Research Article

Vaginal Microorganism Pattern in Premature Rupture of Membrane

Pola Mikroorganisme Vagina pada Ketuban Pecah Dini

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Abstract

Objective: To determine the pattern of vaginal microorganisms in pregnant women and risk factors for premature rupture of membranes (PROM) in Manado.

Methods: This is a case-control study. Samples was 40, divided into 20 pregnant with PROM and 20 control samples. Samples were taken from Kandou Manado Hospital and network hospitals within the inclusion criteria. Samples were taken from vaginal swab for microorganism culture.

Result: The highest age distribution in the case of PROM was in the reproductive age group of 20-35 years, 19 people (95%, p = 1,000). Primiparas were dominant in the PROM were 11 people (55%, p = 0.204). The distribution of BMI mostly from the overweight and obesity groups of 11 people (55%, p = 0.527). In the PROM, 19 people (95%, p = 0.605) did not smoke. *Escherichia coli* was the most microorganisms in swab vaginal of PROM: 7 people (29.17%).

Conclusions: The most microorganisms found in PROM are *Escherichia coli, Enterobacter cloacae* and Staphyloccocus aureus and in non-PROM are Klebsiella oxytoca, Staphylococcus epidermidis, Staphylococcus saprophyticus and Pseudomonas fluorescens. There was no relationship between BMI, age, parity, smoking, and gestational age with the incidence of PROM in this study.

Keywords: PROM , vaginal microorganisms pattern, vaginal swab.

Abstrak

Tujuan: Untuk mengetahui pola mikroorganisme vagina pada kultur vaginal swab wanita hamil dan faktor risiko pada KPD di Kota Manado.

Metode: Studi ini bersifat case control. Total sampling 40 sampel dibagi menjadi 20 sampel hamil dengan KPD dan 20 kontrol. Sampel diambil dari RSUP Kandou Manado dan RS jejaring yang memenuhi kriteria inklusi. Semua pasien dilakukan pengambilan sampel swab vagina untuk dilakukan kultur mikroorganisme.

Hasil: Hasil sebaran usia pada KPD paling banyak pada usia reproduksi 20-35 tahun yaitu 19 orang (95%, p = 1,000). Primipara dominan pada KPD yaitu 11 orang (55%, p = 0,204). Hasil sebaran IMT paling banyak kelompok overweight dan obesitas yaitu 11 orang (55%, p = 0,527). Pada KPD sebanyak 19 orang (95%, p = 0,605) tidak merokok. Mikroorganisme terbanyak pada swab vagina KPD adalah Escherichia coli sebanyak 7 orang (29,17%).

Kesimpulan: Mikroorganisme terbanyak pada KPD adalah Escherichia coli, Enterobacter cloacae dan Staphyloccocus aureus dan pada non KPD adalah Klebsiella oxytoca, Staphylococcus epidermidis, Staphylococcus saprophyticus dan Pseudomonas fluorescens. Tidak ada hubungan antara IMT, usia, paritas, merokok, dan usia kehamilan dengan kejadian KPD pada penelitian ini.

Kata kunci: hMG, infertilitas, fertilisasi in vitro, rFSH, stimulasi ovarium.

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INTRODUCTION

Maternal Mortality Rate in 2012 is 359 per 100,000 live births.[1] The causes of maternal death are bleeding 60-70%, pre-eclampsia and eclampsia 10-20%, and infections 10-20%. 23% infection in pregnancy can be caused by premature rupture of membranes.[2] Premature rupture of membranes (PROM) can cause maternal complications such as chorioamnionitis, sepsis, placental abruption and endometritis. Genital infection of mother can occur in the event of PROM due to rupture of the membranes will make it easy for bacteria to enter the uterus and multiplied.[3]

The cause of PROM is multifactorial. Risk factors include depending on the socioeconomic, previous history of PROM, smoking and infection.[4] Intrauterine infection is the most common cause of complications in pregnancy, such as PROM. Microorganisms can enter the amniotic fluid or the fetus through several channels, such as ascending from the vagina and cervix, through hematogenous from the placenta (transplacental infection), retrograde from the abdominal cavity through the fallopian tube and from invasive procedures such as amniocentesis.[5]

This study aimed to determine the pattern of vaginal microorganisms in pregnant women and risk factors for PROM in Manado.

METHODS

This is a case-control study conducted in the Obstetrics and Gynecology Department, Faculty of Medicine, Sam Ratulangi University/Prof. Dr. R.D. Kandou General Hospital, Manado. The study was conducted from June 2019 to September 2019.

