

# The roles of beta-human chorionic gonadotropin, creatinine and urea in vaginal washing fluid in the diagnosis of premature rupture of membranes

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## Abstract

**Objective:** In this study, we aimed to test the roles and safety of urea, creatinine and beta-human chorionic gonadotropin for the diagnosis of premature rupture of membranes (PROM) by considering that fetus urine is significant for amnion formation, urea and creatinine concentrations being high in fetus urine and beta-human chorionic gonadotropin (beta-HCG) concentrations are high in amniotic fluid.

**Methods:** This study was conducted on 150 pregnant women who referred to the obstetrics and gynecology clinic. The study group consisted of 75 pregnant women between 27 and 42 weeks of gestation whose diagnosis of membrane rupture was confirmed and amniotic fluid came apparently. The control group consisted of 75 pregnant women between 27 and 42 weeks of gestation who did not have complaints of water break and vaginal discharge, and whose membrane rupture was ruled out. After administering 10 cc 0.9% NaCl to posterior fornix by sterile injector, a total of 6 cc washing fluid was collected. Urea, creatinine and human chorionic gonadotropin were examined on the same day on collected fluid and both groups were compared statistically.

**Results:** Beta-HCG, urea and creatinine values in vaginal washing fluid of the cases with premature rupture of membranes were statistically and significantly high compared to the control group ( $p<0.05$ ). When designating *cut-off* value of beta-HCG in vaginal bleeding fluid as 50 mIU/dL, sensitivity, specificity, and positive and negative predictive values were 85.33%, 93.33%, 92.75% and 86.41%, respectively.

**Conclusion:** In our study, we obtained the most valuable data in premature rupture of membranes (PROM) by beta-HCG values in vaginal washing fluid. Similar results were obtained by other PROM diagnosis tests currently used such as insulin-like growth factor binding protein-1 (IGFBP-1), placental alpha microglobulin and prolactin in the vaginal fluid. When these tests are compared, it is seen that beta-HCG test in vaginal fluid is cheaper and easier, and thus it becomes more practicable.

**Keywords:** Premature rupture of membranes, human chorionic gonadotropin.

## Özet: Erken membran rüptürü tanısında vajinal yıkama sıvısında beta-insan koryonik gonadotropin, kreatinin ve ürenin yeri

**Amaç:** Bu çalışmada, amniyon oluşumunda fetüs idrarının önemli bir yeri bulunması, fetüs idrarında da üre ve kreatinin konsantrasyonunun yüksek olması ve amniyon sıvısında beta-insan koryonik gonadotropin (beta-HCG) konsantrasyonlarının yüksekliğini göz önüne alarak, bu parametrelerin erken membran rüptürü (EMR) tanısındaki yeri ve güvenilirliğini test etmeyi amaçladık.

**Yöntem:** Bu çalışma kadın hastalıkları ve doğum kliniğine başvuran 150 gebe üzerinden yapıldı. Membran rüptürü tanısı kesinleştirilen, amniyotik sıvının belirgin olarak geldiği, 27-42. haftalar arasında 75 gebe çalışma grubunu oluşturdu. Su gelmesi ve vajinal akıntı şikâyeti olmayan, membran rüptürü ekarte edilmiş, 27-42. haftalar arasındaki 75 gebe ise kontrol grubumuzu oluşturdu. Posterior fornixte steril enjektör ile 10 cc %0.9 NaCl verilmesini takiben toplam 6 cc yıkama sıvısı toplandı. Toplanan sıvılardan aynı gün üre, kreatinin, insan koryonik gonadotropin çalışılmış olup her iki grubun istatistiksel karşılaştırması yapıldı.

**Bulgular:** Erken membran rüptürlü olguların vajinal yıkama sıvısındaki beta-HCG, üre ve kreatinin değerleri kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksek idi ( $p<0.05$ ). Vajinal yıkama sıvısındaki beta-HCG *cut-off* değerini 50 mIU/dL olarak belirlediğimizde sensitivite, spesifite, pozitif ve negatif prediktif değerleri sırasıyla, %85.33, %93.33, %92.75, % 86.41 olarak bulduk.

