



# Assessment of the Diagnostic Accuracy of Eotaxin-2 as a Marker for Preterm Prelabour Rupture of Membranes

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## Abstract

**Background/Aim:** Preterm pre-labour rupture of the membranes could be caused by the change in local cytokines concentration due to inflammatory or infectious conditions. Cytokines, such as chemokine generated by immune cells that have been activated are the most extensive category of biochemical factors implicated in the development of preterm pre-labour rupture of the membranes (PPROM). Among this chemokine, eotaxin serves as a specific protein that attracts eosinophils. Aim of this study was to assess the diagnostic accuracy of eotaxin-2 as a marker for the diagnosis of PPRM.

**Methods:** A case-control study was conducted in the Department of Obstetrics and Gynaecology at Bagdad Teaching Hospital, Iraq during a period of 10 months. The study sample included 90 pregnant women and was divided into three groups, the first group: 30 women with PPRM, the second group: 30 women with preterm labour and intact foetal membranes and the third group: 30 women at term pregnancy. For all women in the study blood samples were taken for measurement of eotaxin-2 at the same gestational age.

**Results:** The eotaxin-2 level was found to be lower in cases of PPRM than in preterm labour and term cases. The sensitivity of eotaxin-2 was 83.3 %, specificity 70 % and negative predictive value 89.4 %.

**Conclusion:** Eotaxin-2 might be considered as a marker for diagnosis of PPRM.

**Key words:** CCL24 chemokine; Preterm rupture of membranes; PROM; Cytokines.

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## Introduction

Prelabour rupture of the membranes (PROM) refers to the occurrence of foetal membrane rupture before the initiation of labour. The occurrence of premature rupture of membranes after 37 weeks of gestation, which is often regarded as term, is estimated to be about 8 %–10 % in pregnancies.<sup>1</sup> If PROM occurs before 37 completed weeks of gestation (between 24 to 36+6 weeks), it is referred to as preterm pre-labour rupture of

the membranes (PPROM). PPRM occurs in approximately 3 % of pregnancies overall and occurs in up to 30 % of preterm deliveries.<sup>2</sup>

The rupture of membranes occurs due to a range of circumstances that eventually result in the expedited weakening of the membrane. The aforementioned phenomenon is attributed to an elevation in cytokine levels within the local

environment, that contributes to an escalation in intrauterine pressure.<sup>3</sup> In contrast to the occurrence of membrane rupture during labour at term, the aetiology of PPRM is mostly associated with inflammatory processes that are often connected to infection. Cytokines, such as chemokine generated by immune cells that have been activated are the most extensive category of biochemical agents implicated in the development of PPRM. These cytokines have the potential to serve as important biomarkers for the identification of preterm labour.<sup>4</sup>

Eotaxin is a polypeptide consisting of 74 amino acids with a molecular weight of 8.4 kDa. It is classified as a member of the chemokine family (CC) and is encoded by a gene located on chromosome 17. The compound selectively interacts with the CCR3 receptor, which is mostly found on eosinophils, basophils and Th2 cells. *In vitro*, this substance is a very effective chemoattractant for eosinophils. It induces the respiratory burst in eosinophils.<sup>5</sup> Another study demonstrates that the expression of CD11b is increased, leading to enhanced adherence of eosinophils to endothelial cells.<sup>6</sup> The expression of messenger RNA (mRNA) for eotaxin is consistently seen in tissues that are abundant in eosinophils, such as the small intestine and colon.

Eotaxin-2 plays a vital role in inflammatory reaction which is an important mechanism mediating PPRM. Thus, aim of this study was to investigate the diagnostic accuracy of eotaxin-2 as a marker for PPRM.

## Methods

A case-control study was conducted in the Department of Obstetrics and Gynaecology at Baghdad Teaching Hospital, Iraq during a period of 10 months (from January to October 2022). The study included 90 pregnant women and was divided into three groups, first group (PPROM): 30 women with PPRM (pregnant with rupture membrane at gestation from 24 to < 37 weeks); second group (PTL): 30 pregnant women with preterm labour from 24 to < 37 weeks with intact foetal membranes; third group (Term): 30 pregnant women in labour at term gestation  $\geq$  37 weeks with intact foetal membranes.

Prior to data collection, verbal consent was sought from each patient and the information collected was kept confidential. The process included the removal of personal names and their replacement with unique identifying numbers. All sensitive information was stored securely on a laptop protected by a password and the data were used only for research reasons.