The study population was pregnant women who came to the Obstetric clinic for ANC or Emergency room Prof. Dr. R.D. Kandou General Hospital, Manado and Pancaran Kasih Hospital Manado. The study sample was pregnant women who came to the emergency room at the Department of Obstetrics and Gynecology, Prof. dr. R. D. Kandou Manado and Pancaran Kasih Hospital Manado within the inclusion criteria and sign the informed consent.

The inclusion criteria were all pregnant women with main complain there was amniotic discharge from the birth canal <24 hours with evidenced by a positive nitrazin test, and women without amniotic discharge from the birth canal as a

control who is willing to participate in the study and signed the informed consent.

The exclusion criteria were pregnant women with obstetric complications, such as antepartum bleeding, decreased consciousness, pregnant women with medical complications, such as heart disease, diabetes mellitus, HIV, malignancy, in treatment using antimicrobials, immunosuppressants, using vaginal wash fluids in the past 2 weeks, have sex in the past 24 hours and who were not willing to participate in the study.

The patient was taken for a vaginal swab sample. Culture was carried out with agar media, continue with identification test. Samples were carried out at Prodia Manado Laboratory. The results called positive if after bacterial breeding the growth of microorganism colonies is found. Data is collected and processed using the statistical data processing software program SPSS version 23.0.

RESULTS

In this study, the most age distribution results were in the reproductive age group of 20-35 years, which were 19 people (95%) in the case of PROM and 19 people (95%) in the case of non- PROM. Chi-square test ($\chi2$) shows that $\chi2$ = 0,000 with p = 1,000 which means there is no relationship between maternal age and the incidence of PROM. The results of the distribution of marital status found that the average patient was married with 18 people (90%) in the PROM group and 19 people (95%) in the non-PROM.

Table 1. Characteristics of Subject

Characteristic	PROM		Non PROM		
	N	%	N	%	*P-value*
Age (years)					1.000
High risk (< 20 and > 35)	1	5	1	5	
Normal (20-35)	19	95	19	95	
Marital status					1.000
Marry	18	90	19	95	
Single	2	10	1	5	
Parity					0.204
Primipara	11	55	7	35	
Multipara	9	45	13	65	
Level of education					0.661
Primary school	0	0	1	5	
Secondary school	2	10	7	35	
High school	14	70	10	50	
University	4	20	2	10	
Occupation					0.288
Housewife	13	65	16	80	
Working	7	35	4	20	

Gestational age (weeks)					0.752
< 37	9	45	10	50	
≥ 37	11	55	10	50	
Body Mass Index					0.527
Normoweight	9	45	11	55	
Overweight/Obesity	11	55	9	45	
Smoking history					0.605
Yes	1	5	3	15	
No	19	95	17	85	
UTI history					1.000
Yes	3	15	2	10	
No	17	85	18	90	
Duration of rupture of membranes (hours)					
≤ 12	12	60	0	0	
>12	8	40	0	0	

Fisher's Exact test shows p = 1,000 which means there is no relationship between marital status and the incidence of PROM.

The results of the distribution of parity in which primipara is more dominant in the PROM are 11 people (55%) and multipara in 13 people (65%). Chi-square test ($\chi2$) shows that $\chi2$ = 1.616 with p = 0.204 which means there is no relationship between parity and the incidence of PROM. The most education distribution results were in the high school group. In the PROM group was 14 people (70%) and in the non-PROM group was 10 people (50%). Fisher's Exact test shows p = 0.661 which means there is no relationship between education status with the incidence of PROM.

The most occupation distribution both groups of PROM and non-PROM are mostly on the housewife group. Chi-square test ($\chi2$) shows that $\chi2$ = 1.129 with p = 0.288 which means there is no relationship between occupation and the incidence of PROM. Education, socioeconomic and poor hygiene, cultural factors, limited health facilities, unhealthy eating habits affect the increased prevalence of infections in the vagina that cause PROM.[6]

The most common distribution of gestational age is in the gestational group ≥37 weeks. Chi-square test ($\chi2$) shows that $\chi2$ = 0.100 with p = 0.752 which means there is no relationship between gestational age and the incidence of PROM. The results of the distribution of body mass index in PROM group mostly came from the overweight / obesity group of 11 people (55%). In the non-PROM group, most came from the normoweight group, with 11 people (55%). Chi-square test ($\chi2$) shows that $\chi2$ = 0.400 with p = 0.527 which means there is no relationship between BMI and the incidence of PROM.

