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Betamethasone effects on the endocervical inflammatory cytokines in preterm labor: A randomized clinical trial

Sedigheh Hantoushzadeh [a], Pouya Javadian [b,*], Bahram Salmanian [b], Tooba Ghazanfari [c], Arezou Kermani [c], Fatemeh Abbasalizadeh [d], Farahnaz Zandevakil [e], Soghra Khazardoost [a]

[a] Department of Perinatology, Vali-e-Asr Reproductive Health Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b] School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[c] Immunoregulation Research Center and Department of Immunology, Shahed University, Tehran, Iran
[d] Department of Gynecology, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Department of Gynecology, Sanandaj University of Medical Sciences, Sanandaj, Iran

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ABSTRACT

Objective: This study aimed to investigate the effect of betamethasone treatment on the endocervical concentration of IL-1β, IL-4, IL-6, and TNF-α in preterm labor patients.
Study design: We studied 68 prime-gravid women in preterm labor between 34 and 37 weeks of gestation without clinical infection. Endocervical concentrations of inflammatory cytokines were assessed; immediately on admission and 48 h after administration of two doses of intramuscular betamethasone (12 mg/kg). Wilcoxon and Mann–Whitney tests along with χ^2 and Student's *t* tests were utilized for statistical analysis.
Results: In the betamethasone group IL-1β and TNF-α significantly decreased ($P<0.001$), and IL-6 and IL-4 increased (*P*: NS). Among patients delivered before or on the 7th day of admission IL-6 and TNF-α were higher at the most significant levels ($P<0.001$) compared to IL-1β and IL-4 (*P*: 0.001, 0.002 in respect).
Conclusion: Betamethasone can help induce the down regulation of endocervical inflammatory cytokines in patients with preterm labor.

1. Introduction

Spontaneous preterm birth due to preterm labor less than 37 weeks of gestational age and preterm premature rupture of the membranes (PPROM) comprise 80% of preterm deliveries. Preterm birth has been described as a multi-factorial circumstance in which genetic and environmental factors are both involved [1,2].

Infection is known to be a predominant cause of preterm labor and PPROM [3]. Therefore pro-inflammatory cytokine concentrations are expected to be higher in such conditions compared to healthy mothers not in labor [4].

Infection or other reasons could disrupt the delicate balance of cytokines, which leads to preterm activation of parturition mechanism. It has been strongly suggested that excessive or aberrant production of inflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6 at the maternal–fetal interface is a disturbing factor for pregnancy [4]. Those cytokines may play a role in labor by increasing the expression of oxytocin receptors on myometrial cells [5]. This is reported to be independent of the infection presence [6].

There is no efficient diagnostic indicator or treatment for preterm delivery [6]. In order to decrease the mortality and morbidity due to the respiratory distress syndrome and intra-ventricular hemorrhage, antenatal glucocorticoids are routinely given to women at risk of preterm delivery before 34 weeks of gestation. This therapy is standard of care, based on the meta-analysis of 18 randomized controlled trials carried out by Crowley [7].

It is believed that glucocorticoids regulate the inflammatory cascade by affecting cytokines' production [8]. Despite our extensive literature review we did not find any study investigating the effect of betamethasone (BTM) on the endocervical inflammatory cytokines among preterm labor (PTL) patients with no history of infection.

The aim of the present study is to evaluate the effect of BTM treatment on the endocervical concentration of IL-1β, IL-4, IL-6, and TNF-α in the women at risk of PTL.

2. Materials and methods

2.1. Study design and participants

This was a double-blind, placebo-controlled, parallel-group randomized clinical trial conducted in Iran. All patients were informed

* Corresponding author at: Maternal–Fetal & Neonatal Research Center and Breastfeeding Research Center, Vali-e-Asr Reproductive Health Research Center, Tehran University of Medical Sciences, Tehran, Iran. Tel.: +98 912 544 2177; fax: +98 21 88606317.
E-mail address: pouyajavadian@yahoo.com (P. Javadian).

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about the study and were provided with written informed consent papers. Women with labor pain starting between 34 and 37 weeks of gestation (according to the routine ultrasound examination in the first trimester) admitted to the obstetric emergency department of Vali-e-Asr Hospital, on June 2006–July 2009 were selected. They were randomly divided into two equal groups.

They were examined for signs of preterm labor: palpable uterine contractions every 5–8 min and Bishop score of 4 and higher associated with cervical dilatation of more than 1 cm and 50% of effacement at least. Those prime-gravid women with preterm labor were included.