Inclusion criteria were: women with singleton viable foetus; gestational age from 24 to less than 37 weeks for first and second groups; gestational age  $\geq$  37 for the third group; any maternal age and parity; patient within 24 h of rupture membrane (for the first group). Exclusion criteria were: women with a history of atopy, asthma and chronic allergy conditions; patient with chronic medical illness; chronic drug users; smoking; obesity; obstetrical complications (preeclampsia, etc).

Patients were selected from the inpatient ward in the Department of Obstetrics and Gynaecology at Baghdad Teaching Hospital, Iraq. All cases were interviewed with a pre-designed questionnaire that informed about the demographic data patient age, weight and height, gravidity, parity and miscarriage, educational level, occupation and residency. Systemic examination and recording of the vital signs was performed and noted, as well as abdominal examination (fundal height, foetal lie and foetal heart rate (FHR), uterine contraction), speculum examination for those with vaginal discharge (suspected PPRM) and per vaginal examination for those with labour without vaginal discharge. Patients were followed till delivery to record the mode of delivery and neonatal outcome.

Blood was drawn from all participants on admission including complete blood count (CBC), random blood sugar test (RBS), renal function tests (RFTs), liver function tests (LFTs), C-reactive protein (CRP) and blood samples for estimation of the level of serum eotaxin-2. Laboratory analysis for eotaxin-2 and obstetric ultrasound were done as well. Five mL of venous blood were drawn from each participant and kept in a serum separator tube (yellow top tube) and allowed the samples to clot undergo 2 h at room temperature or overnight at 4 °C before centrifugation for 15 min at 1000 rpm. Serum was isolated in separate tubes and sent to a private laboratory (authorised by the Iraqi Ministry of Health) for measurement

of serum eotaxin-2, using CUSABIO-human eotaxin-2/CCL24 ELISA kit and introduced into an ELISA analyser and the result was interpreted after a few minutes.

The data were analysed using SPSS 26. Data included mean, standard deviation and ranges. Frequencies and percentages reflected categorical data. Continuous data normality was tested using the Shapiro-Wilk test. ANOVA was used to compare parametric continuous variables, whereas the Kruskal-Wallis test was employed

for non-parametric variables. The Chi-square and Fisher-Freeman-Halton exact tests assessed categorical data statistical significance. Eotaxin-2 was tested for its ability to diagnose PPROM using receiver operator characteristic (ROC) curve analysis. Each cutoff point in the curve was calculated for sensitivity and specificity. The appropriate cutoff threshold was chosen using the Youden J index test. After selecting a cutoff point, the test's PPV, NPV and accuracy were calculated.  $P < 0.05$  was considered statistically significant.

## Results

The mean maternal age was not different between the three groups. The mean gestational age at presentation was lower in both cases of PPROM and PTL than in the term group. The gravidity, parity and rate of miscarriage were not different between the three groups. The occupation, educational level and residency were not different among the three groups. The rate of irregular antenatal care visits was the highest in the PTL group and was high in PPROM group in comparison to the term group (a significant difference was found between term and both PPROM and PTL, while PPROM and PTL were not different statistically), as shown in Table 1.

No difference was found regarding haemoglobin, white blood count (WBC) and body mass index (BMI) among the three groups as shown in Table 2.

Regarding mode of delivery, the rate of vaginal delivery was significantly higher in cases of PPROM and PTL in comparison to the term group ( $P1 = 0.739$  and both  $P2$  and  $P3 = 0.003$ ). Regarding neonatal outcome, both Apgar score and neonatal weight were significantly lower in cases of PPROM and PTL than the term group, as shown in Table 3.

Table 1: Distribution of demographical data

Variables	PPROM group N: 30	PTL group N: 30	Term group N: 30	P-value		
	Mean ± SD	Mean ± SD	Mean ± SD	P1	P2	P3
Age (years)	28.27 ± 7.10	28.17 ± 8.25	27.60 ± 8.40	0.999	0.941	0.962
GA (weeks)	32.83 ± 1.64	32.31 ± 1.70	38.37 ± 0.73	0.463	< 0.001	< 0.001
Gravidity	5.97 ± 2.30	5.40 ± 2.39	6.00 ± 2.27	0.619	0.998	0.582
Parity	4.53 ± 2.33	4.27 ± 2.26	4.70 ± 2.28	0.895	0.958	0.741
Miscarriage	0.43 ± 0.50	0.32 ± 0.35	0.30 ± 0.47	0.560	0.540	0.266
Occupation				0.739	0.927	0.927
Employee	5 (16.70)	6 (20.00)	5 (16.70)			
Housewife	25 (83.30)	24 (80.00)	25 (83.30)			
Education				0.894	0.983	0.983
Illiterate	2 (6.70)	1 (3.30)	1 (3.30)			
Primary	13 (43.30)	14 (46.70)	12 (40.00)			
Secondary	12 (40.00)	13 (43.30)	14 (46.70)			
Higher	3 (10.00)	2 (6.70)	3 (10.00)			
Residency				1	0.894	0.894
Urban	27 (90.00)	27 (90.00)	26 (86.70)			
Rural	3 (10.00)	3 (10.00)	4 (13.30)			
ANC				0.136	0.001	0.001
Regular	10 (33.30)	5 (16.70)	19 (63.30)			
Irregular	20 (66.70)	25 (83.30)	11 (36.70)			

