Maternal plasma nitric oxide metabolites and cervical length assessment in predicting the tocolytic therapy in preterm labor in Isfahan

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Background: Preterm labor (PTL) is the main challenge in prenatal health care, leads to high rate of mortality and increases cost of health services. To evaluate the preterm delivery (PTD)-related risk factors, we decided to measure nitrite oxide metabolites and cervical length (CL) as the diagnostic and predictive tools for PTD in women and response to tocolytic therapy. **Materials and Methods:** In this case–control study, sixty women of 18–35 years with first pregnancy during the 24–34 gestational weeks with PTL in case group admitted to the delivery section of Beheshti Hospital, Isfahan, Iran were included. Sixty women in control group have the same specifications. NO and CL level were assessed, and the collected data were analyzed by SPSS software, version 20 and MedCalc software, version 15.1. **Results:** The two groups were similar regarding maternal and gestational age ($P > 0.05$). Lower level of NO was observed in PTL women with a mean of 35.30 ± 8.27 µmol/L compared to the normal gestation group with a mean of 39.05 ± 10.17 µmol/L ($P = 0.035$). In addition, the diagnostic accuracy of both PTL-predicting factors was determined (NO ≤31, sensitivity 99.7%, specificity 82.5% and CL ≤22, sensitivity 80%, specificity 99.9%). **Conclusion:** As the previous investigations stated, it can be claimed that NO might be the reliable marker for predicting the PTL, and administration of NO synthesis could be a candidate for the future therapeutic target.

Key words: Cervical length, nitrite oxide, preterm delivery, preterm labor

INTRODUCTION

Preterm birth, as one of the pregnancy problems, is the leading cause of neonatal mortality and the second cause of child deaths under the age of 5.[1] It is defined as birth before 37 weeks of gestation, which affects 5–18% of the pregnancies.[2] For the families and health-care systems, short- and long-term morbidity and financial implications of preterm birth have high economic and social costs in terms of neonatal intensive care.[3]

Primary symptoms of preterm labor (PTL) are often mild, and the associated symptoms occur too late, so, it is difficult to diagnose early. Some methods are used to predict preterm delivery (PTD) by clinical symptoms and epidemiological risk factors such as obstetric history, but these are neither sensitive nor specific.[4,5] The presence of a method, enabling to a rapid and accurate diagnosis of actual PTL in pregnancy, could be useful to lower treatment costs and unnecessary interventions.

Nitric oxide (NO), as one of the pivotal metabolic factors, has been implicated in a variety of biological and reproductive processes including oocyte maturation, fertilization, and embryonic progression.[6] NO has a short half-life time and promptly oxidized by nitrite oxide which is changed to nitrite oxide metabolites (NOx), contained to NO_3 (nitrates) and NO_2 (nitrites).[7] In fact, the level of NOx is the marker of NO activity. Assessment

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of NO metabolite can be measured easily from plasma, urine, and vaginal secretions.[8,9]

Short cervical length (CL) is a helpful marker for predicting PTL. Transvaginal ultrasound examination is a reliable method for the CL evaluation.

Even though the effects of tocolytics on neonatal outcomes improvement have been not shown, they are the important interventions in obstetrics to delay PTD.[10,11] The aim of tocolytic therapy is to delay PTD long enough for antenatal corticosteroids to be administered or transporting the mother to a tertiary care center, thereby reducing neonatal morbidity and mortality.[12] The use of tocolytic agents in contemporary obstetric practice should be customized based on the available evidences of efficacy and fetomaternal safety, gestational age, maternal conditions, and drugs' potential complications.[13] Magnesium sulfate is one of the tocolytic agents which is commonly used for tocolytic therapy, and despite maternal side effects, its usage in women at risk of preterm birth helps to protect the child's brain, to reduce the rates of cerebral palsy, and to improve long-term neonatal outcomes.[10-14]

Hence, the present study was aimed to estimate the plasma level of NO metabolites in pregnant women with symptoms of PTL compared to the women with normal pregnancy. However, as the strength of the survey, it was tried to reduce the risk of PTL with magnesium sulfate therapy in two factors of NO and CL comparison and to estimate the diagnostic value of each of the two factors.

MATERIALS AND METHODS

This was a case–control study, and according to the Cochran sample size calculation formula to compare the mean values at the confidence level of 95% and power of 80%, a standard deviation of CL was estimated as 1.55 and the least significant difference between the two groups was considered as 0.8; approximately, sixty women assigned in each normal and PTD groups with simple random sampling method. Samples were selected based on the acceptable criteria among the pregnant women admitted to the delivery section of Shahid Beheshti Hospital in Isfahan (Iran) from February to December 2014.