Women with induced pregnancy, history of smoking, current use of antibiotics, uterine tenderness, systemic diseases, maternal hypertension before or during pregnancy, symptomatic vaginal infection, rupture of membranes, and chorioamnionitis signs were excluded [9]. Patients were followed up until the time of delivery. All patients had no clinical signs of infection during pregnancy as well as the parturition period.

A total number of 68 included patients underwent general and obstetric examination on admission. Routine laboratory tests were requested. Swab samples were prepared during the primary examination for both groups. The first group of 34 cases received 12 mg/kg of BTM intramuscular injection immediately after the sampling, and another dose of BTM was injected after 24 h. Another swab sampling was done again 48 h after the second injection. The other group received saline in similar times. Sampling was conducted in the same manner.

Sampling of endocervical secretions was performed with Dacron swabs kept in touch with the endocervical mucosa for 10 s (the details of sampling are explained elsewhere [10]). The endocervical samples were kept in −70 °C, and transferred to laboratory at −20 °C within 1–2 h. Three milliliters of venous blood sample was taken for analysis from all participating women immediately on admission.

The study was approved by the Biomedical Research Ethics Committee of Tehran University of Medical Sciences.

2.2. Determination of cytokine levels

Human IL-1β, IL-4, IL-6 and TNF-α ELISA Development Kits (R&D Systems, Minneapolis, USA) were used to measure the cytokine levels. Primary antibody was mouse anti-human. Biotinylated goat antihuman was utilized as the secondary antibody. Standards of the kits were diluted with 1% BSA in PBS. The wash buffer and the block buffer were 0.05% Tween 20 in PBS and 1% BSA in PBS respectively [11].

Table 1
Background variables comparing the betamethasone and saline treated groups, measurements are done before any intervention.

	Betamethasone	Saline	P value
	N = 28	N = 30	
Age (years)	24 (16–34)	23 (15–36)	0.543
BMI (kg/m^2)	23.93 (16.53–29.36)	22.27 (15.24–32.39)	0.714
Systolic blood pressure (mm Hg)	115 (100–140)	110 (100–140)	0.335
Diastolic blood pressure (mm Hg)	70 (60–100)	70 (60–100)	0.174
HB (gm/dL)	12.10 (8.70–13.10)	12.30 (9.10–15.30)	0.101
WBC (cells/μL)	8350 (6100–10400)	7600 (5000–13000)	0.618
CRP (mg/L)	2.40 (0.00–9.00)	2.46 (0.73–18.00)	0.493
IL-6 (ng/dL)	235.00 (22.70–898.60)	275.15 (162.70–396.40)	0.181
IL-1β (ng/dL)	191.75 (23.70–404.90)	174.65 (13.23–416.60)	0.602
IL-4 (ng/dL)	15.20 (13.36–60.80)	14.83 (12.00–47.79)	0.756
TNF-α (ng/dL)	13.93 (7.00–65.75)	11.39 (4.21–80.00)	0.081

Values are median (min–max).

Table 2a
Cytokine levels (ng/dL) measured on admission compared to measurements 72 h after, in the betamethasone treated group (N = 28).

	Primary samples			Samples 72 h after			P value
	Median	Min	Max	Median	Min	Max	
IL-6	235.00	22.70	898.60	341.50	19.40	837.60	0.076
IL-1β	191.75	23.70	404.90	134.30	2.38	344.30	0.005
IL-4	15.20	13.36	60.80	15.41	11.50	74.00	0.855
TNF-α	13.93	7.00	65.75	10.80	7.02	14.88	0.001

2.3. Determination of WBC and CRP

C-reactive protein (CRP) quantitative diagnostic kit (high sensitivity CRP; Negar-Darou, Tehran, Iran) was applied to measure CRP in sera by photometric method. The ELISA reader and washer were Stat-Fax 2100 and Stat-Fax 2600 (USA) respectively. The sensitivity of the assay was 0.5 mg/L.

WBC was determined in blood in the routine laboratory of Vali-e-Asr Hospital (Tehran, Iran).

2.4. Statistical analysis

SPPS version 16 (SPSS Inc. Chicago, IL, USA) was used for data analysis. Wilcoxon and Mann–Whitney tests were respectively used for related and independent data that did not have normal distribution on Kolmorogov–Smirnov test. Parametric data was analyzed by Students' t test and χ^2 where needed. P value of <0.05 was considered significant.

3. Results

Ten cases out of 68 patients were excluded from the study due to unexpected delivery, raised blood pressure, or refusal from second time sampling.