P1: PPROM vs PTL, P2: PPROM vs Term, P3: PTL vs Term); PPROM group: 30 pregnant women with rupture membrane at gestation from 24 to < 37 weeks); PTL group: 30 pregnant women with preterm labour from 24 to < 37 weeks with intact foetal membranes; Term group: 30 pregnant women in labour at term gestation ≥ 37 weeks with intact foetal membranes; PPROM: preterm pre-labour rupture of the membranes; GA: gestational age; ANC: antenatal care visits; SD: standard deviation;

**Table 2:** Haemoglobin (HB), white blood count (WBC) and body mass index (BMI) among the three groups

Variables	PPROM group N: 30	PTL group N: 30	Term group N: 30	P-value		
	Mean ± SD	Mean ± SD	Mean ± SD	P1	P2	P3
HB	11.37 ± 0.99	0.99 ± 11.59	11.59 ± 1.16	0.696	0.207	0.053
WBC	8.05 ± 1.70	1.70 ± 8.75	8.75 ± 1.97	0.308	0.521	0.956
BMI (kg/m <sup>2</sup> )	23.29 ± 2.00	22.41 ± 2.06	23.63 ± 2.00	0.224	0.792	0.062

P1: PPRM vs PTL, P2: PPRM vs Term, P3: PTL vs Term); PPRM group: 30 pregnant women with rupture membrane at gestation from 24 to < 37 weeks); PTL group: 30 pregnant women with preterm labour from 24 to < 37 weeks with intact foetal membranes; Term group: 30 pregnant women in labour at term gestation ≥ 37 weeks with intact foetal membranes; PPRM: preterm pre-labour rupture of the membranes; SD: standard deviation; HB: haemoglobin; WBC: white blood cells; BMI: body mass index;

**Table 3:** Neonatal outcome among the three groups

Variables	PPROM group N: 30	PTL group N: 30	Term group N: 30	P-value		
	Mean ± SD	Mean ± SD	Mean ± SD	P1	P2	P3
Apgar score	6.23 ± 1.79	5.90 ± 2.11	8.50 ± 1.04	0.788	< 0.001	< 0.001
BW (g)	2028.87 ± 126.35	1994.70 ± 129.00	3403.33 ± 275.47	0.557	< 0.001	< 0.001

P1: PPRM vs PTL, P2: PPRM vs Term, P3: PTL vs Term); PPRM group: 30 pregnant women with rupture membrane at gestation from 24 to < 37 weeks); PTL group: 30 pregnant women with preterm labour from 24 to < 37 weeks with intact foetal membranes; Term group: 30 pregnant women in labour at term gestation ≥ 37 weeks with intact foetal membranes; PPRM: preterm pre-labour rupture of the membranes; BW: birth weight; SD: standard deviation;

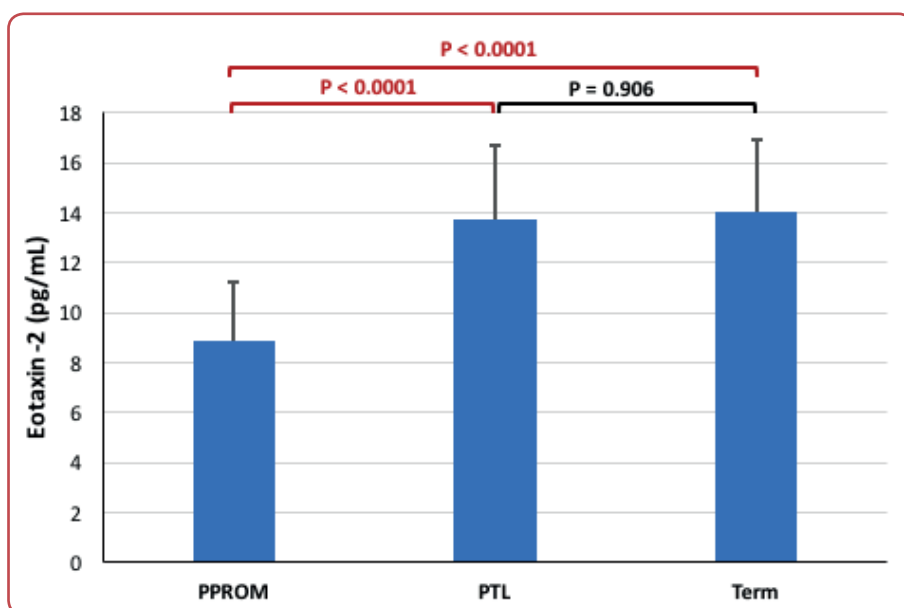
Regarding the neonatal intensive care unit (NICU) admission rates, PPRM and PTL were both associated with significantly higher NICU admission rates in comparison to the term group (P1 = 0.436, P2 = 0.001 and P3 = 0.001).

