The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 8010- 8015

Interleukin-6 Concentration in Maternal Blood as a Sensitive Marker for Impending Preterm Labor and as a Predictor for Early Diagnosis of Bacterial Infection after Premature Rupture of Membranes

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ABSTRACT

Background: Despite numerous recent developments in an evidence-based approach to Premature Rupture of Membranes (PPROM) management, there is no widely accepted set of therapeutic techniques. A model for predicting intra-amniotic inflammatory markers is required to prevent a negative neonatal outcome in pregnant women with PPROM. This model has the potential to enhance pregnancy management and parental counselling for at-risk women. This is supported by the prevalence of bacterial infection in premature birth and its association with intra-amniotic inflammation. **Objective:** The aim of the current study is to determine whether serum interleukine-6 concentrations predict impeding preterm delivery. **Patients and methods:** This case control study included women with a singleton pregnancy, who were admitted to Maternity Hospital and diagnosed with PROM at gestational age from 24 week to 36 weeks. Participants were divided into *Control Group* consisted of 30 women who had at least one normal pregnancy without history of PROM, abortion, still birth, or preterm delivery and *Study Group* consisted of 30 women at high risk of preterm labor with history of at least one preterm labor or mid-trimester abortion. IL-6 was measured in studied groups.

Results: For diagnosis of preterm labor (PTL), the best cutoff point of IL-6 was ≥64.5, with sensitivity 80%, specificity 100% and accuracy 90%. Area under the curve was statistically significant. **Conclusion:** With strong sensitivity and specificity, maternal blood IL-6 can be utilized as a biomarker to identify impending infection in PPROM patients. This information can also help the doctor modify treatment plans to optimize feto-maternal outcomes.

Keywords: Preterm premature rupture of membranes, Preterm labor, Interleukin-6, Bacterial Infection, case control study, Zagazig University.

INTRODUCTION

Preterm PROM refers to spontaneous membrane rupture that happens before 37 weeks of gestation, which is the definition of premature rupture of membranes (PROM), which occurs before the start of labor **(1).**

About 2-3% of all pregnancies experience membrane rupture occurs prematurely (PPROM), which is linked to 30–40% of preterm births. Therefore, it is the main known factor causing preterm birth and all of its side effects, such as respiratory distress syndrome, infections in newborns, and bleeding in the brain intraventricular hemorrhage (IVH) **(2).** Multiple factors appear to contribute to PROM's multifactorial etiology, which includes decreased collagen synthesis, changed collagen structure, and rapid collagen breakdown. Infection and immune response clearly play a part in the beginning and spread of the molecular processes that result in the advancement of PPROM **(3).**

Commonly used laboratory markers including erythrocyte sedimentation rate, the number of white blood cells, the number of neutrophils, or a vaginal bacterial culture cannot be relied upon to indicate early infection. Clinical symptoms including fever and feto-maternal tachycardia typically take time to manifest **(4).**

Interleukin-6 (IL-6) is a marker for amniotic fluid that has been proposed, however it needs an invasive procedure called amniocentesis. The liver releases the acute phase protein C-reactive protein (CRP) in reaction to inflammation. The application of CRP as an early diagnostic test for chorioamnionitis during PPROM is not supported by clinical evidence, and it is not specific for infection. This is primarily due to disagreements on the typical values of CRP at various pregnant gestations and the various thresholds selected at random by various studies **(5).** Recent research has demonstrated that chorioamnionitis causes many cytokines to increase in the maternal serum, fetal cord blood, and amniotic fluid **(6).** When an intrauterine infection takes place, the cytokines that are released within the uterine cavity initially cause a localized inflammatory response. After that, they travel to the mother's bloodstream and liver, where they encourage the production CRP production by hepatocytes or bone marrow targets, which ultimately results in leucocytosis. As a result, cytokine production increases before CRP does **(7).**

Examining cytokine levels is one method for giving prompt information regarding the possibility infection that occurs inside the amniotic sac and early-onset sepsis in newborns. IL-6 is an inflammatory cytokine that alters the host's biochemistry, physiology, and immune system. Human trophoblasts produce IL-6, which is seen beginning in the second trimester and residing in the

Received: 19/7/2022
Accepted: 21/9/2022

amniotic fluid. Additionally, macrophages, amnion, chorion, and decidual cells also generate it [5]. In pediatrics, cytokine measurement in cord blood has proven a helpful method for early identification of neonatal infection [8].

