Relationship between Premature Rupture of Membranes and Collagen Amount in Chorioamnionic Membranes in Term Pregnancy

By Hasaneroğlu, Murat Bakacak, Mehmet Sühhabostancı, Eminesedagüven, Rukset Attar, Özgekızılkale, Gaziyıldırım & Agahan Han

Department of Obstetrics and Gynecology, Turkey

Abstract- Aim: Aim of this study is investigate the collagen amounts that have been deposited within the chorioamnionic membranes of term pregnancies by means of electron microscopy.

Material and Methods: The study group was made up by 20 women with term pregnancies who were diagnosed with premature rupture of membranes (PROM). Control group consisted of 20 women with term pregnancies who had no leakage of amniotic fluid. Electron microscopy was utilized to assess the collagen amount within the tissue samples which were acquired from the chorioamnionic membranes of the patients.

Keywords: electron microscopy, premature rupture of membranes, pregnancy.

GJMR-E Classification : NLMC Code: WJ 190
Relationship between Premature Rupture of Membranes and Collagen Amount in Chorioamnionic Membranes in Term Pregnancy

Hasaneroğlu a, Murat Bakacak a, Mehmet Sühhabostancı b, Eminesedagüvendağgüven c, Rukset Attar c, Özgekızılkale d, Gaziyıldırım e & Agahan Han f

Abstract- Aim: Aim of this study is to investigate the collagen amounts that have been deposited within the chorioamnionic membranes of term pregnancies by means of electron microscopy.

Material and Methods: The study group was made up by 20 women with term pregnancies who were diagnosed with premature rupture of membranes (PROM). Control group consisted of 20 women with term pregnancies who had no leakage of amniotic fluid. Electron microscopy was utilized to assess the collagen amount within the tissue samples which were acquired from the chorioamnionic membranes of the patients.

Results: When the control group was compared with the study group, term pregnancies with PROM were found to have statistically lower quantity of collagen within unit area of chorioamnionic membranes (Mean values are respectively 69.5% ± 26.3% vs 49.6% ± 23.44%; p=0.017; 95% confidence interval: 0.14-0.19) when compared with the study group.

Conclusion: Many factors have been implicated in the etiology of PROM. The present study concludes that the decrease in collagen quantity is statistically significant in chorioamnionic membranes of term pregnant women with PROM.

Keywords: electron microscopy, premature rupture of membranes, pregnancy.

I. Introduction

The fetal membrane (FM) is the membrane surrounding the fetus during gestation and is a structurally soft tissue critical for maintaining a successful pregnancy and delivery (1). The membrane is subjected to applied stresses during pregnancy and must support the bulk loads of the fetus and amniotic fluid as well as tolerate local deformation associated with fetal movement (1). The intact FM is a bilayer structure composed of both the amnion layer and the choriodecidua layer. The choriodecidua layer is thicker than the amnion layer and is cellular (1). The amnion layer is stiff and strong and only accounts for approximately 20% of the FM thickness (1). However, the amnion layer dominates the mechanical response of the intact FM (1). Rupture of fetal membranes is an event integral to the onset and development of labor at term normal pregnancy. Premature rupture of the fetal membranes (PROM) is defined as the rupture of the amniotic membranes with release of the amniotic fluid more than 1 hour prior to the onset of labor (2). PROM affects approximately 10% of women at term leading to an increased risk of maternal and neonatal infection (2). The mechanisms by which term or preterm fetal membrane weaken and rupture are not completely understood although it has been attributed to cellular apoptosis, extracellular matrix remodeling, and stretch-induced physical weakening of fetal membranes (3-5). Weakening of term FM is not homogenously distributed across the entire surface, but is more localized to the area that overlies the uterine cervix (3). This physiologic weak zone is present prior to the onset of labor in the third trimester, as demonstrated from studies of term FM from elective, scheduled, unlabored cesarean sections as well as from spontaneous vaginal delivery specimens (3, 6). This area of altered morphology has been further characterized by the same group of researchers to have increased matrix metalloproteinase (MMP) activity and apoptosis (3, 7).

Collagen is a major constituent of the extracellular matrix of amnion and is responsible for its ability to withstand an applied force. Collagen content in preterm amnions with premature rupture of the membranes was significantly lower than that of preterm amnions without premature rupture of the membranes (8). Meinert et al. have also reported collagen fiber disorganization and that hyaluronan may absorb water, with resultant increases in tissue pressure, causing FM separation and weakness (9).