Regarding eotaxin-2 level, it was found that it was significantly lower in the PPRM group and was different from both PTL and term groups (in other words, eotaxin-2 was marking PPRM cases only in comparison to the other groups) as shown in Figure 1.

Eotaxin-2 was positively correlated with low birth weight ( $p < 0.05$ ) whereas significantly negatively correlated with birth weight > 2000 g ( $p < 0.001$ ) as shown in Table 4.

After the application of ROC curve analysis (Figure 2), it was found that eotaxin-2 equal to or less than 11.6 was associated with 83.3 % sensitivity and 70 % specificity in marking PPRM in preterm cases.

Also, it is worth noting that the negative predic-

**Figure 1:** Eotaxin -2 distribution in PPRM, PTL and Term groups

PPROM group: 30 pregnant women with rupture membrane at gestation from 24 to < 37 weeks); PTL group: 30 pregnant women with preterm labour from 24 to < 37 weeks with intact foetal membranes; Term group: 30 pregnant women in labour at term gestation ≥ 37 weeks with intact foetal membranes; PPRM: preterm pre-labour rupture of the membranes;

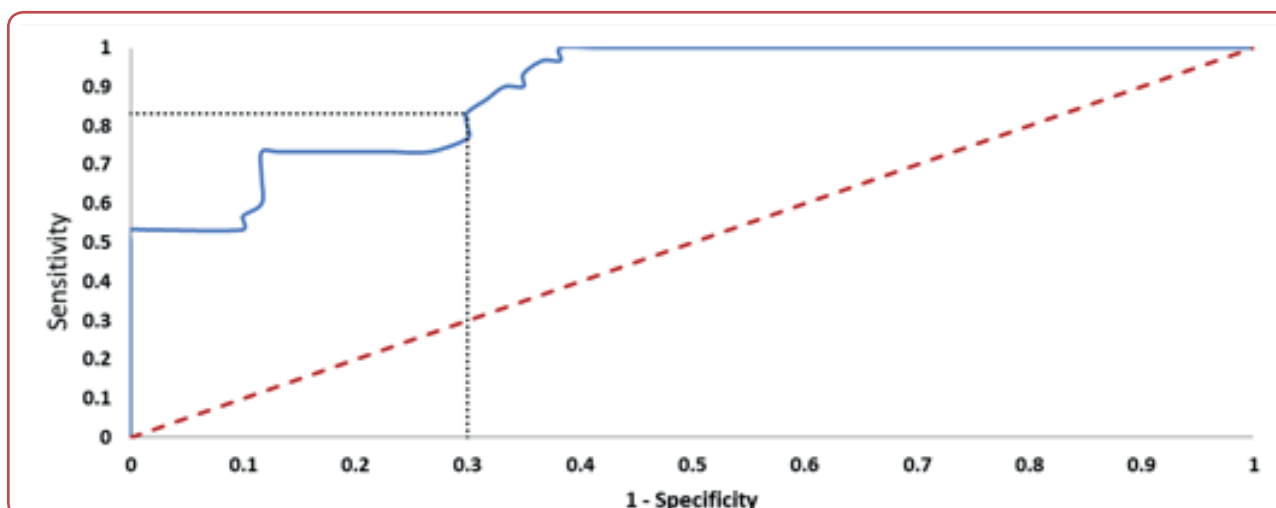


Figure 2: ROC curve analysis of the eotaxin-2 sensitivity and specificity

Table 4: Correlation between birth weight of newborns and eotaxin-2 level in mother