In painful uterian contractions, documented, despite hydration, for 1 h on an external tocodynamometer or regular contractions resulting cervical changes in dilatation and effacement was defined as PTL in case group. Cases with any clinical signs of infection or any other maternal or fetal complications were not selected. Inclusion criteria were age range of 18–35 years, singleton pregnancy, gestational age of 24–34 completed weeks, nulliparous pregnancies with

intact membranes, no history of type 1 or 2 diabetes mellitus, hypertension, cardiovascular disease, and infectious diseases. Control group has the same inclusion criteria. Women with PTL were excluded from the study if they had contraindication by using tocolytic drugs, fetus or amniotic fluid anomaly, uterine or cervical abnormality, multiparity, and emerging with some undesirable conditions during parturition such as pre-eclampsia and abruption.

Women with unreliable gestational age for any reason, whom with multiple pregnancy or problems such as premature rupture of membranes, gestational diabetes, high blood pressure, eclampsia or pre-eclampsia, incompetent cervix, uterus anomaly, use of prescription drugs such as corticosteroids before sampling or during hospitalization, and evidence of chorioamnionitis or other infections were excluded from the study. Those who have contraindications to medications such as tocolysis (fetal asphyxia, fetal growth restriction, and placental problems, such as a placenta previa or abruption) were also excluded from the study (ten females have been excluded from the study because of some problems).[13] In addition, the study was approved by the Isfahan University of Medical Sciences' Ethical Committee with the code of 392348.

CL was measured by an experienced sonographer, using a transvaginal ultrasound (Siemens, Sonoline G40 model, EV9-4 Mhz probe) placed in the anterior fornix of the vagina while the patient's bladder was empty. CL is defined as the distance between the internal to external along the end cervical canal.

Baseline data were collected to assess comparability of the study groups, and in all women, blood samples were collected by venipuncture to measure the plasma level of NO. After that, the women in case group were received standard tocolysis, ampicillin and corticosteroid for fetal lung maturity. The methods used for tocolysis treatment were in this way; use of magnesium sulfate as follows: First, injection of 4 g magnesium sulfate 20% and then 2 g/h continued, after that, they were followed till delivery time to assess the treatment response. The plasma was decanted and stored at −70°C until it was analyzed. NO concentration was measured spectrophotometrically using the Griess reaction with a commercial kit (Madison, Wisconsin, USA, Promega Co.).

Data were analyzed by SPSS software version 20 (SPSS Inc., Chicago, IL, USA), using independent *t*-test, Pearson's correlation, and logistic regression to compare the two groups, as well as in case of the two subgroups with preterm labor and and preterm delivery regarding the data normal distribution approved by Kolmogorov–Smirnov test. In addition, to compare the diagnostic value of NO with the CL, receiver operating characteristic (ROC) analysis

and McNemar test were performed using MedCalc for Windows, version 15.1 (MedCalc Software, Ostend, Belgium). The significance level was <0.5 in the MedCalc software.

RESULTS

In the current study, the mean age of the women with preterm birth risk ($n = 50$) was 23.84 ± 3.94 years, whereas in women with normal status ($n = 60$), it was 24.50 ± 4.42 years. The mean gestational age in the group with preterm birth was 30.58 ± 2.48 weeks, whereas it was 29.91 ± 2.60 weeks in normal group. No significant difference was found between the two groups, and they were similar regarding maternal and gestational age ($P > 0.05$). Furthermore, the level of NO in the normal group, with the mean of 39.05 ± 10.17 μmol/L, was significantly higher than the case group (35.30 ± 8.27 μmol/L) ($P < 0.05$) [Table 1].

On the other hand, among the pregnant women with PTL after magnesium sulfate treatment, ten participants (20%) had PTD (in 26.13 ± 3.24 h after treatment) and forty cases (80%) had term pregnancy (in 17 ± 2.47 h after treatment), indicating the level of NO in the term pregnancy women, with the mean of 37.78 ± 7.09 μmol/L; this has been significantly higher than PTD women (25.38 ± 4.21 μmol/L) ($P < 0.0001$), and the length of cervix in the treated women with the mean of 27.30 ± 1.71 mm has been significantly more than PTD women with the mean of 19.90 ± 2.96 mm ($P < 0.0001$). In addition, in the case group, there was a direct and significant correlation between the length of cervix and the level of NO so that the length of cervix increased with NO level (correlation PTD = 0.795 and correlation PTL = 0.624) [Table 2].

The results of logistic regression to determine the risk of influential factors in preterm birth among the pregnant women at risk of PTD indicated that the level of NO, with impact factor of 0.494 and odds ratio of 0.610 (0.440–0.846%), and also the length of cervix with impact factor of 31.099 and odds ratio of 3.119 (1.23–27.03%), have been influential in the occurrence of preterm birth ($P < 0.05$) [Table 3].

