Suez Canal University Medical Journal Vol. 28 (6), 2025
Pages 9-14

Association between Membrane Echogenicity and Inflammatory Biomarkers among Women with Preterm Premature Rupture of Membranes: A Prospective Cohort Study

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Abstract:

Background: **Premature rupture of membranes (PROM) is a challenging condition in pregnancy, often linked to infections. Early identification of women at risk for preterm delivery is crucial for providing appropriate care.** ***Aim:*** **To investigate the relationship between the echogenicity of amniotic membranes and inflammatory biomarkers in pregnant women presenting with preterm premature rupture of membranes (PPROM).** ***Methods:*** **This prospective cohort study was carried out at the obstetrics and gynecology department of Suez Canal University Hospital. It included 72 pregnant women, aged 20–45 years, with singleton pregnancies diagnosed with PPROM between 28 and 37 weeks of gestation. Transvaginal ultrasound was used to assess the sonographic appearance of amniotic membranes near the internal os. Membranes were categorized as hyperechoic if their echogenicity resembled that of fetal bones (skull, femur, or pelvis) or as normo-echoic otherwise. Serum inflammatory biomarkers, including total leukocyte count and C-reactive protein (CRP), were measured. The study's primary outcome was to assess the association between membrane echogenicity and these biomarkers.** ***Results:*** **The mean patient age was 26.06 years, and the mean gestational age at admission was 32.47 weeks. No statistically significant differences were observed between the hyperechoic and normo-echoic groups regarding total leukocyte count (9.78 ± 2.50 vs. 9.89 ± 2.22, p = 0.610) or CRP levels (3.26 ± 0.91 vs. 3.51 ± 0.89, p = 0.108).** ***Conclusion:*** **Although increased echogenicity of the membranes may indicate potential inflammation, no significant differences in inflammatory biomarkers were found between the two groups in this study.**

Keywords: PROM; Amniotic membranes; Echogenicity; CRP; Intra-amniotic inflammation.

Introduction:

Fetal membranes provide a secure environment for the fetus inside the uterine cavity, and it commonly ruptures at the time of delivery by the uterine activity [1, 2]. Its rupture before 37 weeks is known as preterm premature rupture of membranes (P-PROM) [2], which is the cause of about one third of all recorded preterm deliveries [3], accordingly leads to a vast number of infant and mother related uneventful outcomes [4].

Up to date, the exact cause is not properly determined, and it is considered a multifactorial disorder [1, 5]. A recent focus

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was directed to membrane echogenicity among women with P-PROM, with variable results reported [3, 6]. It has been postulated that an inflammatory process in the choriodecidual interface leads to changes in amniotic membrane features [7]. The current study aimed to evaluate the association between amniotic membranes echogenicity and the inflammatory biomarkers among pregnant women presenting with P-PROM.

When PPROM occurs during the late preterm period (between 34 0/7 and 36 6/7 weeks gestation), the optimal gestational age for delivery varies by regional practice. In 2020 the American College of Obstetrician and Gynecologists (ACOG) published guidelines indicating that both expectant management and immediate delivery were considered reasonable options. Studies that examined the topic of expectant management versus immediate delivery of PPROM after 34 weeks supported immediate delivery based on an observed increased risk of infectious complications without any benefit to neonatal outcome. [8]

No preventive treatment for PPROM has been documented, although a recent study suggests that low-dose aspirin prophylaxis might reduce the prevalence of PPROM in women screened at high risk for preeclampsia [9]. Improved early prediction of women at high risk for PPROM is important for further investigation of potential preventive interventions.

Patients and Methods:

This prospective cohort study was conducted at the obstetrics and gynecology department at Suez Canal University hospital from March 2023 till March 2024. The study recruited women with P-PROM according to predetermined inclusion and exclusion criteria. The inclusion criteria were a) women aged 20 – 45 years, b) single baby, and c) gestational age between 28 and 37 weeks. The exclusion criteria included a) abnormal placentation, b) previously known uterine anomaly, c) cervical cerclage, d) congenital infections, e) fetal anomalies, f) women presenting in labor, and g) evidence of chorioamnionitis.

Diagnosis of P-PROM was based on clinical history and speculum examination (visualization of leaking amniotic fluid from the cervix during sterile speculum examination) [10]. Patients were followed up from the time of diagnoses till the spontaneous onset of labor.

Eligible patients were subjected to:

-Laboratory tests including complete blood count for the total leucocytic count (TLC), and C-reactive protein (CRP).

- Transabdominal ultrasound for fetal viability, presentation, estimated fetal weight, and amniotic fluid index (AFI).
- Transvaginal ultrasound for evaluation of membrane echogenicity close to the internal os. The fetal membranes were differentiated into hyperechoic when their echogenicity mimicked that of the fetal bones (either skull, femur or pelvic bones) or normo-echoic [11].

- Maternal follow up based on daily monitoring of clinical signs of chorioamnionitis (diagnosed by elevated body temperature ≥ 38 degrees together with maternal tachycardia, fetal tachycardia, uterine tenderness, bad odor vaginal discharges, increased total leucocytic count) [12].
- Laboratory tests (white blood cells and C-reactive protein) were recorded on alternate days. The last laboratory results

available before delivery were enclosed in the final analysis.

- Daily cardiotocography (CTG) and ultrasound done twice weekly with doppler studies were done for fetal surveillance.

The sample size included all cases presented with P-PROM during the study period.

