

Study of Nitric Oxide and Lipid Peroxidation in Preterm Labour: A Case-control Study

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ABSTRACT

Introduction: Preterm Labour (PTL) is a major obstetric health problem. It contributes to 75% of neonatal mortality worldwide. According to a World Health Organisation (WHO) report, 15 million preterm birth occur every year. Nitric Oxide (NO), synthesised by three isoforms of NO Synthase (NOS) play physiological role in uterine muscle relaxation and prevention of preterm labour contractions. NO is also demonstrated to react with Reactive Oxygen Species (ROS).

Aim: To assess the level of serum NO, Malondialdehyde (MDA) (end product of lipid peroxidation), total protein, albumin, calcium, and inorganic phosphorus in PTL and also to compare these parameters with full-term labour.

Materials and Methods: A case-control study was conducted in the Department of Biochemistry and Obstetrics and Gynaecology, Government Medical College, Miraj, Maharashtra, India, during the period of November 2014 to May 2015. Study included 60 women with age ranged between 21-31 years and gestational age between 28 to 37 weeks. Out of total 60 women, 30 were full-term labour, served as control and 30 women who had regular uterine contraction before 37 weeks were selected as cases. Estimation of NO (as nitrite) and MDA, Total Protein (TP), albumin, calcium and

inorganic phosphorus was done. Data were analysed by applying a Student's t-test and Pearson correlation test.

Results: The mean maternal age was 26.53±3.42 years for cases and for control 27.63±2.51 years. The mean gestational age in cases were 31.1±2.795 weeks, whereas in controls was 37.26±0.520 weeks. A highly significant decrease in NO (7.1967±1.236 µmol/L and 15.900±3.897 µmol/L in PTL and control group), TP (4.996±0.509 g/dL and 6.906±0.480 g/dL in PTL and control group), Albumin (3.460±0.440 g/dL and 4.406±0.390 g/dL in PTL and control group), calcium (8.240±0.431 mg/dL and 8.950±0.486 mg/dL in PTL and control group) and inorganic phosphorus (2.006±0.532 mg/dL and 3.786±0.537 mg/dL in PTL and control group) concentration was observed in PTL as compared to the controls. Lipid peroxidation (MDA) (10.590±0.8715 mg/dL and 5.6500±0.4725 mg/dL in PTL and control group) was found to be significantly increased in PTL as compared to controls.

Conclusion: There was significant drop in the NO and increase in ROS reflected by increase in serum MDA levels in PTL group as compared to normal full-term labour. These parameters may have role in the diagnosis and prevention of PTL after appropriate larger cohort studies.

Keywords: Calcium, Inorganic phosphorus, Malondialdehyde, Neonatal mortality, Nitric oxide synthase

INTRODUCTION

Preterm delivery is defined as a delivery before completing 37 weeks of gestational age. Preterm birth is a major public health problem contributing to 75% of neonatal mortality worldwide. According to a WHO report, 15 million preterm birth occur every year. The incidence of preterm deliveries in India is 23.6% of the world's total [1-4].

In developed countries, the rate of preterm birth is 7-11% of all pregnancies and it causes 85% of neonatal deaths in normally formed infants without any congenital abnormalities. In India, the incidence of preterm live birth is 7-9% [5,6]. Preterm birth is related to neurological and developmental problems, or other morbidities, and can lead to death. Medical conditions of the mother or foetus, genetic factors, environmental exposure, infertility treatments, behavioural and socio-economic factors, and iatrogenic prematurity are the factors responsible for the preterm birth [7,8]. It leads to socio-economic and global problems for humanity. It is a huge financial burden on healthcare resources, although the costs of immediate neonatal care are high [6]. The diagnosis of PTL is perhaps one of the most difficult and important tasks facing clinicians today [9].