**Sonuç:** Çalışmamızda EMR tanısında en değerli verileri vajinal yıkama sıvısındaki beta-HCG değerleri ile tespit ettik. Günümüzde kullanılan diğer EMR tanı testleri olan vajinal sıvıda insülin benzeri büyüme faktörü bağlayıcı protein-1 (IGFBP-1), plasental alfa mikroglobulin-1, prolaktin ile de benzer sonuçlar elde edilmiştir. Bu testlerle karşılaştırıldığında, vajinal sıvıda beta-HCG ölçümünün daha ucuz ve kolay uygulanabilir olması, bu testin kullanılabilirliğini arttırmaktadır.

**Anahtar sözcükler:** Erken membran rüptürü, insan koryonik gonadotropini.

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## Introduction

Premature rupture of membranes (PROM) is a condition that fetal membranes rupture before labor and amniotic fluid discharges. If the rupture in membranes occurs before 37 weeks of gestation, it is defined as preterm premature rupture of membranes (PPROM). While PROM is seen in 10% of all pregnancies, 80% of the cases occur at term pregnancy.<sup>[1]</sup> Preterm premature rupture of membranes is seen in 3-5% of all pregnancies and it is the most common reason of preterm labor.<sup>[2]</sup> Fetal membranes are more solid in early pregnancy period in terms of rupture. As week of gestation increases, membranes get weaker and uterine contractions, fetal movements and increase of uterus tension facilitates the rupture of membranes.<sup>[3]</sup> Also, the decrease of collagen amount in membranes towards the term increase rupture risk. The most significant complication of PPRM is the preterm labor and associated prematurity.<sup>[4]</sup> It is essential to establish proper PROM diagnosis due to possible complications. The misdiagnosis of membrane rupture causes mis-treatments such as labor induction and unnecessarily prolonged hospitalization. Today, there is no diagnosis method for PROM which is non-invasive and of gold standard. Observing direct fluid discharge from cervical os confirms PROM.<sup>[5]</sup> Amnion presence can be detected at a rate of 93% by nitrazine paper changing its color from yellow to dark blue when it is placed to vaginal posterior fornix, and many factors such as vaginitis, blood and semen increase false positivity of the test.<sup>[6]</sup> With quite high specificity and low sensitivity, the detection of fetal fibronectin in cervicovaginal secretions has been interpreted as the indicator of labor although there is no rupture of membranes.<sup>[7]</sup> Another diagnosis method is the determination of insulin-like growth factor binding protein-1 (IGFBP-1) and placental alpha microglobulin-1 protein in cervicovaginal fluids.<sup>[8,9]</sup> In a study performed, the sensitivity and specificity of immunochromatographic dipstick method used for the determination of IGFBP-1 in vaginal secretions for PROM diagnosis were found as 97%.<sup>[10]</sup> In a study including the determination of placental alpha-microglobulin-1 protein in cervicovaginal fluid as another immunochromatographic method, specificity was found 98.9% and sensitivity as 100%.<sup>[11]</sup>

Beta-human chorionic gonadotropin (HCG) exists in amniotic fluid as well as maternal blood and urine. In a study carried out by the measurement of beta-HCG

level in vaginal fluid for PROM diagnosis at third trimester, sensitivity, specificity, and positive and negative predictive values were found as 100%, 96.5%, 88.9% and 100%, respectively. In the same study, it was emphasized that beta-HCG is a reliable test at second and third trimesters in PROM cases.<sup>[12]</sup> Urea and creatinine are two substances discharged from kidneys. By considering that a major part of amnion fluid consists of fetus urine, the studies have been performed to evaluate the roles of urea and creatinine levels in vaginal washing fluid for PROM diagnosis. In a study, sensitivity, specificity, and positive and negative predictive values were all found as 100% when creatinine and urea cut-off values were considered as 0.6 and 12, respectively.<sup>[13]</sup>

In this study, we aimed to test the roles and safety of urea, creatinine and beta-human chorionic gonadotropin for the diagnosis of PROM by considering that fetus urine is significant for amnion formation, urea and creatinine concentrations being high in fetus urine and beta-HCG concentrations are high in amniotic fluid.