Chorioamnionitis and newborn infection are highly correlated with an elevation in the IL-6 and interleukin-8 found in the amniotic fluid as cytokines. However, due to its intrusiveness and potential adverse effects, amniocentesis performed in succession for the purpose of cytokine analysis in individuals. It does not appear to be a practical option for use on a regular basis given the danger of infection. Asymptomatic intrauterine infections must be detected in PPROM patients might benefit from a quicker and less intrusive approach of monitoring cytokine IL-6 in maternal serum. Because of its recognized function of the endocrine system in the body as a regulator of IL-6, which measures the acute phase response in the liver, is an excellent choice for this assessment in the systemic circulation [7].

In order to determine whether or not cytokines, in particular IL-6, can assist with or perhaps take the place of conventional inflammatory markers in the diagnosis of subclinical chorioamnionitis, additional research is required and aid in the choice of an appropriate time for the delivery of the fetus. These parameters are CRP, temperature, leucocytosis, and fetal heart rate, which are those commonly used for the detection of infection [9].

In order to determine maternal blood IL-6 concentrations in PPROM and their connection to Infectious morbidity during pregnancy, this study was conducted.

The aim of the current study is to determine whether serum interleukine-6 (IL-6) concentrations predict impeding preterm delivery and whether the determination of IL-6 in maternal serum could supplement or replace routine inflammation parameters.

PATIENTS AND METHODS

This case control study included women with a singleton pregnancy, who were admitted to the maternity hospital and diagnosed with PROM at gestational age from 24 week to 36 weeks.

The study group consisted of 30 women at high risk of preterm labor with history of at least one preterm labor or mid-trimester abortion.

The control group consisted of 30 women who had at least one normal pregnancy without history of PROM, abortion, still birth, or preterm delivery.

Inclusion criteria of cases:

1. Women with a singleton pregnancy, who were admitted to the maternity hospital
2. Diagnosed with PROM [10].
3. Gestational age from 24-36 weeks.

Exclusion criteria:

1. Pregnant women with congenital fetal malformation or IUFD.
2. Any criteria of fetal compromise or stress.
3. Women with incompetent cervix and or cerclage.
4. Cases with antepartum hemorrhage, signs of chorioamionitis or signs of active labor.
5. Maternal medical disease.

Methods:

All patients were subjected to the following**:**

- Full history taking.

- Clinical general and abdominal examination.

- Laboratory investigations including: maternal total leucocytic count (TLC) and its differential count Normal leucocyte count varies considerably during pregnancy, C-reactive protein, vaginal swab for culture and sensitivity, urine analysis.

- Follow-up: every patient was monitored clinically on regular basis for temperature, presence of any abdominal pain or tenderness, maternal pulse, presence of any offensive vaginal discharge. Additional laboratory ad ultrasonographic criteria were followed up routinely.

- Delivery: The pregnancy was terminated at the end of the 36th gestational week or when any signs of chorioamnionitis appeared. The decision of the type of the delivery either vaginal or CS was individualized. After delivery, the neonatal outcome was assessed by the pediatrician attending the delivery.

Measurement of Outcomes:

Primary outcome: To determine whether serum interleukin 6 concentration predict impending preterm labor.

Secondary outcomes: To evaluate whether the determination of interleukin 6 routine inflammation parameters in maternal serum could replace.

Ethics consent:

Both the Institutional Review Board (IRB) and the Local Committee of Ethics approved the protocol of this research in the Faculty of Medicine, Zagazig University. A written consent of being informed was collected from all participants prior to their involvement within this study. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical analysis:

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20 for windows. Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as means

and standard deviation (SD), and independent sample t-test/Mann-Whitney U test was used for comparison between groups. Using receiver operating characteristic curves, sensitivity and specificity, of available scores and parameters, were calculated. Analysis of correlation (using Pearson's method): to determine how strongly two quantitative variables are associated. P-value ≤0.05 was considered significant.

RESULTS

Table 1 shows that there were no statistically significant differences between control group and study group regarding mean values of age, GA at PROM and GA at delivery. But there were statistically significant differences between control group and study group regarding parity, abortion and the latent period from PROM to delivery.

Table 1: Demographic & clinical characteristics of study participants in the control group and cases.

