Amniotic Membrane’s Hydroxyproline Content and Preterm Premature Rupture of Membranes

Amnion Zarının Hidroksiprolin İçeriği ve Preterm Erken Membran Rüptürü

ABSTRACT Objective: To determine whether preterm premature rupture of the membranes (PPROM) is caused by a generalised and/or localised reduction in the hydroxyproline content of the amniotic membrane. Material and Methods: This study included 105 parturient women who delivered between September 2005-March 2006 in Zekai Tahir Burak Women’s Health Education and Research Hospital High Risk Pregnancy Department. The first group included 40 parturient women with PPROM. The second group included 65 parturient women with intact membranes having an elective Caesarean section with the diagnosis of previous caesarean section. In the PPROM group (n= 40), 25 women delivered vaginally and 15 women had an emergency Caesarean section for fetal distress at the beginning of labour. The hydroxyproline content of the amniotic membranes of 40 women with preterm premature rupture of the membranes was compared with these of 65 women who delivered by elective Caesarean section. Three regions of the amniotic membrane were examined: 1) the rupture site 2) the midzone area, which was the halfway between the rupture site and placental edge; and 3) periplacental area, which was usually 10-12 cm away from the rupture site. Results: In the rupture site, no significant difference was detected in the hydroxyproline content between the control group (0.17-0.63 mg/mg protein) and the PPROM group (0.26-0.51 mg/mg protein) (p = 0.916). In the midzone area, there was no significant difference between the control group (0.16-1.45 mg/mg protein) and the PPROM group (0.24-0.84 mg/mg protein) (p = 0.462). In the periplacental area there was no significant difference between the control group (0.23-1.35 mg/mg protein) and the PPROM group (0.23-1.13 mg/mg protein) (p = 0.753). Conclusion: Preterm premature rupture of the membranes does not appear to be accompanied by a generalised or localised reduction in the collagen of amniotic membrane.

Key Words: Fetal membranes, premature rupture; hydroxyproline; amniotic membrane

ÖZET Amaç: Preterm erken membran rüptürünn (PEMR) amnion zarının hidroksiprolin içeriğindeki yaygın veya lokalize azalmadan kaynaklanıp kaynaklanmadığını belirlemek. Gereç ve Yöntemler: Çalışmaya Zekai Tahir Burak Kadın Sağlık Eğitim ve Araştırma Hastanesi Yüksek Riskli Gebelik Bölümü’nde Eylül 2005-Mart 2006 arasında doğan 105 kadını içeren ik i grup PEMRsi olan 40 kadın kapsayordu. İkinci grupta daha önce sezaryen geçirmiç kayıtlı olduğu için elekifet sezaryene ait, membranları sağlam 65 kadın vardı. PEMR grubunda (n= 40), 25 kadın vaginally yoldan doğ妈 yaptı ve 15 kadın fetal distres nedeniyle travayın başında acil sezaryene ait. Preterm erken membran rüptürü olan 40 kadının amnion zarının hidroksiprolin içeriği elekifet sezaryen yapılmış 65 kadınını karşılaştırdı. Amniotik membranın üç bölüge incelendi: 1) rüptür bölgesi, 2) rüptür ile plasental ucun ortasında bulunan orta bölge, ve 3) genellikle rüptür bölgesinde 1-12 cm uzakta olan periplasental alan. Bulgular: Rüptür bölgesinde, kontrol grubu (0.17-0.63 mg/mg protein) ile PEMR grubu (0.26-0.51 mg/mg protein) arasında önemli fark saptanmadı (p = 0.916). Orta bölgede, kontrol grubu (0.16-1.45 mg/mg protein) ile PEMR grubu (0.24-0.84 mg/mg protein) arasında önemli fark yoktu (p = 0.462). Periplasental alanda, kontrol grubu (0.23-1.35 mg/mg protein) ile PEMR grubu (0.23-1.13 mg/mg protein) arasında önemli fark yoktu (p = 0.753). Sonuç: Preterm erken membran rüptürününe amnion zarının kollageninde yaygın veya lokalize bir azalma eşlik ediyor gibi görünmemektedir.