Nitric Oxide (NO), a free radical and potent smooth muscle relaxant is catalysed by a group of enzymes known as NOS. Three isoforms of NOS, catalyse the conversion of L-arginine to NO and citrulline. The L-arginine-NO system and three isoforms of NOS (endothelial NOs, inducible NOS, and neural NOS) have been confirmed to be present in the female reproductive system suggesting that these are locally synthesised [10]. NO may be involved in the physiological

activities of reproduction including egg maturation, fertilisation, and cervical ripening, and can react with ROS and molecular oxygen [11]. NO synthesis has been demonstrated to show variable phase dependency, increased production (upregulation) occurring in pregnancy (most probably to maintain quiescence uterus) and decreased production (down-regulation) occurring during labour, thus suggesting a role for NO in tocolysis and prevention of preterm labour [12].

A stressful process of preterm labour, damage during pregnancy and various mechanisms causes the production of free radicals [13]. Oxidative stress depends on the balance between free radical formation and defense mechanisms. The oxidative stress increases lipid peroxidation, MDA is the end product of lipid peroxidation being used extensively as a marker of oxidative stress [14]. Women with PTL have diminished antioxidant abilities to defend against oxidative stress-induced damage [15]. In developing countries before and during pregnancy women consume lower quantity of protein, minerals, and vitamins. This inadequate dietary intake may be responsible for preterm delivery and the low birth weight of neonates [16]. Many studies have evaluated serum NO [10,12,17,18] and MDA [19,20,21] levels independently, but no study was found to evaluate them together with protein, calcium, albumin, and inorganic phosphorus level.

With a view that NO, lipid peroxidation, serum TP, albumin, calcium, and inorganic phosphorus may have potential clinical value to cause preterm labour, the present study was planned to evaluate

the serum NO and MDA, serum TP, albumin, calcium, and inorganic phosphorus in PTL patients and to understand whether the concentrations of these parameters are associated with the risk of preterm labour.

MATERIALS AND METHODS

The present case-control study was carried out in the Department of Biochemistry and Department of Obstetrics and Gynaecology, Government Medical College, Miraj, Maharashtra, India, during the period of November 2014 to May 2015. Institutional Ethical committee approval was taken and informed consent was taken from all the participants.

Inclusion criteria: Women aged between 21 to 31 years, in labour before 37th week of gestation with cervical dilatation >4 cm in last two hours were included as cases. Age matched women in labour with full-term gestation >37 weeks. (The term early term labour is used when the gestational age is in between 37 weeks to 38 weeks and 6 days) [1].

Exclusion criteria: Women with multiple pregnancies, foetal anomalies, abruption placenta, placenta previa, preeclampsia, intrauterine growth retardation, diabetes mellitus, thyroid dysfunction, with bad obstetric history were excluded. Presence of any general disease, signs suggestive of infection were also excluded from the study.

Sample size: This was designed as case-control study on the pilot basis. So according to the feasibility, consecutive 30 women of PTL were planned to include in the study and matching number of controls were recruited during the study period.

Demographic data from all the patients in terms of age, gestational age was collected. About 4 mL of blood was collected from the cases and control groups under aseptic conditions during the first two hours of labour. The blood was allowed to clot and centrifuged at 3000 rpm for five minutes. Separated serum was utilised for the estimation of NO, MDA, TP, albumin, calcium, and inorganic phosphorus. The NO was measured (as nitrite) by the method of Cortas NK and Wakid NW method (calorimetric) and MDA estimation by colorimetric method. Both methods were standardised and standard graphs were plotted. Normal ranges for NO and MDA were tried to dig from the literature but no consensus value was found, routine parameters were interpreted against the reference values established by the American Association of Clinical Chemistry (AACC) [22-26]. Methods and reference ranges for biochemical parameters are shown in [Table/Fig-1].

