OXIDATIVE STRESS IN PRE-ECLAMPSIA – MDA & IMA AS BIOMARKERS

Biochemistry

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ABSTRACT

BACKGROUND: Pre-eclampsia (PE) is a disorder of pregnancy and a common cause of foetal and maternal death. MDA & Ischemia Modified Albumin (IMA) has emerged as a marker in different diseases where ischemia is the origin or consequence behind disease pathology but studies have been few.
Reliable biochemical markers for identifying patients at risk of PE can have a better impact on maternal health and better management.
MATERIALS & METHODS: 30 patients with PE were selected for the study and compared with 30 pregnant healthy controls. IMA and Malondialdehyde (MDA) with other routine biochemical markers were estimated in these patients.
The results were then statistically analysed.
RESULTS: IMA levels were found significantly raised in PE patients as compared to normal pregnant controls (p value<0.001). A significant correlation was also found between IMA levels and MDA levels in PE (r= 0.3)
CONCLUSION: IMA which is generated by hypoxia/ischemia driven oxidative stress is also raised in PE and hence can be used as a biomarker in PE.
Further studies are needed to establish the relationship between IMA and the disease process and its association with severity of disease.

KEYWORDS

Eclampsia, Ischemia Modified Albumin, Malondialdehyde

INTRODUCTION

Pre-eclampsia is a disorder characterized by development of hypertension to the extent of 140/90 mmHg or more with proteinuria after 20th week in a previously normotensive and non-proteinuric women. It occurs in approximately 6-8% of all pregnancies, 10% of first pregnancies and 20-25% of women with a history of chronic hypertension. Pre-eclampsia is one of the most common causes of prematurity accounting for one fourth of all very low birth weight infants with birth weight less than 1500 grams. Recurrence risk is of 32% for pre-eclampsia and 46% for pre-eclampsia superimposed on pre-existing chronic hypertension.

Oxidative stress has been implicated as one of the aetiologies of pre-eclampsia. Many other theories regarding its pathogenesis such as increased insulin resistance, immunological tolerance between maternal and foetal tissue etc. are also postulated. Defective endovascular invasion of cytotrophoblast into the spiral arteries is also implicated as a causal factor in the pathogenesis of the disease.[1]

National High Blood Pressure Education Programme (NHBPEP 2000) classifies hypertension during pregnancy into five types-
a) Gestational Hypertension
* Elevation of blood pressure ≥ 140/90 mm Hg noted for the first time during pregnancy after 20 weeks of gestation.
* No proteinuria.

b) Pre-eclampsia
* Elevation of blood pressure ≥ 140/90 mm of Hg noted for the first time during pregnancy after 20 weeks of gestation.
* Proteinuria of ≥300 mg/24 hours by dipstick method in a random urine sample.

c) Eclampsia
* Eclampsia is defined as development of seizures that cannot be attributed to other causes in women with pre-eclampsia.

d) Pre-eclampsia superimposed on chronic hypertension
* New onset of proteinuria ≥300 mg/24 hours in hypertensive women but no proteinuria before 20 weeks of gestation.

e) Chronic hypertension
* defined as presence of BP≥140/90 mm Hg before pregnancy or diagnosed before 20 weeks gestation or hypertension first diagnosed after 20 weeks gestation and persisted after12 weeks postpartum.

Pre-eclampsia is associated with impaired endovascular trophoblast invasion and inadequate remodelling of uterine spiral arteries leading to hypoxic intrauterine environment and generation of hypoxic environment and generation of oxidative free radicals or reactive oxygen species (ROS). Increased ROS accumulation accompanied by depletion of antioxidant defence mechanism is considered as a hallmark of Pre-eclampsia.

As placental hypoxic conditions are found in Pre-eclampsia and oxidative stress is implicated in its pathogenesis, maternal serum Ischemia Modified Albumin (IMA) as well MDA were thought can be used as potent biomarker of pre-eclampsia.

Ischemia reperfusion injury generates ROS which alters the N-terminal region of serum albumin, which impairs its capacity to bind to the transition metals. This chemically modified albumin is called as Ischemia Modified Albumin. Serum Sodium was associated with blood pressure regulation and was seen as a causative agent in the etiopathogenesis of pre-eclampsia. Thus, with a moderate restriction of sodium in the diet during pregnancy, the incidence of Pregnancy Induced Hypertension (PIH) can be reduced.[2,3]

AIMS & OBJECTIVES
To determine and compare the values of IMA in pre-eclamptic primigravida and healthy pregnant females.

To correlate MDA levels with IMA in the subjects to study the effect of oxidative stress

MATERIALS AND METHODS
This cross sectional study was conducted taking women with gestational hypertension and pre-eclampsia as cases and healthy pregnant females were taken as controls from April 2017 to April 2018. The cases and controls were selected from tertiary care hospitals in local geographical area.

Written informed consent was taken from each study subject.

Selection of study subjects
* Based on inclusion and exclusion criteria total 60 study subjects (30 cases and 30 controls) were selected for the study. A proforma was used to record relevant information and patient's data.

* **CASES =** 30 women with hypertensive disorders of pregnancy were selected on the basis of definition given by National High

Blood Pressure Education Programme (NHBPEP 2000).

