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Maternal serum Interleukine-6 and Interleukine-8 levels in preterm labor

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Abstract

Cytokines may be implicated in the patho-physiological mechanism of preterm and term labor. Many studies indicates cytokines as predictors of preterm delivery and explain partially mechanism of preterm uterine contraction. The objective of this study was to find out whether preterm labor is associated with raised maternal serum concentration of intreleukine-6 and interleukine-8 levels. Fifty-pregnant women in preterm labor (group A), 25 term pregnant women in labor (group B), and 25 women preterm not in labor (group C). The serum cytokines levels were measured by enzyme linked immunosorbant assay (ELISA). The result showed that IL6 and IL8 significantly higher during preterm labor and term labor in compares with preterm group did not in labor. No statistical difference was found in cytokines levels between women in preterm labor with rupture membranes and those with intact membranes. There was a significant higher serum concentration of IL6 in late preterm labor (34weeks-36+6days) compared with early preterm labor (24weeks-33weeks+6days). In conclusion; our data presented that higher serum cytokines levels (IL-6 &IL-8) in patient with preterm labor and term labor than preterm not in labor. The increase of IL-6 &IL-8 level during labor can be related with the possible role of these cytokines in the immunological mechanism of labor beginning.

Keywords: Cytokines; IL-6; IL-8; Preterm birth

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Introduction

Preterm birth (PTB) is delivery of fetus between 24 weeks and completed 36 weeks of gestation (36+6) [1], is a common obstetric problem, and occurs in 8-11% of all pregnancies, it is a major cause of perinatal morbidity and mortality [2]. This obstetric complication is responsible for 75-80% of all neonatal deaths [3]. Infection is the underlying cause of preterm labor and preterm

prelabor rupture of membranes (pPROM) in about 25-40% of the cases. The frequency of infection in preterm birth is inversely related to the gestational age [4]. The common source of infections are: intrauterine infection, either overt or subclinical, lower genital tract infection or colonization, and distant infections, such as periodontitis [5].

Bacterial invasion of the chorio-decidual space associated with recruitment of leukocytes followed by cytokine production including interleukine-6, interleukine-8 and tumor necrosis factor alpha (TNF-&) [6]. these pro-inflammatory cytokines may induce the synthesis and release of prostoglandins from amnion, chorion, decidua, and myometrium. This in turn, leads to uterine contractions, cervical dilatation, membrane exposure, and greater entry of microbes into uterine cavity. Cytokines have also been found to stimulate production of matrix-degrading enzymes (metalloproteinase) that soften the cervix and weaken the chorioamniotic membranes leading to their rupture [7].

Reliable methods for the diagnosis of early or asymptomatic infection are therefore needed, a less invasive and more practical procedure is the measurement of these cytokines in maternal serum. If infection is the predominant cause of preterm labor and pPROM then proinflammatory cytokine concentrations would be expected to be higher in these conditions compared to healthy patients not in labor.

Cytokine release due to intrauterine infections seems to play an important role in a considerable percentage of preterm deliveries [8]. Interleukin (IL) are a group of naturally occurring proteins (cytokines) that mediate communication between cells. Interleukins regulate cell growth, differentiation, and motility. They are particularly important in stimulating immune responses, such as inflammation [9] Fifteen different types of interleukins are known, Interleukin-1 and tumor necrosis factor (TNF)-α are considered to be the prototypic pro-inflammatory cytokines, interferon (IFN)-γ, IL-6 and IL-8 are other cytokines considered to posses pro-inflammatory properties [10]. The objective of this study was to find out whether preterm labor is associated with raised maternal serum concentrations of IL-6, IL-8 and whether the measurement of these cytokines can be used to detect early intrauterine infection in preterm labor.

Patients and methods

The study took place in Al-Zahra'a Teaching Hospital from May to October 2013 after approval by the hospital ethics committee. women enrolled in the study were divided into three groups:
Group 1: consisted of women at 24-36+6 weeks of gestations who were admitted to the hospital in preterm labor (n=50).

Group 2: consisted of healthy women at 24-36+6 weeks of gestation, not in labor recruited from our outpatient clinic (n=25).

Group 3: consisted of women who were in normal term labor(n=25).

Labor was diagnosed when the woman had regular painful uterine contractions occurring at least twice per 10min for at least 1 hour, confirmed by cervical effacement or dilatation. Gestational age was assisted by the last menstrual period and/or from ultrasound fetal measurement carried out in the first or early second trimester when available. The exclusion criteria were:- Women who had more than one fetus, evidence of preeclampsia ,urinary tract or respiratory tract infections, fever, were currently using antibiotics or who presented with clinical sign of chorioamnionitis ((define as maternal temperature of more than 37.8c, uterine tenderness , mal odorous vaginal discharge, maternal tachycardia (>100 bpm), fetal tachycardia (>160 bpm) or maternal leukocytosis)).

Blood 5cc was withdrawn in a plain tube immediately upon admission of women in a labor room and before receiving of any treatment. Blood from preterm women not in labor was with draw in the outpatient clinic. The blood was immediately centrifuged and the separated serum was stored at -70c. Full blood counts, and urine examinations were also performed for all women.

Interleukins measurements

IL-6 and IL-8 measured using ELISA. Commercial enzyme-linked immunoassays were used according to the manufacturers recommendations to measure IL-6 & IL-8 (Ray Bio Human IL-8 ELISA Kit) and (ANOGEN Human IL-6 ELISA Kit),enzymatic reaction was evaluated with the automated micro plate reader, results were expressed as pg /ml. The lower cut off value of the IL-6 & IL-8 kit was 2.0 pg /ml.