Anahtar Kelimeler: Fetal membranlar, prematür rüptür; hidroksiprolin; amnion amniyos

Pretterm premature rupture of the membranes (PPROM) is defined as rupture of the membranes before 37 weeks of gestation. PPROM is the leading identifiable cause, occuring in 1% of all pregnancies. Prematurity is the third leading cause of prenatal death. PPROM has a variety of causes. Intrauterine infection is thought to be a major predisposing factor. The membrane’s tensile strength depends on the collagen that makes up the extracellular matrix (ECM). The strength of the fetal membranes is thought to be influenced by both synthesis and degradation of the components of the extracellular matrix. The fibrillar collagens (type I, III, and V) are presumed to be the critical components lending tensile strength to the amnion. Hydroxyproline is the main amino acid constituent of collagen and it is usually measured in studies.

It is thought that PPROM may be caused by a collagen deficiency. This could either be generalised throughout the membranes, or localised to a particular area. A generalised deficiency could be caused by an abnormal gene expression which may explain the high recurrence rate of PPROM. A localised deficiency may result from the breakdown of collagen by bacterial collagenases. This has been shown to produce membrane weakening in vitro. Recent studies hypothesise that matrix metalloproteinases and in particular metalloproteinase 9 could contain some specific enzymatic properties involved in the rupture of membranes following infection.

In a previous large study on membrane hydroxyproline, Skinner et al. found that the mean amniotic hydroxyproline content was significantly reduced in cases with PROM (47.6 mcg/mg) compared to 102 controls with timely membrane rupture (51.8 mcg/mg) (p<0.02). Kanayama et al.’s study supported this finding. MacDermott and London, on the other hand, found that there was no association (p>0.5) between the hydroxyproline content (either per mg or per cm² amnion) and the timing of membrane rupture. In view of these conflicting results, a further study was considered appropriate. An additional goal of our study was to investigate whether PPROM was associated with a generalised or localised reduction in the amniotic membrane's hydroxyproline content. Samples were taken from three different regions of the amniotic membrane in order to answer this question.

Following approval from the Zekai Tahir Burak Women’s Health Education and Research Hospital’s Ethics Committee and informed written consents were obtained from the patients, the amniotic membranes were collected.

### MATERIAL AND METHODS

The study included 105 parturient women who delivered between September 2005-March 2006 in Zekai Tahir Burak Women’s Health Education and Research Hospital High Risk Pregnancy Department. Two groups of women were investigated. The first group included 40 parturient women with PPROM. The second group included 65 parturient women with intact membranes having an elective Caesarean section (C/S). Both groups were between 26 and 38 weeks of gestation. Infection was excluded by clinical parameters and microbiological investigations on the basis of vaginal swabs and body temperature. Histological chorioamnionitis was not investigated further. Amniotic membrane samples were taken following delivery in both groups. In the PPROM group (n=40), 25 women delivered vaginally and 15 women had an emergency C/S for fetal distress at the beginning of the labour. The placentae were collected within half an hour of expulsion. Membranes were washed with ice cold saline and all clots were removed. The entire fetal membrane was cut in a manner that location and orientation of each tested piece was maintained relative to each other, to the placental disc and to the area of fetal membrane previously overlying the cervix (for C/S fetal membrane specimens). For the C/S patients, the fetal membrane’s weak zone overlying the cervix was visually identified. After laying the placental membranes flat, the area previously overlying the cervix was identified and a series of cuts were made starting from this region and extending to the placental disc. This allowed the membranes to lay completely flat. Serial secondary cuts were made along a line extending from marked area/tear line (which would include the initial point of rupture for vaginal deliveries) extending to the placental disc to obtain tissue test pieces.
the collection of fetal membranes, amnion and chorion were manually separated. Fetal membranes were sampled using a Babcock clip. On average, an area of 3 x 3 cm² around the clip was taken. Three regions of the amniotic membrane were examined: 1) the rupture site (R) which included the initial point of rupture for vaginal deliveries or marked area which was visually identified as fetal membrane weak zone overlying the cervix 2) the midzone area (M), which was halfway between the rupture site (for vaginal deliveries)/marked area (for C/S deliveries) and placental edge; and 3) periplacental area (P), which was usually 10-12 cm away from the rupture site/marked area. Before processing, the samples were stored at -80°C. No chemical substances were added until analysis.