Parameter	Groups			
	Group I (Control) N=30	**Group II (Study) N= 30**	**Test**	**P value**
Age (year)	**Mean** ± SD			
	27.9 ± 6.87	29 ± 4.35	t=0.874	0.386 NS
	Median (range)			
Parity	2 (1-4)	2 (2-4)	MW= -2.329	**0.020***
	Median (range)			
Number of previous abortion	0.5 (0-2)	2 (1-5)	MW= -5.013	**0.001****
GA at PROM (weeks)	31.2 ± 1.29	32.4 ± 2.58	t=1.249	0.217NS
GA at delivery (weeks)	33.02 ± 3.73	33.8 ± 2.83	t=0.181	0.857NS
Latent period from PROM to delivery	2.13 ± 1.63	1.4 ± 1.03	t=2.075	**0.042***

Table 2 shows that there were no statistically significant (P >0.05) differences between control group and study group regarding CRP and IL6 levels and neonatal outcome, delivery, PTL and infection**.**

Table 2: Statistical comparison between control group and cases regarding mean values of CRP, IL6, neonatal outcome, delivery, PTL and infection.

Parameter	Groups			
	Group I (Control) N=30	**Group II (Study) N=30**	**test**	**P value**
CRP	**Median (range)**			
	9 (4-56)	12 (6-58)	MW= 0.267	0.789 NS
IL6	**Median (range)**			
	7.5 (3-62)	10 (3-71)	MW= 0.519	0.604 NS
Neonatal outcome	**Number (%)**			
Good	19 (63.3%)	15 (50%)	X^2= 1.099	0.577 NS
NICU	9 (30%)	12 (40%)		
Died	2 (6.7%)	3 (10%)		
Delivery				
CS	16 (53.3%)	20 (66.7%)	FET	0.430 NS
VD	14 (46.7%)	10 (33.3%)		
Preterm labor (PTL)				
No	9 (30%)	8 (26.7%)	FET	0.621 NS
Yes	21 (70%)	22 (73.3%)		
Infection				
No	18 (60%)	18 (60%)	FET	1.000 NS
Yes	12 (40%)	12 (40%)		

For diagnosis of PTL, The best cutoff point of IL-6 was ≥64.5, with corresponding sensitivity 80%, specificity 100% and accuracy 90%. The area under the curve was highly statistically significant (**Table 3**).

Table 3: Diagnostic performance of IL-6 in the prediction of PTL.

Cutoff point	≥64.5
AUC (95% CI)	0.944 (0.889 -1.000)
Sensitivity	80%
Specificity	100%
Positive predictive value	100%
Negative predictive value	83.3%
Accuracy	90%
P value	<0.001**

For diagnosis of infection, The best cutoff point of IL-6 was ≥64.5, with corresponding sensitivity 82.8%, specificity 100% and accuracy 91.7%. The area under the curve was highly statistically significant (**Table 4**).

Table 4: Diagnostic performance of IL-6 in the prediction of infection.

Cutoff point	64.5
AUC (95% CI)	0.953 (0.900 -1.000)
Sensitivity	82.8%
Specificity	100%
Positive predictive value	100%
Negative predictive value	86.1%
Accuracy	91.7%
P value	**<0.001****

For diagnosis of PTL, The best cutoff point of CRP was ≥8.5, with corresponding sensitivity 86.7 %, specificity 66.7% and accuracy 76.6%. The area under the curve was highly statistically significant (**Table 5**).

Table 5: Diagnostic performance of CRP in the prediction of PTL.

Cutoff point	8.5
AUC (95% CI)	0.882 (0.782-0.982)
Sensitivity	86.7%
Specificity	66.7%
Positive predictive value	72.2%
Negative predictive value	83.3%
Accuracy	76.6%
P value	<0.001**

For diagnosis of infection, the best cutoff point of CRP was ≥8.5, with corresponding sensitivity 86.2 %, specificity 64.5% and accuracy 75%. The area under the curve was highly statistically significant (**Table 6**).

Table (6): Diagnostic performance of CRP in the prediction of infection.

Cutoff point	8.5
AUC (95% CI)	0.883 (0.779-0.986)
Sensitivity	86.2%
Specificity	64.5%
Positive predictive value	69.4%
Negative predictive value	83.3%
Accuracy	75%
P value	<0.001**

Table 7 reveals that there was highly statistically significant positive correlation between serum IL-6 and CRP on one hand and PTL and infection on the other hand.