The purpose of this study was to investigate the collagen amounts that have been deposited within the choriodecidua layer of term pregnancies by means of electron microscopy.
II. Materials AND Methods

Patients were recruited for this study with informed consent, using a protocol approved by the institutional review board. The study group was made up by 20 women with term pregnancies (gestational age ≥ 37 weeks) who were diagnosed with PROM due to the observation of amniotic fluid pooling on the posterior vaginal wall during sterile speculum examination. On the other hand, control group consisted of 20 women with term pregnancies who had no leakage of amniotic fluid. All of the women were at more than 37 weeks gestation, did not have diabetes, pre-eclampsia, meconium stained liquor or any fetal abnormalities and did not smoke or have an infection.

Because FMs are heterogeneous over their surface in histologic, biochemical, and physical characteristics, and there is a weak zone in the FM overlying the cervix we used methodology that would allow us to determine the exact location and orientation of each cut piece of FM relative to both the placental disk and the “cervical” weak zone (see reference 3 for details). A circular area 10 cm in diameter centered on the weakest point along the tear line was used to define the weak zone of each FM (3). After fixation using 2.5% glutaraldehyde for 48 hours, fragments were washed in phosphate buffer, 0.1 M, pH 7.3, fixed with 1% osmium tetroxide solution. Fixed tissue stained in azure II – methylene blue for light microscopy investigation with Leica DC200 and DCR-RCM (Leica, Wetzlar –Germany). Thin sections were stained with uranyl acetate and lead citrate and then viewed and photographed with high resolution transmission electron microscope (JEOL JEM-1200EX-BIO).

Statistical analysis was performed using SPSS 13.0 software. For comparison of two groups of variables independent t-test, Mann-Whitney U test was used. Calculations were performed on 95% confidence interval. The values obtained, when appropriate mean ± standard deviation (range: minimum-maximum) or number (percentage, %) was expressed as. p <0.05 was considered statistically significant.

III. Results

The study included a total of 40 cases, PROM proved to be 20 patients in group 1 (study group) and amniotic fluid flow were not proven 20 patients in group 2 (control group) were included. Between study and control groups for age, gravidity, parity, gestational age based on last menstrual period and ultrasonography findings no statistically difference was found. The demographic characteristics of the study and control groups are shown in Table 1.

Physical examination findings like fever, tachycardia, uterine tenderness, foul-smelling vaginal discharge, leukocytosis, elevated sedimentation rate, elevated serum C-reactive protein levels as the physical findings EMR which suggest intrapartum infection identified more frequently at study group than the control group. Although no statistically important differences were found. Physical examination findings of the study and control groups are shown in Table 2.

For the study group collagen ratio in the choriodecidua membrane per unit area was found to be significantly lower than the control group (mean values 69.5% ± 26.3%, 49.60% ± 23.44% respectively, p = 0.017, 95% confidence interval: 0.14 to 0.19).

IV. Discussion

Membrane rupture appears not to be entirely a result of physical forces alone, since in 10% of term labor and 40% of premature labor membrane rupture occurs before contractions begin (10). PROM is often a complication for many years, although its pathophysiology is still unclear. Spontaneous preterm birth due to preterm labor and PROM is associated with a variety of clinical characteristics. Possible causes include local amniotic membrane defects, vaginal, cervical or intraamniotic infections especially for group B streptococcal colonization, cervical insufficiency, polyhydramnios, multiple pregnancy, placental abruption, placenta previa, trauma, smoking, zinc and copper deficiency.

The mechanical response of the fetal membranes is a combination of the mechanical responses of the individual chorion and amnion components. Toppozada et al. showed that the specific contraction causing rupture of membranes was rarely the most forceful contraction that had been experienced up to that time (11). They argued that prior contractions weakened the membrane so that it subsequently gave way with less force (11). Collagen dissociation in the cervical region of the membranes leading up to parturition has been observed and is thought to represent the site of initial rupture (12). Vadillo-Ortega showed that collagen of the amniotic membrane structure was decreased, whereas, with the solubility of collagen was found to increase collagen degradation activity (13). The possibility that PROM is caused by a deficiency of the different collagen types has been investigated (8, 14). Results of gel electrophoresis examination of amniotic membranes with collagen type III / II, III / IV and III / total collagen by ratios of PROM patients were found to be significantly lower other preterm patients (8). Alterations in membrane protease activity may be involved in the formation of this structurally weaker zone which has also been observed in PROM leading to the hypothesis that protease activity may be elevated in PROM membrane (15). Furthermore, it has been shown that non-weak membranes can be...
transformed into weak membranes by in vitro incubation with cytokines (16).