Parameter	Method	Reference range
Serum Nitric Oxide [22]	Colorimetric method (Najwa K Cortas and Nabid W Waked method)	Not applicable
Serum MDA [23]	Colorimetric method	Not applicable
Serum Total Protein [24]	Biuret method, End-Point	6-8.3 (g/dL)
Serum Albumin [25]	BCG dye method, End point	3.5-5.5 (g/dL)
Serum Calcium [25]	OCP method, End point	8.6-10.2 (mg/dL)
Serum Inorganic Phosphorus [26]	Colorimetric method	2.5-4.5 (mg/dL)

[Table/Fig-1]: Methods and reference ranges for biochemical parameters. OCOO: O-cresolphthalein complexone, BCG: Bromocresol green

STATISTICAL ANALYSIS

The data was analysed by Graph-pad software version 9. The numerical data were presented as mean±standard deviation. The statistical significance was calculated using the Student's t-test. The correlation between the parameters were assessed by Pearson's correlation test. The two-tailed p-value <0.001 was considered to be highly significant.

RESULTS

In present study, the mean maternal age was 26.53±3.42 years for cases and for control 27.63±2.51 years. The mean gestational age

in cases were 31.1±2.795 weeks, whereas in controls 37.26±0.520 weeks [Table/Fig-2].

S. No.	Parameters	Case group (n=30) Mean±SD	Control group (n=30) Mean±SD	p-value
1	Maternal age (years)	26.53±3.42	27.63±2.51	0.08
2	Gestational age (weeks)	31.1±2.795	37.26±0.520	<0.001

[Table/Fig-2]: General characteristics of women with PTL and full-term labour. Student's t-test. Level of significant p-value <0.05

The mean NO level in the case group was significantly lower 7.1967±1.236 µmol/L than in controls, 15.900±3.897 µmol/L (p-value <0.001). The mean Lipid peroxidation (MDA) level in the case group was significantly higher than controls, (p-value <0.001). It was observed that serum protein, albumin, serum calcium as well as phosphorus levels were significantly lower in cases as compared to controls (p-value <0.001) [Table/Fig-3].

S. No.	Biochemical parameters	Case group (n=30) Mean±SD	Control group (n=30) Mean±SD	p-value
1	Nitric oxide (µmol/L)	7.1967±1.236	15.900±3.897	<0.001*
2	Lipid peroxidation (MDA) (mg/dL)	10.590±0.8715	5.6500±0.4725	<0.001*
3	Total protein (g/dL)	4.996±0.509	6.906±0.480	<0.001*
4	Albumin (g/dL)	3.460±0.440	4.406±0.390	<0.001*
5	Calcium (mg/dL)	8.240±0.431	8.950±0.486	<0.001*
6	Inorganic phosphorus (mg/dL)	2.006±0.532	3.786±0.537	<0.001*

[Table/Fig-3]: Comparison of biochemical parameters in cases and control group. Student t-test, p<0.001 *highly significant

A significant positive correlation was found between NO and LPO (MDA) in cases (correlation coefficient r=0.560, p-value=0.001). A significant positive correlation (r=0.824, p-value <0.001) was found between protein and albumin in cases. A significant negative correlation was found between calcium and inorganic phosphorus in cases; (r=-0.395, p-value=0.031) and in control group (r=-0.402, p-value=0.02) [Table/Fig-4].

Parameters	LPO (MDA)		Albumin		Inorganic phosphorus		
	r-value	p-value	r-value	p-value	r-value	p-value	
Cases	NO	0.560	0.001	-0.113	0.552	-0.246	0.190
	Total protein	-0.181	0.338	0.824	<0.001	0.033	0.862
	Calcium	-0.167	0.378	-0.147	0.439	-0.395	0.031
Control	NO	0.047	0.804	-0.441	0.015	0.109	0.568
	Total protein	-0.082	0.667	0.549	0.002	-0.34	0.058
	Calcium	-0.034	0.859	0.056	0.768	-0.402	0.02

[Table/Fig-4]: Correlation of biochemical parameters in cases and control group. p-value <0.05 considered significant

DISCUSSION

Preterm Labour (PTL) and preterm birth are one of the most common neonatological problem and it may lead to infant morbidity and mortality and variety of physical diseases [10]. There is a still lack of understanding about the exact mechanisms involved in the initiation of PTL in spite of extensive research. Research studies planned to find new predictors of PTL in today's need. This will help in a better understanding of the pathophysiology of PTL and its management. New markers for predicting preterm birth may help identify women at risk of PTL and the development of more effective interventions. Therefore, present study attempted to study the potential clinical value of certain biochemical parameters i.e., serum NO, lipid peroxidation, TP, albumin, calcium, and inorganic phosphorus in PTL. In this study, significantly decreased concentration of NO was observed (p-value <0.001) in cases than controls. These findings

were supported by the previous observations of Koleva IM et al., they also found NO radical production and oxidative stress/nitrosative stress increases with advancing gestation during preterm birth and decrease in Threatened Preterm Labour (TPL) groups [10].