- **CONTROLS =** 30 healthy pregnant females.
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- **INCLUSION CRITERIA**
- 30 diagnosed cases of pre-eclampsia in the age group of 20-45 years.
- Pregnant females of $\geq$ 20 weeks of gestation with blood pressure of $\geq$140/90 mm of Hg noted first time during pregnancy on $\geq$2 occasions at least 6 hours apart with proteinuria of $\geq$ 1+ by dipstick method in a random urine sample was considered to have pre-eclampsia.
- Control = healthy sex matched 30 controls were taken.

EXCLUSION CRITERIA
- History of chronic hypertension that was present before pregnancy.
- History of diabetes mellitus and/or who are on insulin therapy.
- Subjects taking anti-hypertensive drugs.
- Liver disease patients.

Collection of Blood samples
- About 4 ml of blood was drawn under aseptic precautions from selected subjects with overnight fasting of 12 hours.
- 3 ml of blood was collected in a red vacutainers for serum IMA (maternal).
- The blood samples were centrifuged at 3000 rpm for 10 minutes to obtain the serum.

Parameters to be Measured
- The parameters which are to be measured in the cases and controls is maternal serum MDA and IMA levels.

Measurement of serum Ischemia Modified Albumin
- It is done according to Bar Or et al 2000. Known amount of cobalt was added to the serum sample and unbounded cobalt was measured by the intensity of coloured complex formed after reacting with dithiothreitol by spectrophotometer at 470 nm.[4,5]

RESULTS
Table 1 – Demography of subjects & IMA levels

Parameter	Case	Control	P-value
Age	27.7 ± 4.99	26.93 ± 4.55	0.5367
IMA	0.468 ± 0.075	0.279 ± 0.047	<0.0001*

*significant difference

The IMA variables were noted on cases as well as control. All the variables are continuous in nature. T-test for two independent samples has been applied to data as the samples are independent. It has been observed that cases and controls were matched for age and there was no significant difference found in age of cases and control. IMA levels were found to be higher in cases compared to control. The difference of total bilirubin turned out to be highly significant statistically. (Table 1) Though there was no statistically significant increase in MDA in cases compared to controls but positive correlation was seen between MDA levels and IMA levels. (r value = 0.3)

DISCUSSION
Pre-eclampsia is associated with defective placental growth leading to failure of conversion of small diameter high resistance vessels to large diameter low resistance vessels leading to oxidative stress due to ischemic reperfusion injury and generation of free radicals. Eclampsia is the end stage of pre-eclampsia characterized by generalized seizures. Pre-eclampsia and eclampsia complicate 2%-8% of pregnancies and overall 10%-15% of direct maternal deaths are associated with these conditions.[6]

Biomarkers can not only allow detection of patients at risk but can also help in grouping patients into different categories according to their severity of PE for timely intervention. Many different biophysical and biochemical markers have been investigated based upon pathophysiology of the disease but their reliability in predicting pre-eclampsia has been inconsistent.

Serum IMA has been observed to be significantly elevated in diseases where oxidative stress is because of the disease process. In the present study a significant increase in serum IMA was seen in pre-eclamptic women compared to normal pregnant women. There occurs

haemodilution in pregnancy leading to decrease in plasma albumin concentration so ischemia modified albumin was normalized to albumin by calculating ratio of IMA/Albumin. These observations were in accordance with the studies done by Akolekar R et al and Papageorghiou et al. Placental hypoxia causing ischemic reperfusion injury results in the generation of free radicals which leads to increased MDA levels leading to alteration of NH_2 terminus of human serum albumin resulting in decreased binding of albumin to cobalt compared to normal pregnant control.[7,8]

However in a limited study done by Roberts JM et al serum IMA was found elevated in normal pregnant controls compared to the non-pregnant controls (p=0.015) but the IMA levels in pre-eclampsia were similar to those of normal pregnant controls (p= >0.05). The discrepancy in these studies could possibly be explained by smaller number of patients and differences in severity of pre-eclampsia.[9]

Significant increase in serum MDA was also observed in our study which was in line to the observations of Ebru Di Uotila ,Yoneyama Y et al. These findings on maternal serum MDA provides further evidence that increased lipid peroxidation may play an important role in pathophysiology of pre-eclampsia. There was a positive correlation between the maternal serum IMA and MDA as well as serum IMA/ALB in pre-eclampsia suggesting that there occurs increase in oxidative stress due to ischemia in PE. This is in accordance with the work of Debasis Roy et al who suggested that increased IMA levels might have resulted from increased oxidative stress whether due to ischemia reperfusion injury or other mechanisms linked to reduction in blood flow.[10,11]

CONCLUSION
IMA is seen to be significantly increased in PE. MDA levels correlate positively with IMA concentration. This suggests that measurement of MDA, the oxidative biomarker, may be useful in monitoring pregnancies with respect to the development of pre-eclampsia and their further management.

IMPLICATIONS
IMA and MDA can be very useful in monitoring pregnancies for Pre-eclampsia as well as in guiding the treatment of pre-eclampsia for better management and patient care.

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