Birth weight (g)	R-value	P-value
≤ 2000 g	0.715	< 0.050
> 2000 g	-0.527	< 0.001

R: correlation coefficient;

Table 5: Predictive ability of eotaxin-2

Parameter	Value
AUC	0.891
95 % CI	0.825-0.956
Cutoff point (pg/mL)	11.60
Sensitivity	83.30 %
Specificity	70.00 %
PPV	58.10 %
NPV	89.40 %
Accuracy	74.40 %
OR	11.67

AUC: Area under the curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; OR: odds ratio;

tive value of eotaxin-2 was 89.4 % making it good for the prediction of ruling out PPRM cases. A level of eotaxin-2 equal to or less than 11.05 was related to 11.67 times increased risk of PPRM than those with a higher level of eotaxin-2, as shown in Table 5.

## Discussion

One of the primary roles of foetal membranes is to provide protection to the developing baby during its growth and developmental processes.

Additionally, these structures provide mechanical and immunological defence mechanisms, serving as a protective barrier against microbial infiltration. The compromise in the immunological and mechanical qualities of the foetal membranes facilitates the invasion of microbes from the genital canal. This invasion triggers the activation of the host's inflammatory response, which subsequently leads to the mechanical disruption of collagen via collagenolytic processes. Consequently, the weakening of the membranes occurs, ultimately resulting in PPRM.<sup>7</sup> The mean maternal age was not different between the three groups, this was intentionally selected to eliminate selection bias. Gafner et al<sup>8</sup> and Sae-Lin et al<sup>9</sup> found that maternal age had no effect on the rate of PPRM.

Mean gestational age was not different between the PTL and the PPRM groups, but it was different from the term group. There was no difference regarding gravidity and parity. Sae-Lin et al<sup>9</sup> (n = 1280) found that nulliparous cases had more risk of PPRM than control. This difference may be attributed to the small sample size used in the current study, which could open the door for type II error. The rate of previous miscarriages was not different between the three groups. Oliver-Williams et al<sup>10</sup> found that previous miscarriages increase the risk of both preterm labour and PPRM. Again, this could be attributed to the small sample size selected in the current study.

The mean BMI was not different between the three groups, this selected intentionally to eliminate the effect of BMI on eotaxin-2. Lutsiv et al<sup>11</sup> and Torloni et al<sup>12</sup> found that increased BMI had adverse outcomes on both mother and neonate

with increased rate of both preterm labour and PPRM. Occupation was not different statistically between the three groups. Although, studies found that occupations associated with strenuous activity associated with an increased rate of PPRM as found by Cai et al.<sup>13</sup> This may be attributed to small number of employee cases in the current study. Educational level was not different between the three groups, with similar results found by Pius et al.<sup>14</sup> The residency was not different among the three groups, on the other hand, Zhou et al.<sup>15</sup> and Gat et al.<sup>16</sup> found that rural residency had a higher rate of PPRM and attributed this difference to the environmental factors in increasing the risk. Irregular antenatal care visits were more prevalent in cases of preterm labour and PPRM in comparison to control. Sae-Lin et al.<sup>9</sup> found similar results.

Regarding neonatal outcome, cases of PTL and cases of PPRM were both associated with lower Apgar scores, with smaller birth weights in comparison to term neonates. This is attributed to prematurity that increased the chance of prolonged NICU admission rate than term neonates. Similar results were found by Esteves et al.,<sup>17</sup> Lovreen et al.<sup>18</sup> and Pius et al.<sup>14</sup> The mean eotaxin-2 levels was found to be lowest in cases of PPRM (lower than PTL and term cases). Cases of PTL and term had similar mean eotaxin-2 level, this result could be interpreted that a low level of eotaxin-2 is related to the membrane rupture process rather than to the preterm process (as preterm and term cases were not different). Similar result was found by Raba et al.<sup>19</sup> This decrement in the level of eotaxin-2 could be part of a larger chronic inflammatory cascade that is associated with a decrease in some cytokines as suggested by Lee et al.<sup>20</sup>

## Conclusion

The mean eotaxin-2 level was found to be lowest in cases of PPRM (lower than PTL and term cases). Eotaxin-2 might be of help in the diagnosis of PPRM especially in those with symptoms of rupture membrane without clinical evidence of vaginal discharge.

## Ethics

The study was approved by the Ethical Committee of Scientific Council of Obstetrics and Gynaecology and Iraqi Board for Medical Specialisations with the registration No (EAC-7458), dated 16 November 2021.

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## Conflicts of interest

The authors declare that there is no conflict of interest.

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## Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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