Research Article

Leukocyte Esterase Activity (LEA) for Identifying Chorioamnionitis Cases

Leukocyte Esterase Activity (LEA) untuk Mengidentifikasi Kasus Korioamnionitis

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Abstract

Objective: To compare sensitivity and specificity of LEA to histopathology examination in diagnosing chorioamnionitis.

Methods : We compared diagnostic tests in dr. Mohammad Hoesin hospital, Palembang, from September 2015 to April 2016. Ninety-one pregnant women were included. LEA and histopathology examination were carried out with neonatal sepsis as main outcome. Data were analysed by SPSS version 21.0 and Med-calc statistic.

Results: Chorioamnionitis was detected in 54 (77.1%) patients with gestational period ≥37 weeks and in 16 (22.9%) patients with gestational period <37 weeks. Duration of membrane rupture was significantly associated with chorioamnionitis (p = 0.001 and p = 0.011). Neonatal sepsis was also significantly associated with chorioamnionitis in both groups (p = 0.014 and p = 0.036). LEA value with cut-off point >0.5 was able to significantly predict chorioamnionitis with 98.6% sensitivity and 95.2% specificity, providing better accuracy in diagnosing chorioamnionitis in preterm pregnancy group.

Conclusions: LEA had a very good predictive value for chorioamnionitis with better accuracy in diagnosing chorioamnionitis in preterm pregnancy.

Keywords: chorioamnionitis, histopathology, leukocyte esterase activity, neonatal sepsis, salafia criteria.

Abstrak

Tujuan: *Membandingkan sensitifitas dan spesifisitas diagnosis korioamnionitis antara pemeriksaan Leukocyte esterase activity (LEA) terhadap histopatologi.*

Metode: *Penelitian uji diagnostik dilakukan di RSUP dr. Mohammad Hoesin Palembang selama periode September 2015 – April 2016, 91 perempuan hamil yang memenuhi kriteria inklusi selanjutnya dilakukan pemeriksaan LEA, Histopatologi dan luaran sepsis neonatorum. Data kemudian dianalisis dengan menggunakan software SPSS versi 21.0 dan Med-calc statistic.*

Hasil: *Korioamnionitis terdeteksi pada 54 (77,1%) pasien dengan usia gestasi ≥37 minggu dan 16 (22,9%) pada usia gestasi <37 minggu. Durasi lamanya pecah ketuban akan berpengaruh secara signifikan terhadap kejadian korioamnionitis (p = 0,001 dan p = 0,011), Sepsis neonatorum juga secara signifikan berhubungan dengan kejadian korioamnionitis pada kedua kelompok (p = 0,014 dan p = 0,036). Kadar LEA dengan cut off point >0,5 secara signifikan mampu memprediksi kejadian korioamnionitis dengan sensitivitas 98,6%, spesifisitas 95,2% dan nilai akurasi yang lebih baik ditemukan dalam penegakan diagnosis korioamnionitis pada kelompok kehamilan preterm.*

Kesimpulan: *Pemeriksaan LEA memiliki kekuatan prediksi yang sangat baik terhadap kejadian korioamnionitis dengan akurasi yang lebih baik dalam mendiagnosis korioamnionitis pada kehamilan preterm.*

Kata kunci : *korioamnionitis, histopatologi, leukocyte esterase activity, sepsis neonatorum, kriteria salafia*

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INTRODUCTION

Chorioamnionitis or infection of foetal membranes is defined as inflammation or infection in placenta, amnion and/or chorion.[1] About 1-4% delivery in United States is complicated by chorioamnionitis. Chorioamnionitis is found as complication in 40-70% preterm delivery with preterm premature rupture of membrane and is found in 1-13% term delivery.[2,3]

Risk factors of chorioamnionitis are young age, long duration of preterm rupture of membrane, null parity, low socioeconomic status, multiple vaginal examinations, prolonged internal foetal monitoring, bacterial vaginosis, Streptococcus group B colonisation, and meconium aspiration. Chorioamnionitis diagnosis is established by the use of clinical, laboratory, and histopathologic criteria. Clinically and using laboratory numbers, chorioamnionitis symptoms and signs are maternal fever reaching more than 38˚C or 100.4˚F, and one of these criteria: maternal leucocytosis (leukocyte >15,000/mm^3), maternal tachycardia (>100/minute), persistent foetal tachycardia (>160/minute), abdominal pain, atonic uterus, and cloudy and purulent amniotic fluid with foul odour.[1,2,4]