Further, Diejomaoh MFE et al., concluded that a decreased NO metabolites levels in active spontaneous PTL and the active phase of induced labour at term. NO may play a role in the maintenance of uterine quiescence in early pregnancy as well as during labour [12]. Bansal RK et al., have studied the expression of iNOs in human myometrium and found a large decline in iNOs expression in PTL patients [17]. Shahshahan Z et al., concluded that the lower level of NO was observed in PTL compared with term labour. NO play a role in uterine quiescence during pregnancy increased NO production has been reported in the inflammatory process due to increased macrophage activity and the activation of proinflammatory cytokines [18].

The exact mechanism by which PTL occurs is not known, however, several factors including oxidative stress may be the underlying factor for deranged NO production. The imbalance between ROS and antioxidant capacity causes oxidative stress. The oxidative stress increases lipid peroxidation, MDA is the extensively used end product of lipid peroxidation as a marker of oxidative stress which is being used in this study [19,27]. In the present study, the marker for lipid peroxidation (MDA) was significantly increased (p -value <0.001) in cases than controls. These findings were in accordance with Sari YM and Yerizel E, Ayse A et al., Khan S et al., Verma V et al., they found that the Lipid peroxidation (MDA) level increased in PTL than the full-term labour [19,20,28,29]. Present study also found a significant correlation between these two parameters in cases ($r=0.560$). Present study demonstrated the association of oxidative stress measured as lipid peroxidation with PTL. As mentioned earlier, a significant alteration in the level of NO and LPO products in PTL was found.

Present study result indicates that the serum TP, albumin, calcium and inorganic phosphorus levels are decreased in PTL. Sultana M and Sultana M and Chowdhary P, found low levels of serum total protein and albumin in PTL; similar to the present study [16,30].

The metabolism of phosphorus is largely related to that of calcium. Plasma calcium and inorganic phosphorus have many physiological functions in the human body. Ionic calcium is crucial for many biochemical processes including blood coagulation, neuromuscular excitability, and cell membrane integrity. Ionised calcium is crucial for various biochemical processes such as cellular enzymatic activities, blood coagulation, cell membrane integrity, and many other processes [31]. The present study indicated a significantly decreased (p -value <0.001) concentration of serum calcium as well as inorganic phosphorus in PTL as compared to that of controls. These findings were supported by a studies by Smolarczyk R; Li M et al., Wójcicka-Jagodzińska J et al., they found that lower levels of serum calcium and inorganic phosphorus in PTL [32-34]. Hypocalcaemia in pregnancy may lead to various complications in mothers and foetus. Calcium supplementation has been shown to prevent preterm delivery [32].

Limitation(s)

Limitations of the present study were small sample size and study subjects were included from a single centre.

CONCLUSION(S)

This study concludes that, high levels of MDA and lower levels of NO levels are associated with PTL. A low concentration of protein and albumin was also found in PTL patients. Measurement of biochemical parameters such as NO, and Lipid peroxidation (MDA) may have a role in the diagnosis and prevention of life-threatening emergencies like PTL and its management. It could be better to

confirm these findings in larger multicentric studies to establish the role of the NO and other biochemical parameters in the prevention of PTL. Future cohort studies could include role of NO, status of NOS system, levels of MDA and antioxidant status of mother and its effect on outcome of the pregnancy.

Authors' contribution: MNC is the first author who collected the cases and run the tests and wrote a manuscript, JG conceptualised the idea of the research, SJ helped to standardise methods of estimation, AY helped to write a manuscript and ANN overviewed the overall manuscript writing and proofreading.

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