Research Article

The Identification of Placental Alpha Micro Globulin-1 (Amnisure®) as a Method to Identify Rupture of Membrane

Deteksi Placental Alpha Micro Globulin-1 (Amnisure®) sebagai Metode dalam Identifikasi Ketuban Pecah

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Abstract

Objective: We aimed to do a study in the use of the identification of Placental Alpha Micro Globulin-1 or PAMG-1, in the form of Amnisure® test, as a method to diagnose rupture of membrane (ROM), compared with other conventional method (direct visualization and nitrazine test).

Method: We used a cross-sectional design. Every pregnant woman who came to our hospital with gestational age of 14 to 42 weeks complaining of membrane rupture was recruited. Sterile speculum examination and nitrazine test was performed for every patient. Amnisure® was utilized, using vaginal swab from posterior fornices. Data analysis was performed with SPSS version 17.

Results: We recruited 20 patients to join our study. Mean age, parity and gestational age was 28.5 years, parity one and 35.5 ± 3.4 weeks of gestation. Amnisure® test was positive in 14 patients. With nitrazine as standard for ROM diagnosis, the sensitivity for Amnisure® was 85% and the specificity was 83.3%. The positive predictive value was 92.3% and negative predictive value was 71.4%.

Conclusion: For every positive vaginal pooling, nitrazine and Amnisure® will be tested positive. Several studies using Amnisure® have shown similar results. Role of Amnisure® seemed evident in cases of uncertainty such as chronic ROM and severe oligohydramnios due to ROM. Positive results in presence of intact membranes which suggested micro-perforations of the membrane still need further research. Much still needed to be done before implementing Amnisure® in our country, especially in the matter of cost effectiveness.

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Keywords: amnisure®, PAMG-1, ROM

Abstrak

***Tujuan**: Mengadakan penelitian untuk menguji metode deteksi ketuban pecah menggunakan Placental Alpha Micro Globulin-1 (Amnisure®) dibandingkan dengan metode konvensional (tes valsava dan tes lakmus/tes nitrazin).*

***Metode**: Penelitian ini merupakan studi potong lintang. Setiap wanita hamil yang datang ke rumah sakit kami dengan usia gestasi 14 hingga 42 minggu dan keluhan ketuban pecah diikutkan dalam penelitian. Pemeriksaan spekulum steril untuk melihat tes valsava dan tes lakmus dilakukan pada setiap pasien. Tes Amnisure® dilakukan dengan apusan dari forniks posterior. Analisis data dilakukan dengan SPSS versi 17.*

***Hasil**: Sebanyak 20 pasien diikutsertakan dalam studi kami. Rerata usia, paritas dan usia gestasi adalah 28,5 tahun, paritas satu dengan usia gestasi 35,5 ± 3,4 minggu. Tes Amnisure® positif pada 14 pasien. Dengan tes lakmus sebagai standar bagi diagnosis ketuban pecah, sensitivitas Amnisure® adalah 85%, spesifisitas 83,3%. Nilai prediksi positif dan negatif adalah 92,3% dan 71,4%.*

***Kesimpulan**: Untuk setiap tes valsava positif, nilai positif akan di temukan pada tes lakmus dan tes Amnisure®. Beberapa studi menggunakan Amnisure® menunjukkan hasil serupa. Peran Amnisure® tampaknya jelas pada kasus sulit seperti ketuban pecah kronik, oligohidramnion berat karena ketuban pecah. Hasil positif dalam keadaan selaput membran intak, yang menandakan adanya mikro-perforasi memerlukan penelitian tambahan. Studi lebih lanjut diperlukan sebelum mengimplementasikan penggunaan Amnisure® di Indonesia, terutama jika menyangkut isu tentang efektivitas biaya.*

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***Kata Kunci**: amnisure®, ketuban pecah, PAMG-1*

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INTRODUCTION

Rupture of membrane, especially in preterm cases, is associated with infections and adverse neonatal outcomes.[1,2] Review by Teune et al on 29,375,675 infants found that outcomes such as respiratory distress, intraventricular hemorrhage and death were larger in preterm infants.[3] Spontaneous membrane rupture occurred in 35% of 27,771 late preterm births at Parkland hospital.[4] Incidence of preterm birth in Dr. Cipto Mangunkusumo hospital from January till December 2011 was between 5 to 15%. Survival rate for infants born within 28 to 32 weeks gestational age was 65.8% to 77.6% in our hospital (unpublished data). Granovsky found that preterm and small for gestational age infants were even at more risk for developing retinopathy, bronchopulmonary dysplasia, necrotizing enterocolitis and death.[5]