## Methods

This study was conducted on 165 pregnant women who referred to Obstetrics and Gynecology Clinic of İzmir Tepecik Training and Research Hospital between April 2009 and October 2009. Fifteen patients were excluded from the study due to vaginal bleeding, vaginitis, amniotic fluid with meconium and maternal urine mixing with amniotic fluid. Seventy-five patients between 27 and 42 weeks of gestation whose diagnoses of membrane rupture were confirmed by IGFBP-1 measurement in vaginal fluid, nitrazine test and ultrasonography and whose amniotic fluid discharged clearly were included in the study group (Group 1), and 75 patients between 27 and 42 weeks of gestation who had no complaints for water break and vaginal discharge, whose membrane rupture was ruled out were included in the control group (Group 2). All patients underwent vaginal examination by sterile speculum after the anamnesis procedure. The samples were collected as a total of 6 cc washing fluid after administering 10 cc 0.9% NaCl to posterior fornix by sterile injector, and they were examined in the same day. Same researcher collected the samples and carried out the ultrasonography in order to eliminate the difference between observers. Demographic data of the patients included in the study were recorded before collecting samples.

**Table 1.** The demographic data of the patients and their beta-HCG, urea and creatinine values in vaginal bleeding.

	Group 1 (n=75) Mean±SD (min-max)	Group 2 (n=75) Mean±SD (min-max)	p value
Age (year)	29.38±5.6 (19-42)	29.81± 4.7 (21-40)	0.614
Parity	1.61±0.99 (1-6)	1.49±0.77 (1-5)	0.413
Week of gestation	37.1±3.35 (27-42)	37.26±2.9 (27-42)	0.755
Beta-HCG (IU)	214.68±134.49 (12-521)	23.93±17.49(2-105)	<0.001
Urea (mg/dL)	8.67±7.3 (1.6-27.3)	2.57±1.71 (0.3-7.1)	0.001
Creatinine (mg/dL)	0.58±0.59 (0.1-2.8)	0.25±0.20 (0.1-1.2)	<0.001

Statistical Package for the Social Sciences (SPSS) for Windows 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. In intergroup comparisons, independent samples t test was used for the analysis of data with normal distribution. The results were evaluated within 95% confidence interval and according to  $p < 0.05$  significance level.

## Results

Our study included 150 cases and their ages were ranging between 19 and 42. No statistical difference was found when both groups were compared in terms of age, parity and the week of gestation (**Table 1**).

Beta-HCG values in vaginal washing fluid of Group 1 cases were statistically and significantly higher than the values of Group 2 (**Table 1**). When designating cut-off value of beta-HCG as 50 mIU/dL, sensitivity, specificity, and positive and negative predictive values were 85.33%, 93.33%, 92.75% and 86.41%, respectively.

Urea and creatinine values in vaginal washing fluid of Group 1 cases were statistically and significantly higher than the values of Group 2 (**Table 1**). When we designated cut-off value of urea as 10 mg/dL, sensitivity, specificity, and positive and negative predictive values were 26.66%, 100%, 100% and 57.69%, respectively; when we designated cut-off value of creatinine as 0.3 mg/dL, sensitivity, specificity, and positive and negative predictive values were 46.66%, 94.66%, 89.74% and 63.96%, respectively (**Tables 3 and 4**).

## Discussion

Preterm premature rupture of membranes is one of the most common reasons for fetal morbidity and mortality. Therefore, establishing correct diagnosis of PPROM is essential to prevent possible complications and to

decrease hospitalization period in case of misdiagnosis. Fluid drainage from cervical os is observed during speculum examination in the most of the patients referring to hospital with vaginal discharge complaint. Remaining patient group which includes the patients who have no fluid discharge during the examination is the group difficult to establish PROM diagnosis. Since methods such as fern test and nitrazine (pH) test which have been used widely so far are not sufficiently reliable and there is no method defined as a non-invasive golden standard yet, new tests have been researched such as