The primary outcome measure was to evaluate the association between membrane echogenicity and the inflammatory biomarkers.

Statistical analysis:

Gathered information was processed using SPSS version 23 (SPSS Inc., Chiago, IL, USA.). Quantitative data was expressed as means ± SD while qualitative data was expressed as number and percentages (%). Normality of data was tested using Shapiro-Wilk test and statistical tests were used accordingly. A probability value (p-value) <0.05 was considered statistically significant.

Results:

We have recruited a total of 72 pregnant females presented with preterm premature rupture of membranes and based on transvaginal ultrasound findings, 40 of them were classified as having normal echoic maternal membranes and 32 were classified as having hyperechoic membranes**.**

Both groups were matched regarding maternal age and gravidity with mean age 26.06 years. Most of the studied females in both groups were multigravida. At admission the mean gestational age was 32.47 weeks and was found to be significantly higher among females with hyperechoic membranes**.** The estimated fetal weight was significantly higher among females with hyperechoic membranes **(Table 1).**

Table 1: Patients' Characteristics

Variable		Total	Echogenicity of membranes		p-value
			Hyperechoic (n=32)	Normoechoic (n=40)	
Maternal age (years)	Mean ± SD	26.06 ± 4.9	27.06 ± 5.58	25.25 ± 4.33	0.1 (NS)
	Range	20 – 37	20 – 37	20 – 37	
Gravidity	Primi-gravida	27 (37.5%)	13 (40.6%)	14 (35%)	0.6 (NS)
	Multigravida	45 (62.5%)	19 (59.4%)	26 (65%)	
Gestational age at admission (weeks)	Mean ± SD	32.47 ± 2.45	33.3 ± 2	31.8 ± 2.6	0.01*
Gestational age at delivery (weeks)	Mean ± SD	34.2 ± 2.2	34.01 ± 2.17	34.36 ± 2.24	0.5 (NS)
Admission to delivery interval (days)	Mean ± SD	14.3 ± 11.8	7.1 ± 5.6	20 ± 12.3	0.001*
Estimated fetal weight (Kg)	Mean ± SD	1.9 ± 0.49	2.08 ± 0.39	1.7 ± 0.5	0.002*

*Statistically significant difference NS: no statistically significant difference

There was no difference in the TLC between women with hyperechoic and normo-echoic membranes (9.78 ± 2.50 and 9.89 ± 2.22, respectively. P value 0.610).

additionally, no significant difference was reported between maternal temperature and heart rate between both groups. No patient had fever upon admission. There was no significant difference in the CRP level between both groups (p value 0.108) **(Table 2).**

Table 2: Comparison between the two studied groups according to Lab parameters

Variable		Total	Echogenicity of membranes		p-value
			Hyperechoic (n=32)	Normoechoic (n=40)	
WBCs count ($x10^3$ µl)	Mean ± SD	9.84 ± 2.33	9.78 ± 2.50	9.89 ± 2.22	0.610[t]
Temperature (C°)	Mean ± SD	36.65 ± 0.52	36.78 ± 0.49	36.55 ± 0.53	0.060[t]
Maternal heart rate (beat / min)	Mean ± SD.	89.69 ± 6.37	90.0 ± 5.54	89.45 ± 7.02	0.711[t]
C-reactive protein (mg/dl)	Mean ± SD.	3.40 ± 0.90	3.26 ± 0.91	3.51 ± 0.89	0.108[u]
Cervical length (cm)	Mean ± SD	32 ± 1.7	31.8 ± 1.6	32.12 ± 1.77	0.7 (NS)

SD: Standard deviation t: Student t-test. U: Mann Whitney test

Discussion:

The most prevalent place for membrane rupture is the part overlying the internal cervical opening. It showed different structural configuration, making it more liable to disruption, and is heavily exposed to bacteria [13]. However; this theory does not apply to all cases with P-PROM [14].

Hyperechoic membranes were revealed in 32/72 cases (44.4%) presenting with P-PROM. The existence of membranes with increased echogenicity was explained by the existence of possible inflammation in women diagnosed with echogenic membranes. This inflammation was accompanied with specific biochemical and histological alterations that caused advanced echogenicity of the membrane [15]. Another study reported evidence of edema and exudation of the collagen presented in the various layers of the membrane [16, 17]. Additionally, decreased water content, and the aggregation of inflammatory products and collagen degradation were reported [11].

There was no difference in the inflammatory biomarkers between both groups. Fever was not reported in any one of the participants. This agreed with previous results where no difference was reported in the initial TLC, and CRP levels among women with echogenic membranes and normo-echoic membranes. Also, no difference was reported in their maximum level [18]. Another study reported that clinical indicators for inflammation were insensitive and inaccurate [19]. This finding would be rendered to the fact that recent guidelines do not support the use of these markers alone in the diagnosis of possible infection [20, 21]. Additionally, earlier studies did not recommend the use of CRP in predicting infection among women with PROM [22]. An earlier study mentioned that CRP levels were highly diagnostic for subclinical infection among women with PROM [23].

Strength and limitations: The study was conducted as a prospective cohort study. The main limitation of the current study is small sample size; Interleukin 6 and 8 are none done. Further studies are required to determine the association between inflammatory biomarkers and histological evidence of infection. Additionally, the correlation between membrane echogenicity and histological evidence of infection is warranted.

Conclusion:

There was no association between inflammatory biomarkers and membrane echogenicity among women with preterm premature rupture of membranes.

Conflict of interest: None

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