The demographic data is summarized in Table 1. There was no significant difference between two groups considering age, BMI, plasma HB, systolic and diastolic blood pressure. WBC and CRP levels also did not show any significant differences. CRP median value was 2.40 mg/L ranging from 0.00 to 9.00 in the BTM treated group versus 2.46 mg/L (0.73–18) in the group who received saline (P: 0.493). The median value of WBC count in the BTM group was 8350 cells/μL (6100–10400) compared to 7600 cells/μL (5000–13000) in the saline group (P: 0.618). The cytokine levels did not show any significant difference between the two groups prior to treatment (P: NS, all groups) (Table 1).

In the BTM treated group, IL-1β significantly decreased 48 h after the second BTM administration (P: 0.005). TNF-α showed the same tendency and decreased in the 2nd measurement (P: 0.001). Interestingly IL-6 revealed a contrary trend and markedly increased but did not reach statistical significance (P: 0.076). IL-4 slightly increased but it could not reach statistical significance as well (P: 0.855) (Table 2a).

In the control group who received saline regimen, none of the aforementioned cytokines showed significant changes 48 h after second saline administration. IL-6 hardly increased and IL-1β was

Table 2b
Cytokine levels (ng/dL) measured on admission compared to measurements 72 h after, in the saline treated group (N = 30).

	Primary samples			Samples 72 h after			P value
	Median	Min	Max	Median	Min	Max	
IL-6	275.15	162.70	396.40	280.55	250.00	535.60	0.237
IL-1β	174.65	13.23	416.60	210.00	76.61	276.00	0.237
IL-4	14.83	12.00	47.79	15.20	13.00	35.44	0.813
TNF-α	11.39	4.21	80.00	11.76	7.41	18.01	0.845

Table 3
Number of the patients delivered within and after a 1 week period of admission compared between the betamethasone and saline treated groups.

	Betamethasone	Saline	P Value
>1 week n(%)	21 (75%)	16 (53%)	0.086
≤1 week n(%)	7 (25%)	14 (47%)	
Total	28	30	

also higher in small values. IL-4 showed a slight decrease and TNF-α was noticeably lower (*P*: NS, all groups) (Table 2b).

Of all the patients, 36% experienced delivery in ≤1 week period after primary cytokine measurement. The number of deliveries within a week was lower in the BTM treated group compared to the saline treated group but could not reach the statistical significance level, 25% versus 47% (*P*: 0.086) (Table 3).

Regardless of BTM administration, cytokine levels were significantly higher in the patients who delivered before or on the 7th day of admission in comparison to the patients who delivered after the 7th day. It was higher either in the primary or the second measurements (Tables 4a and 4b). In the BTM treated group, IL-6 and TNF-α showed the most significant level ($P<0.001$) in comparison to IL-4 (*P*: 0.002) and IL-1β (*P*: 0.001).

4. Discussion

This study assessed the possible effect of BTM on a number of pro-inflammatory cytokines which are associated with PTL. To the best of our knowledge, this is the first study investigating the effect of BTM on the endocervical inflammatory cytokines in women at risk of PTL. With regard to our findings, IL-1β and TNF-α were significantly decreased after BTM treatment compared to the control group who were treated by saline. Cytokine levels also showed significant differences between the patients who delivered within and after a week following the initial sampling.

There is an increasing evidence to support the effective connection of pro-inflammatory cytokines like IL-1β and TNF-α with preterm parturition in a group of non-infected women [12]. Pro-inflammatory cytokines such as IL-1β and TNF-α increase the production of matrix metalloproteinase (MMP)-1, MMP-3, MMP-9, and cathepsin S which leads to induction of cervix ripening [13]. Clearly, IL-1β and TNF-α are produced at the site of local inflammation and their synergism is a commonly reported phenomenon [14]. IL-6 may increase the expression of oxytocin receptors on myometrial cells in the labor time contributing to an increase in their responsiveness to oxytocin [5].

The in vivo effects of BTM on pro-inflammatory cytokines are poorly understood [15–17]. Although, glucocorticoids usually regulate the inflammatory response by inhibiting cytokine production, however, their twofold effects are reported in animal studies [18].

We found that BTM significantly decreased the endocervical concentration of IL-1β and TNF-α which are known to have a major role in PTL. IL-4 did not show any significant difference. Interestingly, endocervical concentration of IL-6 was increased on second sampling in the BTM group, but not in significant levels. These findings were also reported in other studies. Kramer et al. showed that antenatal BTM suppressed IL-6 production 15 h after administration in preterm lambs but gradually increased after 2 days [8].