**Table 2.** The distribution of patients to the groups when B-HCG level is considered as 50 IU.

Beta-HCG	Group 1 (PROM+) n(%) (n=75)	Group 2 (PROM-) n(%) (n=75)
≥50	64 (85.3)	5 (6.7)
<50 IU	11 (14.7)	70 (93.3)

**Table 3.** The distribution of patients to the groups when urea level is considered as 10 mg/dL.

Urea	Group 1 (PROM+) n(%) (n=75)	Group 2 (PROM-) n(%) (n=75)
≥10 mg/dL	20 (26.7)	0 (-)
<10 mg/dL	55 (73.3)	75 (100)

**Table 4.** The distribution of patients to the groups when creatinine level is considered as 0.3 mg/dL.

Creatine	Group 1 (PROM+) n(%) (n=75)	Group 2 (PROM-) n(%) (n=75)
≥0.3 mg/dL	35 (46.7)	4 (5.3)
<0.3 mg/dL	40 (53.3)	71 (94.7)

insulin-like growth factor binding protein-1 (IGFBP-1), alpha-fetoprotein, prolactin, fibronectin, beta-HCG and placental alpha microglobulin-1. In addition to these tests, the presence of urea, creatinine, AST and ALT in the vaginal washing fluid which were proved to exist in amniotic fluid have become the subjects of new researches in PROM diagnosis. In our study, we also researched the role of beta-HCG, urea and creatinine values in vaginal washing fluid for PROM diagnosis.

A major part of amniotic fluid includes fetal urine at second and third trimesters. Urea and creatinine filtered out of fetal kidneys pass to the amniotic fluid through urine. Beta-HCG is a substance produced by trophoblastic tissue, exists at various concentrations in maternal blood, urine and amniotic fluid, and easy to detect.<sup>[14]</sup> Presence of these three substances in vaginal secretions at low concentrations and the increase of vaginal concentration after amniotic drainage at PROM mean that it would be useful to use these substances in the diagnosis of PROM. In our study, beta-HCG values in vaginal washing fluid of Group 1 cases were statistically and significantly higher than the values of Group 2 (**Table 1**). Similarly, urea and creatinine values in vaginal washing fluid of Group 1 were statistically and significantly higher than the values of Group 2 ( $p=0.001$  and  $p<0.001$ , respectively).

In the study of Cooper et al., when cut-off value of beta-HCG in vaginal washing fluid for PROM diagnosis was designated as 50 mIU/dL, sensitivity, specificity, and negative and positive predictive values were found as 96%, 79%, 95% and 84%, respectively.<sup>[14]</sup> In our study, When designating cut-off value of beta-HCG in vaginal bleeding fluid as 50 mIU/dL, sensitivity, specificity, positive and negative predictive values were 85.33%, 93.33%, 92.75% and 86.41%, respectively.

In the study carried out by Kafalı and Öksüzler, the authors designated urea cut-off value in the vaginal washing fluid as 12 mg/dL and creatinine cut-off value as 0.6 mg/dL and they found sensitivity, specificity and negative and positive predictive values as 100%.<sup>[13]</sup> In our study, we found completely different results. When we designated cut-off value of urea in vaginal bleeding fluid as 10 mg/dL, sensitivity, specificity, and negative and positive predictive values were 26.66%, 100%, 100% and 57.69%, respectively. When we designated cut-off value of creatinine as 0.3 mg/dL, sensitivity, specificity, and negative and positive predictive values were 46.66%, 94.66%, 89.74% and 63.96%, respectively.

## Conclusion

In our study, we obtained the most valuable data in PROM by beta-HCG values in vaginal washing fluid. We found that we can use beta-HCG for the diagnosis of PROM when we designated the cut-off value of beta-HCG as 50 mIU/dL. Similar results were also reached by IGFBP-1 in vaginal fluid, placental alpha microglobulin-1 and prolactin tests which have been currently used as other PROM diagnosis tests.<sup>[15-17]</sup> When these tests are compared, it is seen that beta-HCG test in vaginal fluid is cheaper and easier, and thus it becomes more practicable.

**Conflicts of Interest:** No conflicts declared.

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