Possible strategies for membrane repair or prevention of premature weakening is important for physiology of fetal membrane rupture. Artal demonstrated that membranes placed in pseudo-amniotic fluid become weaker over 24 h but remain unchanged if a mixture of pharmacological enzyme inhibitors is included (17). Harmali et al. have demonstrated improved membrane tensile strength with iatrogenic defects after application of a fibrin sealant, but that the membrane strength remains less than that of unruptured membrane segments (18). Although gross differences in proteolytic activity were not seen when comparing PROM and normal membranes at term it is possible that differences involving individual proteases are being masked. Milwidsky et al. report no general proteolytic differences between amniotic fluid collected from PROM patients and controls and yet a number of studies report differences when examining specific proteases (19).

V. Conclusion

Premature rupture of membranes often encountered in the practice of obstetrics, which may cause significant maternal and fetal complications, but its etiopathogenesis is not yet completely unresolved. Many factors have been implicated in the etiology of PROM. The most important of these, is the deterioration of the fetal membrane stability. In maintaining the integrity of fetal membranes, the amount of collagen content is vital. The amount of collagen and its content have role for the pathophysiology of PROM. Therefore, the amount and changes in the content of collagen play an important role for pathophysiology of patients with PROM. Investigations to be carried out about the amount and content of collagen in patients at risk for PROM can be focused on the development of treatment methods.

Table 1: Demographic Characteristics of Study and Control Groups

<table>
<thead>
<tr>
<th>variables</th>
<th>Study Group (n=20)</th>
<th>Control Group (n=20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>24.85±5.47 (18–39)</td>
<td>24.4±4.88 (16–34)</td>
<td>0.986</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.45±1.95 (1–7)</td>
<td>1.90±0.96 (1–4)</td>
<td>0.699</td>
</tr>
<tr>
<td>Parity</td>
<td>0.80±0.95 (0–3)</td>
<td>0.50±0.60 (0–2)</td>
<td>0.403</td>
</tr>
<tr>
<td>Gestational age for LMP (week)</td>
<td>38.82±1.42 (36–40)</td>
<td>39.64±0.69 (38.3–40.5)</td>
<td>0.390</td>
</tr>
<tr>
<td>Gestational age for USG (week)</td>
<td>37.59±1.04 (35–40)</td>
<td>37.56±1.06 (36–40.3)</td>
<td>0.392</td>
</tr>
<tr>
<td>Cigarette smoking (%)</td>
<td>yes</td>
<td>2 (%66.7)</td>
<td>1 (%33.3)</td>
</tr>
<tr>
<td>Cigarette smoking (%)</td>
<td>no</td>
<td>18 (%48.6)</td>
<td>19 (%51.4)</td>
</tr>
</tbody>
</table>

LMP: last menstruel period, USG: Ultrasound
*a Fisher's exact chi-square test. b Mann-Whitney-U test.

Table 2: Physical Examination Findings related to the study and control groups

<table>
<thead>
<tr>
<th>variables</th>
<th>Study Group (n=20)</th>
<th>Control Group (n=20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine tenderness yes</td>
<td>1 (%100)</td>
<td>-</td>
<td>0.500</td>
</tr>
<tr>
<td>Uterine tenderness no</td>
<td>19 (%48.7)</td>
<td>20 (%51.3)</td>
<td></td>
</tr>
<tr>
<td>Foul smelling vaginal discharge yes</td>
<td>1 (%100)</td>
<td>-</td>
<td>0.500</td>
</tr>
<tr>
<td>Foul smelling vaginal discharge no</td>
<td>19 (%48.7)</td>
<td>20 (%51.3)</td>
<td></td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.52±0.35 (36–37)</td>
<td>36.41±0.30 (36–37)</td>
<td>0.282</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>96.80±19.52 (72–146)</td>
<td>91.55±18.22 (66–140)</td>
<td>0.455</td>
</tr>
<tr>
<td>White blood cell count (/dL)</td>
<td>12475.5±2806.6</td>
<td>11727±2934.5</td>
<td>0.208</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (mm/hour)</td>
<td>33.8±15.91 (19–80)</td>
<td>26.9±9.18 (16–53)</td>
<td>0.112</td>
</tr>
<tr>
<td>C-reactive protein (ng/ml)</td>
<td>33.6±36.64 (0–96)</td>
<td>32.4±30.68 (0–96)</td>
<td>0.699</td>
</tr>
</tbody>
</table>

*a Fisher's exact chi-square test. b Mann-Whitney-U test.

References: