Erythrocyte aggregation is increased in preterm premature rupture of the membranes

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Abstract

Objective: To assess the degree of erythrocyte aggregation in the peripheral blood of women with preterm premature rupture of the membranes (PPROM).

Study design: This was a prospective case control study. Twenty patients with preterm premature rupture of the membranes and matched controls were recruited at the Lis Maternity Hospital. A slide test and image analysis were used to quantitate the degree of erythrocyte aggregation. Hematological indices and markers of inflammation such as the erythrocyte sedimentation rate and C-reactive protein were also compared.

Results: The vacuum radius (VR) of the study group was significantly higher than in the control group at 14.8 ± 1.6 μm versus 10.0 ± 1.0 μm, respectively (P = 0.03). Other hematological indices were not changed significantly between the groups.

Conclusions: We found an increase in erythrocyte aggregation in the peripheral blood of patients with preterm premature rupture of the membranes. We used this sensitive marker of inflammation to further support the theory that PPROM is an inflammatory state.

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1. Introduction

Premature rupture of the membranes (PROM), defined as rupture of the chorioamniotic membranes prior to the onset of labor, is commonplace. It occurs at a reported incidence of 3–18.5% of pregnancies [1]. Preterm PROM (PPROM), defined as the occurrence of PROM before term, i.e. 37 full gestational weeks, accounts for 25% of PROM cases [1]. Although very common, the etiology and pathogenesis of PROM remains uncertain, and most investigators believe that it is multifactorial. One of the most important pathogenic factors is believed to be inflammation. The evidence supporting the role of inflammation in PROM and particularly in preterm PROM is strong [2,3]. An inflammatory process in the chorioamniotic membranes [2] and elevation of proinflammatory cytokines [4] have been found in cases of preterm PROM.

A simple slide test and image analysis have been used to quantitate the state of erythrocyte aggregation/adhesiveness [5]. The state of erythrocyte aggregation/adhesiveness was found to be increased in infectious and inflammatory states such as sepsis and acute myocardial infarction [6–9]. The degree of erythrocyte aggregation has been studied using this technique in obstetrical cases, and was found to be elevated in pregnancy-induced hypertension [10].

We chose to investigate the relationship between red cell aggregation and preterm PROM using this simple slide test. An increase in red cell aggregation could serve as additional...
evidence to support a role for inflammation in the setting of rupture of the membranes prior to delivery.

2. Methods

Twenty women with PPROM admitted to our hospital between June 2000 and March 2001, were recruited to the study. Patients with an established inflammatory state or signs of infection such as fever leukocytosis of 20,000 cells/mL or above were excluded. We also excluded patients in active labor upon arrival.

Seventeen patients matched for age and gestational week were recruited as a control group.

Patients from the two groups were healthy, did not take any medications and had normal, uneventful pregnancies until the occurrence of PPROM or recruitment for the study.

Subjects gave informed consent and the procedure was approved by the local institutional review board according to the ethical standards for human experimentation.

Results are given in mean ± standard error (S.E.). Significant differences were calculated using paired t-test. All statistics were performed using SPSS for windows version 8.0 (SPSS Inc., Chicago, IL, USA).

2.1. Laboratory tests

Blood samples were analyzed for cell count as well as the erythrocyte sedimentation rate (ESR) and fibrinogen concentration by the method of Clauss [11].

The blood cell count was determined by using the Coulter STKS electronic cell analyzer and quantitative C-reactive protein (CRP) was determined by laser nephelometry and specific anti-human CRP antibodies.

2.2. The ERYTHROSENSE™ technology

The ERYTHROSENSE™ biomarker is based on the previously described erythrocyte adhesiveness/aggregation test [7]. Venous blood from the antecubital vein was obtained upon admission. Blood was drawn into a syringe containing sodium citrate (one volume of 3.8% sodium citrate and three volumes of whole blood). One drop of the citrated whole blood was trickled onto a slide inclined at an angle of 30° and allowed to run down by gravity, leaving a fine film. The slides were left to dry in that position, at room temperature. A technician, who was blinded to the clinical and laboratory results of the patients, scanned the slides by using an image analysis system (INFLAMET™ Inflamet Ltd., Tel Aviv, Israel).

2.3. The INFLAMET™

For the analysis of the slides, we used an image analysis system (INFLAMET™) as previously described by Fusman et al. [12].

The variable that was used to describe the state of erythrocyte aggregation was the vacuum radius (VR). Vacuum radius is the distance in microns formed between the red cell aggregates. This parameter is elevated with increased aggregation (Fig. 1).

3. Results

Patients with PPROM and gestational week matched controls were aged a mean of 30.3 ± 1.5 years versus 30.7 ± 1.5 years (P = 0.85) and were pregnant, a mean of 31.8 ± 0.7 weeks versus 29.9 ± 1.1 weeks (P = 0.13), respectively. Mean BMI was 27.8 ± 1.9 and 25.5 ± 0.9 (P = 0.24) in PPROM patients and in controls, respectively.

Fig. 1. A typical blood smear for aggregation studies obtained from a patient with increased aggregation (above) and a matched control (below).
Hematological indices such as hemoglobin, red cell count, white cell count and neutrophil percent as well as platelet count did not differ significantly in the two groups (Table 1).

Indices of inflammation such as erythrocyte sedimentation rate and C-reactive protein also demonstrated no significant difference. ESR was 50.5 ± 5.7 mm/h in the PPROM patients versus 39.8 ± 3.4 mm/h in the controls ($P = 0.13$). CRP was 10.3 ± 1.3 mg/L in PPROM patients and 11.1 ± 3.5 mg/L in the controls ($P = 0.82$).

Fibrinogen, an acute phase reactant, was increased in the PPROM patients: 423 ± 32.9 mg/dL as compared to the controls: 353 ± 15.8 mg/dL, although this difference did not prove statistically significant ($P = 0.15$).

The vacuum radius of the study group was significantly higher than in the control group at 14.8 ± 1.6 μm versus 10.0 ± 1.0 μm, respectively ($P = 0.03$).

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PPROM</th>
<th>Controls</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g%)</td>
<td>11.3 ± 0.3</td>
<td>11.4 ± 0.3</td>
<td>0.76</td>
</tr>
<tr>
<td>WBC ($\times 1000$/mL)</td>
<td>11.4 ± 0.6</td>
<td>12.2 ± 1.0</td>
<td>0.44</td>
</tr>
<tr>
<td>PLT ($\times 1000$/μL)</td>
<td>198 ± 12.8</td>
<td>225 ± 18.7</td>
<td>0.23</td>
</tr>
</tbody>
</table>

### 4. Discussion

Erythrocyte aggregation indices are relatively new markers of inflammation described in the past few years [5–7] and studied in the context of various medical conditions with inflammatory mechanisms. In particular, obstetrical cases such as pregnancy-induced hypertension [10] and gynecological cases such as pelvic inflammatory disease [13] have been shown to increase erythrocyte aggregation.

A possible explanation for the association between erythrocyte aggregation and inflammation is that acute phase proteins such as fibrinogen and CRP, synthesized by the liver in acute inflammatory states, promote erythrocyte aggregation. Fibrinogen, specifically, has been shown to have a major role in the induction of erythrocyte aggregation in peripheral blood [14], and its synthesis is promoted in the presence of elevated levels of IL-6 [15]—an inflammatory cytokine shown to be involved in the processes of both PROM and normal labor [16].

Although extensively studied, the pathogenesis of both term and preterm PROM has only been partially elucidated; various inflammatory markers and cytokines have been shown to be involved, although the inciting factor remains to be determined [2–4].

This study clearly demonstrates enhanced erythrocyte aggregation in patients with preterm PROM, as evidenced by a significantly higher vacuum radius in peripheral blood slides. Our findings provide strong evidence for an association between an inflammatory state and PPROM. We demonstrated increased aggregation in cases of PPROM, where other markers of inflammation such as leukocyte count and ESR were not significantly different. This was similarly found in a previous study investigating inflammatory markers in PID [13]. We speculate that aggregation parameters have increased sensitivity for inflammatory states compared to routine indices of inflammation.

Aggregation indices may support the clinical diagnosis of an inflammatory state, however larger scale studies are needed to assess the sensitivity and specificity of these parameters as independent predictors in the diagnosis of PPROM.

In conclusion, we found an increase in erythrocyte aggregation in the peripheral blood of patients with PPROM. We used this sensitive marker of inflammation to further support the theory that PPROM is an inflammatory state.

### References