Membrane rupture was associated with inflammation, proven by positive amniotic culture in 32 to 35% patients.[6] Presence of membrane rupture will determine further management of the patient.[7-9] Thus, we need to stress out the importance of firm diagnosis of this condition in every day practice. Several markers for infection has been established[8-10] Kiefer et al proposed an amniotic inflammatory score to predict preterm parturition syndrome.[8] Agustin and Romero assessed the value of fetal fibronecton as predictor for spontaneous preterm delivery in multiple gestations.[9] Sarah Lee et al measured level of interleukin-6 trans signaling molecule soluble GP130 and soluble IL-6 receptor in amniotic fluid of women with preterm rupture of membranes and signs of inflammation.[10]

Establishment or membrane rupture can be done by several methods. Visualization by vaginal pooling at posterior fornices, nitrazine test, and fern test are some examples. According to the guidelines from Royal College of Obstetricians and Gynecologist, the diagnosis is best made by history suggestive of membrane rupture, followed by demonstration of fluid pooling.[11] However, in cases in which these conditions are not met, other test might be helpful.[12] Identification of Placental Alpha Micro Globulin-1 (PAMG-1) is a novel method for diagnosis of membrane rupture. In this study, we present our preliminary results of comparison between Amnisure® test with other conservative test in management for membrane rupture.

METHODS

Every pregnant woman with gestational age from 14 weeks up to 42 weeks gestational age and chief complaints of membrane rupture who came to our hospital were asked to participate in our study. Gestational age was determined by addressing last menstrual period, and/or data from earliest ultrasound findings, combined with clinical and ultrasound result at present. History taking from the patient will ask whether she felt her membrane ruptured/not, sensation of water leaking out from her and duration of this feeling.

Every woman will undergo sterile speculum examination. Presence or absence of vaginal pooling at posterior fornices is assessed for each patient. Swabs of the posterior fornices was taken twice for nitrazine test and Amnisure® test. For nitrazine test, we used standard dipstick for pH determination. Positive nitrazine test was determined when pH level of vaginal fluid/secretion was alkaline. A swab for Amnisure® was taken and agitated in diluents vial, which contain 0.9% NaCl, 0.01% Triton X_{100}, 0.05% NaN_3, for one minute. The test strip was then dipped directly to the diluents vial, achieving the results within ten minutes, as shown in Figure 1. Two strips indicated positive results for membrane rupture. Diagnosis of membrane rupture was based on history taking, sterile speculum examination and nitrazine result. Standard for diagnosis of rupture of membrane on our hospital was the combination of these three findings. We have yet to adopted Amnisure® as our standard of diagnostic method.

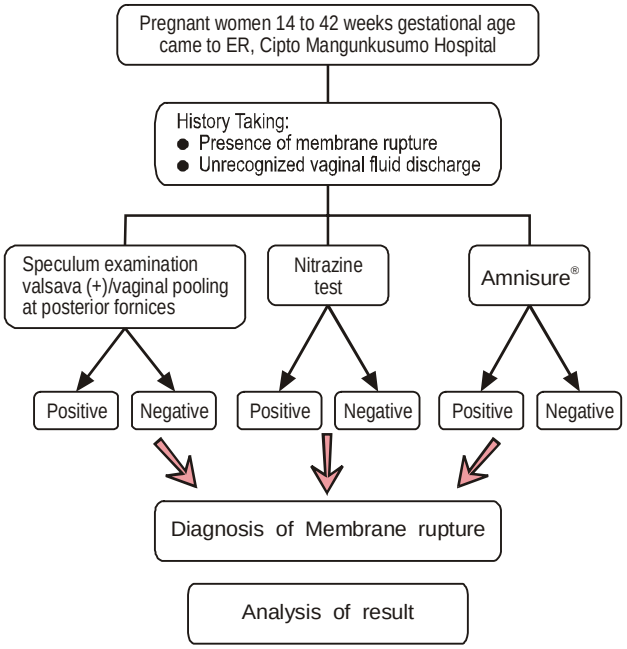

Figure 1. Schematic representation of study scheme.

Data analysis after completion of sample taking was conducted with Statistical Package for Social Science (SPSS Inc) Version 17.0. The mean, sensitivity and specificity were measured. Tabulation of data was performed as well and presented herein.