Finally, ROC analysis indicated that discriminative value of NO level and the length of cervix in relation to preterm birth in tocolytic therapy group (PTL and delivery term) were lower or equal with 31 μmol/L, with a sensitivity of 99.70 (69.0–100%) and a specificity of 82.50 (67.2–92.6%), and in the cervix length, it was lower than or equal to 22 mm with a sensitivity of 80 (44.4–96.9%) and a specificity of 99.90 (91.1–100%). In other words, sensitivity and features of these factors had a high capability in the discriminative evaluation of preterm birth. The NPV was high in the two methods (NPV in NOx = 98.8 and CL = 95.2), while positive predictive value (PPV) was lower

Table 1: Clinical characteristics of pregnant women

Variable	PTL (*n*=50)	Normal (*n*=60)	*P*
Maternal age (year)	23.84±3.94	24.50±4.42	0.091
Gestational age (week)	30.58±2.48	29.91±2.60	0.173
NOx (μmol/L)	35.30±8.27	39.05±10.17	0.035*

*: level significant less than 0.05. NOx = Nitric oxide; PTL = Preterm labor

Table 2: Clinical characteristics of women with preterm labor in two groups: term and preterm

Variable	PTL (*n*=40)	Preterm delivery (*n*=10)	*P*
NOx (μmol/L)	37.78±7.09	25.38±4.21	<0.0001*
CL (mm)	27.30±1.71	19.90±2.96	<0.0001*

*: level significant less than 0.05 NOx = Nitric oxide; CL = Cervical length; PTL = Preterm labor

Table 3: Multivariate analysis of prognostic factors for preterm delivery in women with preterm labor

Variable	β	OR (95% CI)	*P*
NOx (μmol/L)	0.494	0.610 (0.440-0.846)	0.003*
CL (mm)	31.099	3.119 (1.23-27.03)	<0.001*

Use of logistic regression with adjusted maternal age and gestational age. *: level significant less than 0.05. CI = Confidence interval; OR = Odds ratio; NOx = Nitric oxide; CL = Cervical length

than the length of cervix in measuring the NO level (PPV in NOx = 58.8 and CL = 99.9), and also, the level under the curve was more than 0.70 in both the groups. Therefore, it seems that these two factors can be considered as good prognostic criteria for preterm birth ($P = 0.0001$) [Table 4].

On the other hand, comparative analysis of ROC, using the McNamar test in two discriminative criteria of NO level and length of cervix, indicated that the area under curve of NO level (area under receiver operating characteristic curve [AUC] =0.952) had no significant difference with CL (AUC = 1.00) (difference between areas = 0.047, standard error = 0.028, and 95% confidence interval = −0.008–0.103; $P = 0.095$). In other words, the accuracy and value of both NO and CL have been assessed similarly. Therefore, both of them can be appropriate discriminative criteria regarding the preterm birth [Figure 1].

DISCUSSION

PTL, as one of the most common problems in perinatology, may lead to many neonatal disorders such as morbidity and mortality and variety of physical diseases and imposes us the unwanted heavy costs for hospital administration and caretaking of preterm infants.[14,15]

To prevent PTL, many evaluations have been performed, but it represents major unsolved problems in obstetrics. Many investigations have been done to specify the possible reasons through which we can identify the susceptible pregnant women.

Table 4: Comparison between cervical length and nitric oxide diagnostic methods in women with preterm labor

Methods	95% CI		+PV	−PV	AUC	*P*
	Sensitivity	Specificity				
NOx ☐31 (μmol/L)	99.70 (69.0–100)	82.50 (67.2–92.6)	58.8	98.8	0.952	0.0001
CL ☐22 (mm)	80 (44.4–96.9)	99.90 (91.1–100)	99.9	95.2	1.00	0.0001

NOx = Nitric oxide; CL = Cervical length; +PV = Positive predictive value; −PV=Negative predictive value; AUC = Area under receiver operating characteristic curve; CI = Confidence interval; OR = Odds ratio
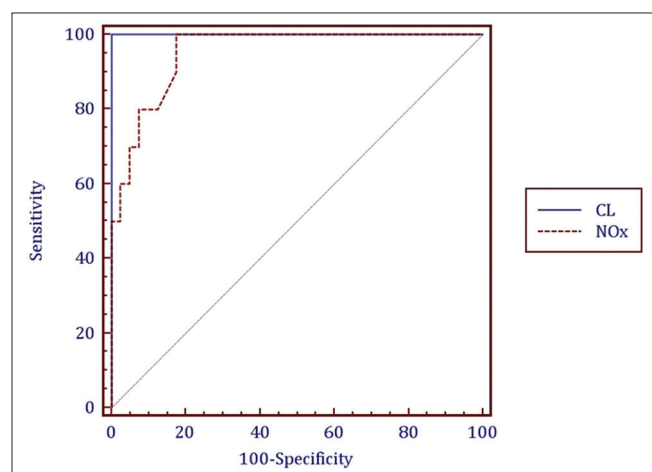
Figure 1: Receiver operating characteristic curve cervical length and nitric oxide diagnostic methods