HYDROXYPROLINE SPECTROMETRIC METHOD

The tissues were homogenised in a 1:10 proportion with PBS buffer and centrifuged at 1000 rpm for 13 minutes at 4°C. The hydroxyproline content was calculated per milligram by biochemistry department.

STATISTICAL ANALYSIS

All data were analyzed using the SPSS statistical package 11.5 for data analysis. Descriptive statistics was used to show the median (min–max). Friedman test was used to compare the three areas within each group (C, M and P areas) and Mann–Whitney U test was used to compare the two groups (controls versus PPROM cases). A p-value <0.05 was considered as statistically significant.

RESULTS

The mean gestational age of patients in PPROM group was 30 (27-35) weeks while mean gestational age of patients in the control group was 38 (36-38) weeks. Twenty five percent of the patients in PPROM group were nulliparous while 43% of patients in the control group were nulliparous. Thirty five percent of the patients in PPROM group and 24.5% of the patients in control group were smokers. The ratio of the patients who reported PPROM in their previous pregnancies was 37.5% in the PPROM group and 8% in the control group, as shown in Table 1.

As shown in Table 2, site no significant difference was detected in the hydroxyproline content between the control group (0.17-0.63 mg/mg protein) and the PPROM group (0.26-0.51 mg/mg protein) in the rupture (p= 0.916). In the midzone area, there was no significant difference bet-

<table>
<thead>
<tr>
<th>TABLE 1: Clinical and obstetrical characteristics of the PPROM group and the control group.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPROM Group (median/min-max)</strong></td>
</tr>
<tr>
<td>Gestational Age</td>
</tr>
<tr>
<td>Maternal Age</td>
</tr>
<tr>
<td>Nulliparity</td>
</tr>
<tr>
<td>Smokers</td>
</tr>
<tr>
<td>History of PPROM at previous pregnancy</td>
</tr>
</tbody>
</table>

Values are presented as median (range) or number (%).

<table>
<thead>
<tr>
<th>TABLE 2: Comparison of the hydroxyproline values within and between the two groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Group</strong></td>
</tr>
<tr>
<td><strong>Median</strong></td>
</tr>
<tr>
<td>S Area</td>
</tr>
<tr>
<td>P Area</td>
</tr>
<tr>
<td>M Area</td>
</tr>
<tr>
<td>P Value</td>
</tr>
</tbody>
</table>

* Comparison between the groups.

** Comparison within the groups.
between the control group (0.16-1.45 mg/mg protein) and the PPROM group (0.24-0.84 mg/mg protein) (p = 0.462). In the periplacental area there was no significant difference between the control group (0.23-1.35 mg/mg protein) and the PPROM group (0.23-1.13 mg/mg protein) (p = 0.753) (Figure 1).

No significant difference was found when the hydroxyproline content of the rupture site in the PPROM group was compared with that of the mid-zone area and periplacental area.

**DISCUSSION**

Collagen forms the main component of the amniotic membrane’s extracellular matrix and its presence is thought to be crucial for the maintenance of the membrane’s mechanical integrity and stress tolerance. Membranes that rupture before delivery have a zone of extremely altered morphology within them, a region that is characterized by dissociation of collagens. This region can be found within the postdelivery tear and is believed to act as the rupture-initiation site. However, we have not found generalised or localised reduction in the amnion’s any hydroxyproline content. Besides this, no differences were found in the hydroxyproline contents of between the control group and the PPROM group in the three regions studied. This implies that there is not a generalised reduction in the amnion’s hydroxyproline content. In addition, the authors concluded that hydroxyproline concentrations did not differ between rupture and nonrupture-site samples within the same patient type, however there was a significantly lower hydroxyproline concentration in PPROM amnion compared to control tissue.