Table 7: Correlation of serum IL-6 and CRP with PTL and infection.

Parameters	IL-6		CRP	
	(r)	P value	(r)	P value
PTL	0.792	<0.001**	0.777	<0.001**
Infection	0.819	<0.001**	0.800	<0.001**

DISCUSSION

Regarding demographic and clinical characteristics in this study, There were no differences between the control group that were statistically important (P >0.05) and cases regarding mean values of age, GA at PROM and GA at delivery. However, there were statistically significant (P <0.05) differences between control group and cases regarding parity, abortion and the latent period from PROM to delivery.

In agreement with our study, **Ali** [11] demonstrated that in their study, there were 50 pregnant women with PPROM and 50 healthy women who were the same age and BMI as the PPROM women. There were no differences in the groups in terms of age or number of children, as shown by the P-values (0.48 and 0.802). Even so, In terms of gestational age, there were statistically significant differences between the two groups (P-value 0.0001).

Our data revealed that, No statistically significant differences between the groups were found control group and cases regarding CRP and IL6 levels and neonatal outcome, delivery, PTL and infection.

In consistent with our result, **Bahar *et al.*** [12] In a study of pregnant women with ruptured and intact membranes, researchers found no difference in the amount of IL-6 in the mothers' blood when the babies' bacterial cultures were positive or negative [1.85 (SD 1.4) pg/ml vs. 1.75 (SD 1.1) pg/ml; P= 0.861].

In disagreement with our study, **Ali** [11] reported that plasma IL-6 mean value was (18.88±0.31 pg/ml vs 5.99±0.12 pg/dl) in women with PPROM and healthy controls, respectively (P < 0.0001). They also found that the elevated level of IL-6 in at admission, the study group was considered a predictor of PPROM and a symptom of microbial invasion.

Furthermore, the best cutoff point of CRP was ≥8.5, with corresponding sensitivity 86.7%, specificity 66.7% and accuracy 76.6%. The area under the curve was statistically very important (P= 0.001).

For diagnosis of infection, our result suggested that the best cutoff point of IL-6 was ≥64.5, with corresponding sensitivity 82.8 %, specificity 100% and accuracy 91.7%. The area under the curve was highly statistically significant.

And the best cutoff point of CRP was ≥8.5, with corresponding sensitivity 86.2 %, specificity 64.5% and accuracy 75%. The area under the curve was statistically very important (p<0.001).

Qiu *et al.* [13] and **Pu *et al.*** [14] reported in consistent with our data that the level of serum IL-6 could as it had proved a useful biomarker for early identification of neonatal sepsis with PROM, PPROM can predict infection in patients.

Martinez-Portilla *et al.* [7] found that the best cut-off point for predicting bacterial infection was maternal blood IL-6 values 19.5 pg/dL, which showed an OR of 15 and a 67.7% detection rate at a 15% FPR.

Similarly to our study, many studies had reported found IL-6 levels in women with PPROM were greater in their serum [5,15,16].

In the current study, there was highly statistically (P <0.001) significant positive correlation between serum IL-6 and CRP on one hand and PTL and infection on the other hand.

Ali's study [11] showed that pregnant women with a history of premature birth and a high plasma IL-6 level were more likely to have PPROM.

Cytokines produced in the uterine cavity in situations of intrauterine infection initially trigger a local inflammatory response. They subsequently pass through the mother's bloodstream and liver to encourage hepatocytes or bone marrow targets to produce C-reactive protein (CRP), which results in leukocytosis. Thus, an increase in cytokines occurs before an increase in CRP [5].

Although early and precise diagnosis of chorioamnionitis is crucial, it is constrained by the inability to assess placental abnormalities prior to delivery. Studies have discovered a rapid, incredibly sensitive, and precise method to identify chorioamnionitis by analyzing cytokines in amniotic fluid, namely interleukin-6 (IL-6). Women with PPROM had high levels of IL-6, a well-known marker of infection and inflammation, in their blood, amniotic fluid, and vaginal secretions [15,17].

CONCLUSIONS

According to the current study, maternal blood IL-6 can be used as a biomarker to accurately and reliably foretell

the onset of infection in PPROM, allowing the physician to make adjustments to the care strategies to enhance feto-maternal outcomes in patients with PPROM.

Funding: None.

Conflicts of interest: The authors affirm that they have no conflicts of interest.

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