Statistical analysis

Using SPSS for windows version 15.0 soft ware. Data were express as mean, intergroup comparisons were made with student's t-test, the Mann-Whitney U-test was used for the comparisons of demographic characteristics. *P*-value below 0.05 was considered statistically significant.

Results

A total number of 100 pregnant women were divided in to three groups: fifty of them were in preterm labor (group A), twenty five were in term labor (group B) and the last twenty five were preterm not in labor (group C). The demographic characteristics of them were shown in table-1 below.

Characteristics	Group A Preterm labor (n=50)	Group B Term labor (n=25)	Group C Preterm not in labor (n=25)
	Median (Range)	Median (Range)	Median (Range)
Age (years)	24(18-39)	28(18-44)	23(16-40)
Gestational age (weeks)	33(26-36)	38(37-41)	32(26-36)
Nulliparous (%)	36%	32%	40%
Multiparous (%)	64%	68%	60%

Table-2 shows the cytokines levels of IL-6 and IL-8 in the three groups of women. There is significant difference between the three groups regarding the IL-6 and IL-8 levels which is higher in group A (73.01 pg/ml, 68.36 pg/ml respectively) and lower in group B (32.72 pg/ml, 38.45 pg/ml) and lowest in group C (2.9 pg/ml, 2.81 pg/ml).

Cytokines (pg/ml)	Group A Preterm labor (n=50)	Group B Term labor (n=25)	Group C Preterm not in labor (n=25)	P value
	Mean±SD	Mean±SD	Mean±SD	
IL-6	73.01±43.48	32.72±21.29	2.9±1.6	<0.001
IL-8	68.36±59.5	38.45±25.84	2.81±1.66	<0.001

Group A (the women with preterm labor) were further subdivided according to their membrane status whether rupture membrane or intact. Table 3 shows the cytokine levels in the two subgroups. Those preterm labor with intact membranes had IL-6 and IL-8 levels 72.4 pg/ml and 67.1 pg/ml respectively, and those with ruptured membrane the IL-6 level was 73.6 pg/ml and the IL-8 level was 69.68 pg/ml. There were no significant differences in IL-6 and IL-8 levels whether the membranes were ruptured or not.

Cytokines (pg/ml)	Intact membrane	Rupture membrane	*P* value
	Mean ±SD	Mean ±SD	
IL-6	72.4385±37.15315	73.6292±50.26357	0.924
IL-8	67.1385±60.41171	69.6875±59.78461	0.882

Table-3. Cytokine concentration among group A according to membrane status.

The preterm labor group divided according to the gestational age into early preterm 26-33 weeks and late preterm 34-37 weeks. In table 4 below shows the cytokines levels between the early and late preterm groups, there is significant difference in IL-6 which is higher in late preterm than early one while there is no significant difference in IL-8.

Cytokines	26-33 weeks	34-37 weeks	P value
	Mean ±SD	Mean ±SD	
IL-6	59.4375±38.34138	97.1389±42.48740	0.002
IL-8	68.0438±56.78057	68.9278±65.78620	0.960

Table-4. Cytokine concentration in early and late preterm among group A.

Discussion

preterm labor is an important obstetric problem around the world, because it is a significant cause of neonatal morbidity and mortality. Despite effort to prevent preterm delivery, difficulties in understanding the underlying pathology, insufficient diagnostic methods, and no efficient treatment methods have led to an increase in preterm deliveries. Our study showed that maternal serum IL-6 and IL-8 levels were significantly higher ($P<0.05$) in preterm labor than women in term labor which in turn higher than those with preterm women not in labor. The increase of IL-6 and IL-8 during labor can be related with the possible role of these cytokines in the immunological mechanism of the labor beginning [11].

Daianu *et al* [12] found that IL-6 play a very important role in the maturation process of the cervix being considered a biomarker for the assessment of the premature birth, and the value of IL-6 at 24 hours was the strongest predictor of the premature birth. Von Minckwitz *et al* [13] also reported an elevation in the concentration of IL-6 & IL-8 in women with preterm labor. Arababadi *et al* [14] found elevated levels of IL-6 in maternal serum with preterm birth.

Our result not in agreement with Ahmed *et al* [8] who found that there was no statistically significant difference in maternal cytokines concentration measured between women in preterm labor compared to preterm not in labor and women in term labor. Yüksel *et al* [15] also found no differences in IL-6 level between preterm and term women. Smith M. *et al* [16] found decreased level of IL-6 in preterm labor compared with term labor.

We further divided the preterm labor patients into ruptured (pPROM) or intact membrane

groups, for both IL-6 and IL-8 the difference between the two groups was non-significant. Zhang Wangl [17] found that maternal serum IL-6 & IL-8 levels were higher in patients of premature rupture of membrane than those of control. This is probably because they use normal term women as control while we use preterm in labor with intact membrane compared with pPROM.

Our results showed significant differences in the level of IL-6 between the different gestational ages of preterm labor, the IL-6 was higher in late preterm labor than early preterm labor while IL-8 level showed no differences. This is not in agreement with finding of Samira Behoudi *et al* [18] who found that IL-6 is higher in early preterm labor.

In conclusion; higher serum cytokines levels (IL-6, IL-8) in patient with preterm labor and term labor compared with preterm not in labor patients. The increase of IL-6 and IL-8 levels during labor can be related with the possible role of these cytokines in the immunological mechanism of labor beginning.

Recommendations

- Large studies are required to find the exact role of IL-6&IL-8.
- Measuring of IL-6 & IL-8 in patients with risk factors for preterm labor may be done as screening program to discover patient who may develop preterm labor.
- Because of great advance in immunotherapy this may be of benefit to reduce risk of preterm labor if immunological theory is proved to be the most likely cause.

Competing interests

The authors declare that there is no conflict of interest.

Author Contributions

All authors wrote, read and approved the final manuscript.

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