Chorioamnionitis may give rise to bad consequences to mother and foetus. Maternal complications of chorioamnionitis are heightened delivery risk with C-section, infected delivery wound, pelvic abscess, bacteraemia, and postpartum bleeding. About 10% of mother with chorioamnionitis and positive blood culture is related to Streptococcus Group B and E. coli infection. Though late complication from this bacteraemia is rarely found. Different from maternal complications, chorioamnionitis foetal complications may end fatally and include meningitis (3%), necrotizing enterocolitis[4-6] pneumonia (10-21%), sepsis (7-28%), intracranial haemorrhage (22-24%), and respiratory distress syndrome (62-63%) with mortality reaching about 25%. Long-term complication in neurologic development, such as cerebral palsy, may also be related to chorioamnionitis.[1,5,7,8]

One of the challenges in diagnosing chorioamnionitis is the lack of ideal biomarker to help establishing the diagnosis. Early detection of chorioamnionitis in patients with premature rupture of membrane or preterm premature rupture of membrane will be very helpful in patient management to decrease perinatal morbidity and mortality.

Leukocyte esterase activity (LEA) examination is one of the available dipstick test used in diagnosing urinary tract infection (UTI).[9,10] Now, LEA is also used to diagnose lower genital tract infection. Leukocyte esterase is released by polymorphonuclear cells as a response to infection, this enzyme then reacts to components in dipstick which will hydrolise indoxyl carboxylic acid ester into indoxyl which further reacts with diazonium salt, producing purple on the dipstick.[10,11]

Hoskins IA et al. in their study regarding LEA in early detection of chorioamnionitis found that LEA, as a predictor of chorioamnionitis, had sensitivity, specificity, positive predictive value, and negative predictive value of 91, 95, 95, and 91%, respectively.[9-11]

Histopathologic chorioamnionitis diagnosis is focused on identification of neutrophils in the membrane this finding is still considered the gold standard in establishing chorioamnionitis diagnosis.[12,13] All of these provoke us to compare LEA with histopathology examination in chorioamnionitis and the outcome of perinatal infection.

METHODS

This study was a comparative study which involves 91 pregnant women consecutively with singleton, live pregnancy ≥22 weeks, all kind of parity, and not in labour or in latent phase of delivery (cervical dilatation when admitted <4 cm). These women consented to be recruited into our study by signing written informed consent. We conducted this study in Obstetrics and Gynecology Department of Dr. Moh. Hoesin General Hospital, Palembang, from September 2015 to April 2016.

Women with singleton, live pregnancy ≥22 weeks, premature rupture of membrane, not in labour or in latent phase of delivery (cervical dilatation when admitted <4 cm), all parity, and suspected chorioamnionitis who consented to be recruited into our study by signing a written

informed consent were included.

Exclusion criteria for our study were twin pregnancy, hypertension in pregnancy, infectious diseases due to systemic disorder, vaginal bleeding excluding bloody show, foetal distress, dead foetus, and history of amniocentesis or chorionic villus sampling.

Basic data were collected from all patients fulfilling inclusion criteria. These data included identity, gestational period, parity, education level, occupation, smoking record, physical examination results, obstetrics examination results, nitrazin test result, and Leukocyte Esterase Activity (LEA) examination results, and record of delivery. After delivery, neonatal examination was carried out, starting from identity, gender, body weight, body length, APGAR score, and physical and laboratory examination to identify neonatal sepsis. Placental histopathology examination was also carried out which included maternal chorion, amnion membrane, umbilical cord, and foetal chorion to determine chorioamnionitis histologically according to Salafia criteria.

Data were analysed using SPSS version 21.0. Descriptive data were analysed using the Pearson Chi-square test, Fisher exact test, and Chi-square test. Cut off point of LEA was displayed using Receiving operating characteristic (ROC) curve. Sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio were calculated using Med-calc statistics.

RESULTS

Demographic characteristics of study samples are illustrated in Table 1. The majority of the subjects in both groups were in the age group of 20-35 years (81.4% and 85.7%) which was an ideal reproductive age range; the majority of women were having term pregnancy (77.1% and 85.7%); most of the women were primigravid (52.9% and 52.4%); most of the subjects were private sector employees (94.3% and 85.7%); most of the subjects were high school educated (54.3% and 61.9%); and there was no subject with smoking habit. Using chi-square analysis, there was no significant difference between the two groups in all these variables. This showed that chorioamnionitis in pregnant women with premature rupture of the membrane was not influenced by demographic characteristics in both groups.