In the current study, we evaluated NO in PTL and normal women. Previous studies have revealed that because NOx is involved in cervical ripening, it is a good marker for PTD.[16] We found the significant relationship between both groups for NOx level. We observed bigger amount of NOx in normal pregnant women. Likewise, in a study done by Zhou *et al.*, they found the similar result for the level of NOx in their both groups.[17] It has been shown that NO normally generates in the uterus and inhibits the uterine contractility. The changes in NO and its effects were consistent with the theory that NO plays a role in uterine quiescence during pregnancy. Hence, a change in this system at term or preterm could play a role in the inhibition of labor and delivery.[18]

Besides, the lower level of NOx was observed in preterm compared with delivery term in PTL women. Likewise, Väisänen-Tommiska *et al*. in their comprehensive investigation found the same result.[19] In addition, Sladek *et al*. found that NO level in preterm was lower than term group.[20] It is supposed that reducing the level of NO during the last stage of pregnancy triggers the initiation of labor consequently and stimulates the contraction of uterine.[21] On the other hand, some studies have reported the elevated NOx level in PTL women.[8,20,22-24]

On the other hand, the CL and NOx levels in women with PTL indicated that PTL risk factors have a significant effect on the sample of our study. Our evaluation revealed that NO level and CL were prognostic factors for PTD in PDT

women. Despite finding a positive correlation between NOx level and CL in PTL, other findings reported the opposite relation.[13] The survey performed by Duckitt and Thornton demonstrated that using magnesium sulfate, which causes an elevated level of NO, had no significant effect of PTL.[25] None of the trials addressed the alteration in cervical ripening or prolonged delivery. Interestingly, Smith *et al*. have revealed that NOx donor treatment tended to decrease the risk of birth compared to placebo group.[26] Other probations have reported the advantages of NO donors in PTL.[27,28]

However, CL reports are fluctuating, and still, there is no global standard for it; so, we could only implicitly compare between our findings with others. In many studies, it has been demonstrated that both CL and NOx factors have a noticeable impact on PTL.[15,16,20,22] In addition, it has been shown that NO has a short half-life time and promptly oxidase to NOx, which contains NO_3 (nitrates) and NO_2 (nitrites).[23] In fact, NOx is the marker of NO activity, which is the main biomedical mediators promoting cervical ripening and maintenance of pregnancy.[24]

Finally, we found the cutoff for our both CL and NOx elements. In other studies, there was variable cutoff. In comparison with our assessment (NOx ≤31 [μmol/L]), some studies have reported the higher amount of NO (NOx ≤ 123 [μmol/l]),[13] while other probations have stated the lower NO level compared with our findings.[18] We believe that the fluctuation of NOx level might be related to our different population nature or small sample size, and we encounter with limiting external validation of the result, and more investigations are needed to evaluate the level of NO in bigger population. For CL cutoff, our result revealed that those with equal or lower CL than 22 mm might highly encounter with PTD. Other studies had a variety of cutoff ranges from 5 mm to 35 mm.[16] In addition, NO was an appropriate discriminative criterion to determine the preterm birth so that cutoff in the level of NO is lower than or equal to 31 μmol/L, and the length of the cervix is ≤22 mm.

Whether or not considering the variety of reports regarding NO efficacy, more number of evaluations should be performed to find the exact effect of biochemical materials such as NO on PTL. Apart from the effect of NO on PTL, it should be kept in mind that using magnesium sulfate has

side effects such as nausea and vomiting, and patients taking magnesium sulfate should be tracked for complications such as cardiac arrest or respiratory depression.[14,29,30] All in all, due to small sample size and no recruitment of race variable in our study, we cannot have a meticulous report; therefore, in the future study, these limitations should be considered. According to the previous investigations, we are standing on this claim that NO might be a reliable marker for predicting the PTL, and administration of NO synthesis could be a candidate for the future therapeutic target.

CONCLUSSION

Our findings shown that nitric oxide was appropriate discriminative criteria to determine the preterm birth so that cut off in the level of nitric oxide is lower than or equals to 31 μmol/l and the length of cervix is less than or equals to 22 mm.

Conflicts of interest
The authors have no conflicts of interest.

AUTHORS' CONTRIBUTION

ZSh contributed in the conception, design, and definition of intellectual content of the work, conducting the study, approval of the final version of the manuscript, and agreed for all aspects of the work. MN contributed in the concepts, literature search, clinical studies, experimental studies, data acquisition of the work, preparation, and editing of the manuscript. FEJ contributed in the literature search, experimental studies, data analysis, statistical analysis of the work, and agreed for all aspects of the work.

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