RESULT

This was a preliminary study, with as many as twenty patients participated in this research. Complaints of membrane rupture were evident in all patients. The mean age was 28.5 year old. Half of them were in their first pregnancy (50%). Mean age during presentation was 35.5 ± 3.4 weeks of

gestation. Positive vaginal pooling and nitrazine test was found in 14 patients (70%) and 13 (65%) patients respectively.

Table 1. Comparison of Amnisure® results and Nitrazine test.

	Amnisure		
Nitrazine test	**Positive**	**Negative**	**Total**
Positive	12	1	13
Negative	2	5	7
Total	14	6	20

Amnisure® was found positive in 14 patients. The value for sensitivity, specificity, positive predictive value and negative predictive value were calculated with nitrazine as standard for diagnosis of membrane rupture; with tabulation seen in Table 1. With nitrazine as standard for ROM diagnosis, the sensitivity for Amnisure® was 85% and the specificity was 83.3%. The positive predictive value was 92.3% and negative predictive value was 71.4%.

Table 2. Results of Amnisure® and diagnosis rupture of membrane (ROM).

	Amnisure		
ROM	**Positive**	**Negative**	**Total**
Evident	14	2	16
Non Evident	0	4	4
Total	14	6	20

Patients with positive vaginal pooling will result in positive nitrazine and Amnisure® test (14 patients). One exceptional case is one patient with negative nitrazine and vaginal pooling but positive Amnisure test.

DISCUSSION

Placental alpha micro-globulin 1 (PAMG-1) was first described by dr. D. Petrunin in 1975. It was derived from amniotic fluid. PAMG-1 is a 34-kd protein. It has unique features, which is a high concentration in amniotic fluid, low levels in blood and extremely low levels in cervicovaginal secretions with intact fetal membranes. Baseline level of PAMG-1 with presence of vaginitis or an admixture of blood or serum will rarely reach 3 ng/ml maximum. Concentration ranges of PAMG-1 in amniotic fluid are 2,000 to 25,000 ng/ml. The Amnisure® test employs monoclonal antibodies to detect minute amount of PAMG-1. Sensitivity threshold for PAMG-1 with Amnisure® is 5 ng/ml. Thus, reducing the probability for false positive and false negative results.

Several studies have tested the robustness of PAMG-1 with other methods for membrane rupture. Cousins et al measured Amnisure® on 203 patients with suspected ruptured membranes and found 90 patients were positive.[13] They claimed that sensitivity and specificity of the test to be 98.9% and 100% respectively. Mittal and Romero reviewed the usage of Amnisure® in 113 pregnant women with sonographically short cervix. They found that patients with positive test had a shorter admission-to-SROM (spontaneous rupture of membrane) interval than those with a negative test (43 days versus 78 days, *p* value 0.02).[14] They suggested that the presence of PAMG-1 in vaginal fluid is indicative of micro-perforations of intact membranes. Similar conclusion was taken by Lee et al which conducted Amnisure® testing for women with and without intact membrane.[15] Patients with positive Amnisure® test had a shorter admission-to-delivery interval than those with negative results.

Our study design was similar with Lee et al.[16] They conducted a prospective observational study from March 2005 till February 2006. From 184 patients recruited in their series, conventional diagnostic method of membrane rupture resulted in 76% diagnosis at initial presentation. Amnisure® confirmed initial diagnosis of membrane rupture with sensitivity of 98.7%, specificity 87.5%, positive predictive value of 98.1% and negative predictive value of 91.3%. It seemed appreciable to note that for every patient in our series with positive vaginal pooling, test for nitrazine and Amnisure® will be positive. This is relevant with guidelines from RCOG, in which history taking and sterile speculum examination will usually suffice to determine membrane rupture.[11] In cases which diagnosis of membrane rupture is un-debatable, role of other methods such as Amnisure® seemed questionable. However, rarely, we will find conditions such as long standing membrane rupture, un-

known causes of oligohydramnios or ROM too severe to cause any fluid to leak again from uterine cavity. Presence of PAMG-1 in these cases will resolve the issue of whether ROM occurred or not. Indeed, in our study, only one patient had negative vaginal pooling and nitrazine test but positive Amnisure® test. The patient admitted having sensation of fluid leaking out and had severe oligohydramnios during ultrasound exam. The baby was delivered healthy, with nil amniotic fluid. Presence of infection was proven with increased leucocyte esterase level from cervicovaginal secretion.