It has been reported that [19] IL-6 is predominantly produced by leukocytes, glandular epithelial cells and surface epithelial cells. Leukocytes infiltrate TNF-α. Cervical epithelial cells and leukocytes could be responsible for production of IL-1β which is found in endocervical fluid.

IL-1 and TNF-α are known to initiate the inflammatory response by targeting the endothelium [14]. IL-4 has marked inhibitory effects on pro-inflammatory cytokines [20,21]. IL-6 is commonly used as a marker for activation of inflammation [22], but nevertheless, diminishing the production of pro-inflammatory cytokines places it among the anti-inflammatory cytokines group [23].

It would be too simplistic to say that pro-inflammatory and anti-inflammatory properties of IL-6 could be the reason to justify the twofold effects of BTM. However, considering our limited data, it is difficult to find the reason behind this conflict.

The median concentrations of IL-1β, IL-4, IL-6 and TNF-α were significantly higher in women who delivered within 7 days after admission than in women who delivered after. This data is in agreement with the study which R-M Holst et al. carried out [9]. Although we conducted our study on patients with no history of infection, the significant level of differences in our study was in concordance with theirs. It shows that inflammatory cytokines play an important role in PTL even in the absence of any known source of infection.

In contrast with other cytokines, the significant level of IL-6 difference between the groups who delivered within and after one week of admission ($P<0.001$) was higher in the BTM treated group ($P<0.001$). These data support the twofold effect of BTM on IL-6 concentration in preterm patients.

The inflammatory reaction associated with preterm labor regarding the expression of different cytokines in cervical fluid has been studied in the presence of infection, however there are very few studies investigating the possible role of inflammatory cytokines in patients without signs and symptoms of infection [12,24]. In our study, the induction of cytokines in the cervical fluid of patients without any infection, represents some other mechanisms apart from infection that could be responsible for starting the preterm labor process. While the mechanism that induces the production of cytokines in cervical segment is so important, moreover, the effects of cytokines on ripening and induction of delivery also remain obscure.

5. Conclusion

The development of rapid assay of cytokines in the endocervical fluid of patients clinically at risk of PTL might have beneficial values. A balance between the effects of pro and anti-inflammatory cytokines might be one of the important factors to determine the outcome of PTL. Besides the established benefits of BTM during the process of PTL, our results suggest that it may play a role in the regulation of

Table 4a
Cytokine levels (ng/dL) measured on admission and 72 h after regarding the delivery time, in the betamethasone treated group.

	Primary samples		P value	Samples 72 h after		P value
	>1 week N = 21	≤1 week N = 7		>1 week N = 21	≤1 week N = 7	
	Median (min–max)	Median (min–max)		Median (min–max)	Median (min–max)	
IL-6	186.90 (22.70–898.60)	498.90 (363.40–758.00)	<0.001	292.00 (19.40–709.10)	584.10 (405.00–837.60)	<0.001
IL-1β	140.00 (23.70–329.20)	277.30 (47.65–404.90)	0.008	118.00 (2.38–248.00)	234.00 (151.00–344.30)	0.001
IL-4	14.92 (13.36–17.31)	30.46 (14.90–60.80)	0.008	14.90 (11.50–19.00)	24.44 (15.00–74.00)	0.002
TNF-α	11.99 (7.00–64.00)	17.70 (15.87–65.75)	0.002	10.50 (7.02–12.80)	13.25 (12.10–14.88)	<0.001

Table 4b
Cytokine levels (ng/dL) measured on admission and 72 h after regarding the delivery time, in the saline treated group.

	Primary samples		P value	Samples 72 h after		P value
	>1 week N = 16	≤1 week N = 14		>1 week N = 16	≤1 week N = 14	
	Median (min–max)	Median (min–max)		Median (min–max)	Median (min–max)	
IL-6	261.85 (162.70–298.00)	324.75 (257.10–396.40)	<0.001	257.75 (250.00–326.50)	334.20 (276.40–535.60)	<0.001
IL-1β	61.31 (13.23–238.76)	261.25 (153.90–416.60)	<0.001	170.45 (76.61–256.00)	224.00 (205.00–276.00)	<0.001
IL-4	14.45 (12.00–15.44)	20.26 (13.77–47.79)	<0.001	14.52 (13.00–15.56)	16.24 (14.50–35.44)	<0.001
TNF-α	9.75 (6.02–17.55)	13.73 (4.21–80.00)	0.004	11.10 (7.41–13.50)	12.50 (10.23–18.01)	0.008

inflammatory cytokines as well. However, further investigation is needed to brighten the interactions by which it may act, and to determine the effect of different doses of betamethasone and concomitant administration of other corticosteroids.

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