to oxidation along with production of MDA in both case and control groups.

100 pregnant females were included in this study, 50 preeclamptic women as the case group and 50 normotensive, non proteinuric women as the control group. In this study, there was no maternal age difference between the case and control groups. From this study, it has been noticed that gestational age is not a predisposing factor for preeclampsia. However, the diagnosed preeclampsia patients showed elevated serum LDL cholesterol concentrations despite no alteration of protein content of LDL particles. As LDL particles are highly vulnerable to oxidation and generation of deleterious free radicals, this may initiate endothelial membrane damage and contribute to the compromised production of endothelial vasodilator factors. As pregnancy itself is a hypervolemic and hypermetabolic state, there is concomitantly increased oxidative stress during gestation. The excess intravascular fluid is accommodated within the vascular tree by means of vasodilation significantly contributed by endothelial derived vasodilator factors. Furthermore, in this study, the susceptibility of LDL particles to oxidation is significantly increased (p<0.001) in preeclampsia group in respect to the control group indicating relatively low reserve of total antioxidant capacity to encounter increased oxidative challenges during pregnancy.

CONCLUSION:

The present study showed a significant increase of maternal serum LDL concentration in preeclampsia patients. Study also revealed that LDL particles are more susceptible to oxidative damage in preeclampsia in comparison to normal pregnant women. This indicates there is relatively low antioxidant reserve in pregnant women developing preeclamsia. Such jeopardised antioxidant defence system makes the biomolecules including LDL particles vulnerable to oxidation, as evident in this study by means of increased susceptibility of LDL particles to oxidation. Thus appropriate supplementation of antioxidants during pregnancy or to women planning to become pregnant might be protective against preeclampsia[8].

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