Most samples does not have a history of smoking. 19 people (95%) did not smoke in

PROM group and 17 people (85%) did not smoke in non PROM. Fisher's Exact test showed p = 0.605 which means there is no relationship between smoking status and the incidence of PROM. There is 17 people (85%) in PROM group and 18 people (90%) in non-PROM do not have a history of urinary tract infections. Fisher's Exact test shows p = 1.000 which means there is no relationship between the history of UTI with the incidence of PROM.

Table 2. Characteristics of Culture Results Found in Vaginal Swabs

Microorganism	PROM		Non PROM	
	N	%	N	%
Escherichia coli	7	29.17		
Enterobacter cloacae	4	16.67		
Staphylococcus epidermidis	4	16.67	3	21.43
Klebsiella pneumoniae	2	8.33	1	7.14
Staphylococcus aureus	2	8,33	1	7.14
Staphylococcus saprophyticus	1	4.17	2	14.29
Raoultella ornithinolytica	1	4.17		
Enterobacter aerogenes	1	4.17		
Serratia odorifera	1	4.17		
Sternotrophomonas maltophilia	1	4.17		
Klebsiella oxytoca			3	21.43
Pseudomonas fluorescens			2	14.29
Pseudomonas oryzihabitans			1	7.14
Kluyvera spp			1	7.14
Total	24	100	14	100

In Table 2, 14 variants of microorganisms were found in vaginal swabs of pregnant patients with PROM and non-PROM from a total of 40 patients, namely gram-positive bacteria (Staphylococcus sarophyticus, Staphylococcus aureus, and Staphylococcus epidermidis), gram-negative bacteria (Enterobacter cloacae, Enterobacter aerogenes, Raoultella ausus, Raoultella ausus ornithinolytica, Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Serratia odorifera, Kluyvera spp, Pseudomonas fluorescens, Pseudomonas oryzihabitans and Sternotrophomonas maltophilia). Most microorganisms found in vaginal swabs of pregnant patients with PROM were Escherichia coli (29.17%), as much 7 patients.

Table 3. Comparison of the Microorganisms with Duration of Rupture of Membranes

Microorganism	Duration of Rupture of Membranes			
	≤ 12 hours	%	>12 hours	%
Escherichia coli	6	40.00	1	11.11
Enterobacter cloacae	2	13.33	2	22.22
Staphylococcus aureus	2	13.33		
Raoultella ornithinolytica	1	6.67		
Staphylococcus epidermidis	1	6.67	3	33.33
Staphylococcus saprophyticus	1	6.67		
Enterobacter aerogenes	1	6.67		
Sternotrophomonas maltophilia	1	6.67		
Klebsiella pneumoniae			2	22.22
Serratia odorifera			1	11.11
Total	15	100	9	100

In table 3 we found that results comparison of the distribution of types of microorganisms in the PROM group based on the duration of rupture of membranes is divided into ≤12 hours and> 12 hours. The highest distribution in ≤12 hours came from Escherichia coli, 6 people (40%) followed by Enterobacter cloacae as many as 2 people (33.33%) and Staphylococcus aureus as many as 2 people (33.33%). While the distribution of microorganisms in PROM > 12 hours at most, namely Staphylococcus epidermidis 3 people (33.33%), Enterobacter cloacae 2 people (22.22%) and Klebsiella pneumoniae 2 people (22.22%).

The most number of Escherichia coli microorganisms were in PROM ≤ 12 hours as much as 6 cases (50%). Chi-square test ($\chi2$) shows that $\chi2$ = 2.967 with p = 0.849 which means there is no relationship between the type of germ and rupture of membranes.[7]

Gram-negative bacteria (Escherichia coli and Enterobacteriaceae) are the most frequent organisms in the recto-vaginal area of the mother. Two studies conducted in the United States showed that Escherichia coli was associated with premature birth, especially giving birth before 34 weeks' gestation. Urinary tract infections by Escherichia coli have also been linked to preterm birth.[8]

DISCUSSION

In this study, the most common age distribution results were in the reproductive age group of 20-35 years. According to Surekha S. Mohan et al (2017), in a study of 358 pregnant women with PROM in India, young adults aged 20-30 years (50.1%) experienced the most PROM in pregnancy.[4] This is consistent with the results of the study this. Similarly, in Iran of 200 pregnant women with PROM, found infections occur on average at age 27.6 ± 2.3 years, 3 and in Uganda of 87 pregnant women with PROM, was found most at the age of 20-34.99 years (84%).[9] Distribution of parity showed that primipara was more dominant in the PROM, this is in accordance in India of 120 pregnant women with PROM, the highest number was found in primigravida in 86 cases (71.16%).[10]