Table 1. Demographic Characteristics of Subjects

Characteristics	Chorioamnionitis (+)		Chorioamnionitis (-)		
	n	%	n	%	p value
Maternal age (years)					
<20	2	2.9	1	4.8	0.724***
20-35	57	81.4	18	85.7	
>35	11	15.7	2	9.5	
Gestational age (weeks)					
<37 weeks	16	22.9	3	14.3	0.545**
≥ 37 weeks	54	77.1	18	85.7	
Parity					
Nulliparity	37	52.9	11	52.4	0.582*
Multiparity	33	47.1	10	47.6	
Occupation					
Private sectors	66	94.3	18	85.7	0.346**
Civil servant	4	5.7	3	14.3	
Education					
Primary school	6	8.6	0	0	1***
Junior high school	21	30	3	14.3	
Senior high school	38	54.3	13	61.9	
Diploma	1	1.4	4	19	
Bachelor	4	5.7	1	4.8	
Smoking habit					
Yes	0	0	0	0	1***
No	70	100	21	100	

** Chisquare test, P = 0.05, ** Fischer exact test, P = 0.05, *** Pearson Chisquare, P = 0.05*

Influence of length of the period of preterm rupture of the membrane to chorioamnionitis

Table 2 showed that in both groups of preterm rupture of membrane (term and preterm pregnancy), length of the period of preterm rupture of membrane significantly (p = 0.001 and p = 0.011) influence chorioamnionitis, whereas longer period of premature rupture of membrane significantly induces more case of chorioamnionitis.

Table 2. Comparison between Duration of Amniotic Rupture and Chorioamnionitis Insidence in Aterm and Preterm Pregnancy

Chorioamnionitis	PROM Duration (Hours)	PPROM Duration (Hours)	*p-value*
(+)	29.8333 + 54.14821	15.3125 + 19.1406	0.103
(-)	3.3333 + 1.90973	1.3333 + 0.57735	0.004
P	0.001	0.011	

Independent t-test, 95% CI

Table 2 also showed that in those having chorioamnionitis, premature rupture of the membrane in the term pregnancy group had longer mean compared to preterm pregnancy group (29.8 hours vs 15.3 hours), showing that preterm premature rupture of the membrane may faster develop complication – i.e. chorioamnionitis – compared to premature rupture of the membrane in term pregnancy.

Association between chorioamnionitis and neonatal outcome

Most neonates from mother with term pregnancy, preterm rupture of membrane, and chorioamnionitis (57.4%) had developed sepsis meanwhile most neonates from mother with term pregnancy, preterm rupture of membrane, and no chorioamnionitis (77.8%) had not developed sepsis. Statistically, there was significant association between neonatal sepsis and maternal chorioamnionitis, with odds ratio (OR) 4.717 (1.372-16.223) which illustrated that mother with term pregnancy with preterm rupture of membrane and chorioamnionitis had 4.717 times the risk for her neonate to be diagnosed with sepsis compared to mother with term pregnancy with preterm rupture of membrane but no chorioamnionitis. Similar to term pregnancy with premature rupture of membrane, all (100%) mothers with preterm pregnancy and premature rupture of membrane without chorioamnionitis had no neonates with sepsis. Statistically, there was a significant association between chorioamnionitis in mothers with preterm premature rupture of membrane and neonatal sepsis.

Cut off point of LEA examination for chorioamnionitis diagnosis

LEA cut off point as high as 0.5 with 98.6% sensitivity and 95.2% specificity, significantly predicted chorioamnionitis diagnosis (p <0.001) with very high predictive value (AUC 0.985).

Table 3. Comparison of LEA Test to Histopathology Examination in Diagnosing Chorioamnionitis in Term and Preterm Pregnancy

Chorioamnionitis	Sensitivity (%)	Spesificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Term pregnancy					
Maternal chorion	77.6	60	96.26	16.67	76.38
Amniotic fluid	78.12	50	92.6	22.22	75
Umbilical cord	76.19	33,33	88.89	16.67	70.83
Fetal chorion	75	75	83.33	16.67	66.67
Preterm pregnancy					
Maternal chorion	84.2	100	100	0	84.2
Amniotic fluid	88.89	100	100	33.3	89.47
Umbilical cord	83.33	0	93.75	0	78.95
Fetal chorion	84.21	100	100	0	84.21

Comparison of LEA test to histopathology examination in diagnosing chorioamnionitis

Table 3 demonstrated diagnostic value of LEA in establishing chorioamnionitis in both groups. Accuracy rate of LEA was found better in diagnosing chorioamnionitis in preterm pregnancy group.

DISCUSSION

Chorioamnionitis is still being a serious infection problem which will show maternal and foetal impacts. Chorioamnionitis will induce preterm labour which in turn will produce neonate in high-risk of sepsis or even perinatal death. Problems regarding chorioamnionitis do not only involve complications arising from chorioamnionitis but also involve increased ability in early detection of chorioamnionitis using cheap, reliable, non-invasive tools.