The finding of absence of any difference in the hydroxyproline content at the rupture site, when compared to the other two areas in the PPROM group, supports the idea that there is no localised reduction in the amnion’s hydroxyproline content in PPROM. This result goes against common sense because the normal expectation is reduced collagen strength at the rupture site. Epidemiological, clinical, histological, microbiological and molecular biology data have all suggested that each of focal infection or inflammation may play primary or secondary roles in the pathogenesis of PPROM. Histological evidence of inflammatory changes is more often noted at the membrane rupture site. In addition, in a study conducted by Jabareen et al., the uniaxial stress-strain response of nine human term fetal membranes was measured, and as in our study, it was concluded that there was no correlation between maximum stress and collagen or elastin content. The matrix metalloproteinases (MMP) are important enzymes in tissue remodelling. They are capable of breakdown of extracellular matrix (ECM) with a broad range of substrate specificities. This matrix breakdown is a part of tissue remodelling, which also clearly involves matrix deposition, with this MMP activity reflecting local remodelling. MMP are involved in many aspects of reproductive function. The pathophysiology of “spontaneous” PPROM also indicates the importance of ECM remodelling in the inter-layer connective tissue of the fetal membranes by these enzymes. In a study conducted by Saglam et al., matrix metalloproteinase-9 (MMP-9) activity was associated with preterm premature rupture of membranes (PPROM) in cases in which infection is excluded.

An explanation for the absence of a local collagen deficiency in this study could be the exclusion of cases with clinical and pathological evidence of chorioamnionitis. Exposure to bacterial collagenases before and/or after membrane rupture may cause a local collagen deficiency.
Although we did not find any association between PROM and a generalised reduction in the hydroxyproline content, our study had a number of limitations such as the presence of both vaginal and C/S deliveries in study group. In a study it was concluded that the hydroxyproline content was significantly greater in patients who had a vaginal delivery when compared to the cases who had elective C/S. In a recent study, it was demonstrated that vaginally delivered artificial ROM fetal membranes had a weak zone overlying the cervix. Vaginally delivered spontaneous ROM fetal membranes contain a weak zone adjacent to the tear line that exhibits biochemical and mechanical characteristics suggestive of collagen remodeling and apoptosis comparable to those of the artificial-rupture fetal membrane’s weak zone. Additionally in a study conducted or only elective C/S patients, a discrete weak zone was present in term pre labor fetal membranes overlying the cervix, and it had biochemical characteristics consistent with tissue remodeling and apoptosis. Another limitation is the sampling of amnion adjacent to rupture site which might have caused an increase in the thickness of the amnion. This may also reflect higher baseline oxidative stress at the rupture site. Besides these, it would not be possible to know whether any observed reduction in the hydroxyproline content was a cause of the rupture, or alternatively, a consequence of the presence of bacterial collagenases around the rupture site in the latent interval between the membrane rupture and delivery. The latent interval is often prolonged when PROM occurs. There are also a number of changes at the rupture site due to the effect of collagenases after membrane rupture. Vadillo-Ortega et al. demonstrated a correlation between increased hydroxyproline content and latency in 13 cases of PROM at term. In our study, study group and control group had both term and preterm patients. In recent studies, it was concluded that separation of amnion from chorioamnion occurs as part of normal term fetal membrane rupture. Fetal membranes become significantly weaker as a result of this separation.

In conclusion; in this study, no significant difference in the collagen content was demonstrated between the PPROM group and the control group. Moreover, no significant difference was observed in the collagen content between the three regions in the PPROM group. It is concluded that changes in the collagen content of the fetal membrane do not appear to be involved in the mechanism of PPROM.

Future research is warranted to establish whether there is a local reduction in the amnion’s hydroxyproline content in women with PPROM as a result of chorioamnionitis as compared with women with PPROM without chorioamnionitis.

Acknowledgement

The authors would like to thank Professor Hasan Ozan for his support. He helped to organise the biochemical part of the study. In addition, we would like to thank the nurses of the high risk pregnancy unit.

REFERENCES