The results of the distribution of education in general are mostly in the middle school group. This is in accordance with research in India in 50 pregnant women with PROM obtained the most recent education on the matric which is a high school level of 20 people (40%). This is because Indonesia and India have the same socioeconomic level. [11]

Education, socioeconomic and lack of hygiene, cultural factors, limited health facilities, unhealthy eating habits also play a role for the increased prevalence of infections in the vagina that can cause PROM.[6]

The distribution of gestational age at the time of rupture of membranes occur mostly from the pregnancy group ≥ 37 weeks. Research conducted in China comparing 577 (80.8%) women with term PROM and 137 (19.2%) women with preterm PROM stated that there were significant differences in intrauterine infection. Intrauterine infection is a mechanism that often causes premature birth. The mechanism by which intrauterine infection causes preterm labor is related to the activation of the immune system, which reflects four main pathogenogenic processes, activation of the hypothalamic-pituitary-adrenal axis in the mother and fetus, decidualechorioamniotic or systemic inflammation, decidual bleeding, and pathological distension of the uterus.[12]

In obesity hormonal imbalance can occur, so that it can cause a shift in the balance of the normal vaginal flora to pathogenic flora. Also in obesity there is an increase in vaginal moisture so that pathogenic bacteria that cause infections easily develop so that it facilitates spontaneous rupture of membranes. Six hundred and thirty three obese pregnant women with PROM found to experience chorioamnitis more frequently than non-obese patients (p <0.01).[13]

The results of the distribution of smoking history of patients - the average patient does not have a history of smoking. This is consistent with the multicenter study conducted by Robert L. Andres et al (2014) in Utah, United States of

America saying that there is no relationship between smoking and the incidence rate of PROM.[14]

In this study, it was found that the most microorganisms found in vaginal swabs of pregnant patients with PROM were Escherichia coli. Research in Nigeria on vaginal swabs of pregnant women with PROM found that most bacteria were Klebsiella (32.1%) followed by Escherichia coli (19.6%).[15] Research in Iran found the most bacteria in endocervical swab culture were Escherichia coli (24.2%), Staphylococcus coagulase negative (27.2%), Enterococcus and candida (11.7%) [3] and research in India, found the most bacteria were E. E. coli followed by Staphylococcus aureus and 1 (2%) growing Candida species.[11]

Hormonal changes are a trigger for an increase in pH due to changes in the amount and thickness of vaginal secretions during pregnancy. Rapid pH changes, causing the vaginal acid-base balance to be disrupted and the rapid growth of normal and anaerobic microorganisms, resulting in an increase in phagocytic processes and the results of metabolism of microorganisms that can change vaginal albus fluorine found to be alkaline and trigger normal vaginal flora into parasites for cervical mucosa and vagina. This situation will change the quality of fluor albus and can be categorized as pathological fluor albus. Genital tract infection is one of the causes of PROM. One possible mechanism is ascending infection and replicating in the placenta, decidua and membrane.[7] Of the vaginal swabs conducted in this study were found to be entirely normal vaginal flora. Most microorganisms found were Escherichia coli, Enterobacter cloacae and Staphylococcus epidermidis. Where Escherichia coli is a gram-negative microorganism that is non-pathogenic that is commonly found in the human intestine as well as Enterobacter cloacae. According to research in Iran that gram-negative bacteria (Escherichia coli and Enterobacteriaceae) are the most frequent organisms in the recto-vaginal area of the mother. Two studies conducted in the United States showed that Escherichia coli was associated with premature birth, especially giving birth before 34 weeks' gestation. Urinary tract infections by Escherichia coli have also been linked to preterm birth.[16]

CONCLUSION

Most microorganisms found in PROM are Escherichia coli, Enterobacter cloacae and Staphyloccocus aureus. Most microorganisms found in non-PROM are Klebsiella oxytoca, Staphylococcus epidermidis, Staphylococcus saprophyticus and Pseudomonas fluorescens. There is no relationship between BMI, age, parity, smoking, and gestational age with the incidence of PROM in pregnancy in this study.

RECOMMENDATION

This study is to determine the pattern of microorganisms that exist in the vagina in pregnancy with PROM in Manado. Further research is needed with a larger sample size so that it can provide results that are closes to accurate in determining the pattern of microorganisms in the vagina in pregnancy with a PROM in Manado. This study found that most microorganisms in pregnancy with PROM, namely Escherichia coli where the transmission is often in contaminated food or unclean hands. Appeals are given to every pregnant woman to wash their hands before eating and maintain their personal hygiene in the genital area.

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