Our study illustrated that chorioamnionitis in pregnant women with preterm rupture of membrane was not influenced by demographic characteristics. Nordenvall et al. also found similar results in which there was no difference in age, gestational age, parity, and smoking habit which may influence chorioamnionitis. In their study, Nordenvall et al. found mean subject age to be 29 years, gestational age to be 39 weeks, and most were primigravid. Erdemir et al. also found similar things in their study where chorioamnionitis was not influenced by maternal age – in which mean maternal age was found to be 30.1±6.9 years.[14]In their study, Erdemir et al. showed that there was no association between chorioamnionitis and maternal socioeconomic status – there was similar percentage of mothers with good socioeconomic status without chorioamnionitis (66.7%) and those with poor socioeconomic status without chorioamnionitis (33,3%).[15]

Different findings were discovered in a study by Chan et al. in Bangladesh in which it was found that chorioamnionitis was affected by maternal occupation – in this study, subjects working as construction workers and janitors were 1.69 times more likely to have chorioamnionitis.[16]

In our study, demographics revealed that there were 54 subjects (77.1%) with term pregnancy and 16 subjects (22.9%) with preterm pregnancy in whom chorioamnionitis was diagnosed. In contrast with our study, Erdemir et al. found that chorioamnionitis were more likely to be encountered in preterm pregnancy with mean gestational age 27.5±2.5 weeks ($p < 0.01$).

Chorioamnionitis was histopathologically defined in our study as having four positive findings in placental samples according to Salafia criteria. We found that duration of premature rupture of membrane significantly influenced chorioamnionitis diagnosis, in other words, prolonged period of premature rupture of membrane significantly associated with more chorioamnionitis cases. Our study also illustrated that preterm premature rupture of membrane were sooner complicated by chorioamnionitis than premature rupture of membrane in term pregnancy. Similar finding also reported by Popowski et al. in 2011, they showed that premature rupture of membrane in pregnancy <37 weeks were having 1.1 times larger risk to develop chorioamnionitis proven histologically than in pregnancy ≥37 weeks with mean period of rupture of membrane 25 hours since admission to delivery.[17]

Findings regarding latency of rupture of membrane (interval between rupture of membrane to delivery) were discovered by Patil et al. in their study about maternal and foetal outcome in preterm premature rupture of membrane. Patil et al. found that amniotic membranes in preterm pregnancy were more likely to have pathologic defects which would cause higher pressure compared to in term pregnancy – this would indirectly associated with latency period. Patil et al. further stated that there was a inverse correlation between gestational age and duration of latent period, i.e. in those with younger gestational age, latent period would be longer, hence, chorioamnionitis incidence would be higher in preterm pregnancy compared to in term pregnancy.[18]

Though hypothesis stating that chorioamnionitis in term pregnancy with premature rupture of membrane required longer duration of rupture compared to preterm premature rupture of membrane was a coincidence. This was supported statistically by insignifcant p found (p = 0.013). Calculation of duration of rupture of membrane in preterm pregnancy which was started from failed conservative management would also influence our results. We recommended larger sample size in future studies to address high variability in term pregnancy with premature rupture of membrane to produce significant finding.

In our study, there was significant association between chorioamnionitis and neonatal sepsis in both term and preterm pregnancy groups. Statistically, there were 31 subjects (57.4%) with term pregnancy and 12 subjects (75%) with preterm pregnancy developing neonatal sepsis.

A study by Rodrigo et al. in 2013 illustrated a significant association between early onset neonatal sepsis and maternal chorioamnionitis (adjusted relative risk = 6.13; 95% confidence interval = 1.67-2.58; p = 0.006) and periventricular leukomalacia and maternal chorioamnionitis (adjusted relative risk = 24.62; 95% CI = 1.87-324.28; p = 0.015).[19] Similar findings in a previous study, i.e. significant association between chorioamnionitis and high incidence of premature rupture of membrane, sepsis, bronchopulmonary dysplasia, and longer mechanical ventilator use were found by Mu et al. in 2008.[20]

Accuracy rate of LEA was found better in diagnosing chorioamnionitis in preterm pregnancy group. Difference in accuracy rate in the two groups in establishing chorioamnionitis by using these two instruments may be caused by several factors. Small sample size and possibility of other primary infection could not be controlled by us. Though in our study, we conducted a robust patient screening. We also performed history taking, physical examination, obstetrics examination, and laboratory examination to exclude potential primary infection which may induce bias in our study.

CONCLUSION

There was a significant association between duration of rupture of membrane, neonatal sepsis, and chorioamnionitis – i.e. preterm premature rupture of membrane sooner developed complications compared to premature rupture of membrane in term pregnancy. LEA examination had a very encouraging predictive value in chorioamnionitis cases with better accuracy rate found in establishing chorioamnionitis in preterm pregnancy group.

RECOMMENDATION

Further study regarding accuracy of LEA examination with larger sample size and improved effort in controlling other primary infections is needed to reach a more refined result.

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