CONCLUSION

To our knowledge, this is the first study to evaluate the usage of PAMG-1 identification for diagnosis of ROM in Indonesia. Given the natural burden of ROM, especially premature ROM (PROM) and preterm PROM (PPROM), a standardized method with excellent sensitivity and specificity is needed for us to firmly diagnose membrane rupture. In obvious cases, Amnisure® test seemed less likely to be needed. However, in some cases, presence of PAMG-1 may be of great aid in determining diagnosis. Support for evidence of micro-perforation in a seemingly intact membrane with positive Amnisure® test needs further research and evaluations. More study is necessary before we can apply this method in Indonesia, especially when dealing with cost effectiveness analysis.

REFERENCES

1. American College of Obstetricians and Gynecologists Committee on Practice Bulletins-Obstetrics. ACOG practice bulletin no. 80: premature rupture of membranes; clinical management guidelines for obstetrician-gynecologists. Obstet Gynecol 2007; 109:1007-19.
2. Raju TNK, Higgins RD, Stark AR, Leveno KL. Optimizing care and outcome for late preterm (near term) infants: a summary of the workshop sponsored by the National Institute of Child Health and Human Development. Pediatrics. 118(3):1207-14
3. Teune MJ, Bakhuizen S, Bannerman CG, Opmeer BC, van Kaam AH, van Wassenaer AG, Morris JM, Mol BMJ. A systematic review of severe morbidity in infants born late preterm. Am J Obstet Gynecol 2011. 05:374.e1-e9.
4. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY (ed). Preterm birth. In: Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY (ed). William's Obstetrics 23rd edition. USA: The McGraw Hill Companies inc. 2010.
5. Grisaru-Granovsky S, Reichman B, Lerner-Geva L. Mortality and morbidity in preterm small-for-gestational age infants: a population based study. Am J Obstet Gynecol 2011. 205. xe-x.ex.
6. Gomez R, Romero R, Mazor M, Ghezzi F, David C, Yoon BH. The role of infection in preterm labour and delivery. In: Elder MG, Romero R, Lamont RF, eds. Preterm labour. Edinburgh: Churchill and Livingstone 1997: 85-126.
7. Hannah ME, Hodnett ED, Willan A, Foster GA, Di Cecco R, Helewa M. Prelabour rupture of the membranes at term: expectant management at home or in hospital. Obstet Gynecol 2000. 96(4):533-8.
8. Kiefer DG, Keeler SM, Rust O. Amniotic fluid inflammatory score is associated with pregnancy outcome in patients with mid trimester short cervix. Am J Obstet Gynecol 2012. 206:68. e1-6.
9. Conde-Agudelo A, Romero R. Fetal fibronectin as a predictor of spontaneous preterm delivery in multiple gestations: a systematic review and meta-analysis. Am J Obstet Gynecol 2009. S196.
10. Lee S, Buhimschi I, Zhao G, Dulay A, Bahtiyar M, Han C, Buhimschi C. Interleukin-6 trans-signaling molecules soluble GP130 and soluble IL-6 receptor in the amniotic fluid (AF) of women with intraamniotic infection (IAI) and preterm premature rupture of membranes (PPROM). Am J Obstet Gynecol 2009. S196.
11. Royal College of Obstetricians And Gynecologists. Guideline no 44. Preterm prelabour rupture of membrane. November 2006
12. Mercer BM. Chapter 27: premature rupture of the membrane. In: Gabbe SG, Niebyl JR, Simpson JL (ed). Gabbe: Obstetrics Normal and problem pregnancies 5th edition. USA: Churchill Livingstone.
13. Cousins LM, Smok DP, Lovett SM, Poetler DM. Amnisure Placental Alpha Microglobulin-1 rapid immunoassay versus standard diagnostic methods for detection of rupture of membranes. Am J Perinat 2005. 22. 1-4
14. Mittal P, Romero R, Soto E, Cordoba M, Nhan-Chang CL, Vaisbuch E, Bleda J, Chaiworapongsa T, Kusanovic JP, Yeo L, Yoon BY, Hassan SS. A role of placental alpha-microglobulin-1 in the identification of women with a sonographic short cervix at risk for spontaneous rupture of membranes. Am J Obstet Gynecol 2009. Supplement. S196-7.
15. Lee SM, Lee JH, Seong HS, Lee SU, Park JS, Romero R, Yoon BY. The clinical significance of a positive amnisure test in women with term labor with intact membranes. J Matern Fetal Neonatal Med 2009. 22(4):305-10.
16. Lee SU, Park JS, Norwitz E, Park CW, Jun JK, Syn HC. The Amnisure ROM test is superior to conventional clinical assesstment in the diagnosis of rupture of membranes. Am J Obstet Gynecol 2006. SMFM Abstracts. S62.