Research Article

Identification of Microorganisms in Vaginal Swab and Peritoneal Fluid of Women with Endometriosis

Identifikasi Mikroorganisme pada Swab Vagina dan Cairan Peritoneum pada Wanita dengan Endometriosis

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Abstract

Objective: To discover the correlation between microorganisms found in vaginal swab culture and peritoneal fluid culture from laparoscopy in reproductive age women diagnosed with endometriosis.

Methods: This cross-sectional study was conducted in Bunda Hospital and YPK Hospital, Central Jakarta. Thirty-one subjects were included. Data were analysed using SPSS 20.0 for Windows. Bivariate analysis was used to identify the correlation between independent and dependent variables.

Results: From 31 subjects with mean age 34.42±5.056 years old, 87.1% were infertile. Vaginal swab culture was found positive in 83.9% subjects while peritoneal fluid culture was found positive only in 9.6% subjects. There was moderate correlation between chronic pelvic pain and positive vaginal swab culture (r=0.601; p=0.001), but weak correlation between Ca125 and vaginal swab culture (r=0.440; p=0.010). Peritoneal fluid culture had significant inverse correlation with left tubal patency (r=-0.346; p=0.047). There was weak correlation between vaginal swab culture and peritoneum fluid culture with correlation coefficient of 0.13.

Conclusion: Most bacteria found in vaginal swab culture and peritoneal fluid culture were the ones found in the gastrointestinal tract. However, vaginal swab and peritoneal fluid culture were not sufficient to prove the hypothesis that infection has a role in the pathogenesis of endometriosis. Therefore, advance examination such as LPS and PCR might be needed to be done in the future research with cohort study, to overcome the limitation of this study.

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Keywords: ascending bacterial contamination, culture, endometriosis vaginal swab, peritoneal fluid

Abstrak

***Tujuan**: Membuktikan adanya korelasi antara mikroorganisme yang ditemukan pada hasil kultur bilasan vagina dengan mikroorganisme yang ditemukan pada cairan peritoneum hasil laparoskopi perempuan usia reproduksi yang terdiagnosis endometriosis*

***Metode**: Penelitian ini menggunakan desain penelitian analitik potong lintang yang bertujuan untuk melihat adanya hubungan korelasi serta mengetahui tingkat korelasi antara mikroorganisme kultur bilasan vagina dengan mikroorganisme pada cairan peritoneum pasien endometriosis.*

***Hasil**: Hasil kultur bilasan vagina dari 31 subjek penelitian yang diteliti, mikroorganisme terbanyak adalah Enterococcus faecalis (32,3%), Eschericia coli (29,1%), dengan 16,1 % dengan hasil kultur negatif. Sedangkan dari hasil kultur bilasan peritoneum terdapat 3 subjek (9,6%) dengan hasil positif yaitu dengan jenis bakteri Eschericia coli, Enterococcus faecalis, dan Pseudomonas. Terdapat korelasi lemah antara hasil kultur bilasan vagina dengan kultur bilasan peritoneum (r 0,13). Terdapat korelasi sedang antara kultur positif bilasan vagina dengan nyeri pelvik kronis, korelasi lemah antara kultur positif bilasan vagina dengan nilai Ca 125, dan korelasi lemah antara kultur positif cairan peritoneum dengan tuba kiri yang non paten.*

***Kesimpulan**: Sebagian besar bakteri dari bilasan vagina dan bilasan peritoneum pada pasien endometriosis memiliki hasil bakteri dari organ pencernaan. Terdapat korelasi lemah antara hasil kultur bilasan vagina dengan kultur bilasan peritoneum pada pasien endometriosis.*

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***Kata kunci**: bilasan vagina, cairan peritoneum, endometriosis, kontaminasi bakteri asenden, kultur*

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INTRODUCTION

Endometriosis is an abnormal growth of endometrium, consists of glandular epithelium and stroma, outside the uterus.[1] This gynecological condition occurs in 7-10% reproductive age women.[2] In 2009, a study in Jakarta reported that endometriosis is found in 64.6% infertility cases.[3] Endometriosis symptoms such as chronic pelvic pain, dysmenorrhea, dyspareunia, irregular bleeding, gastrointestinal symptoms, fatigue/ anemia can be disturbing.[4] Endometriosis can be found in many organs, with the highest prevalence is in uterus, ovarium, or pelvic peritoneum.[5] It is known that in endometriosis there is an imbalance

of cell proliferation and apoptosis with chronic inflammation process during its course.[6] However, the exact cause and mechanisms in endometriosis have not been understood completely.

There are several hypotheses to its pathogenesis, such as transplantation of uterine endometrium to another site by retrograde menstruation (Sampson's transplantation theory), coelomic metaplasia theory, and mullerian ducts remnant.[4] The several factors contributing to endometriosis growth are hormones, genetics, inflammation, and infection initiation. High endotoxin concentration in menstrual fluid and peritoneal fluid is possibly happened because of ascending bacterial contamination, such as Escherichia coli. This hypothesis expected to be involved in peritoneal endometriosis growth.[4] Lipopolysaccharide (LPS) from Gram-negative bacteria acts as inflammatory mediator that promotes endometrium stromal cell proliferation and invasion through cyclooxygenase 2 (COX 2) and prostaglandin E2 (PGE_2) regulation. PGE_2 affects bacterial growth directly and indirectly.[5,6] Women's lower reproductive tract exposed to many microorganisms allows ascending infection to the upper reproductive tract, leading to endotoxin accumulation in menstrual and peritoneal fluid causing inflammation and growth of endometriosis.[7,8]

Eventhough many literatures discussing infection role in the pathogenesis of endometriosis, the research has not been proven. This study was conducted to discover the correlation between microorganisms found in vaginal swab culture and peritoneal fluid culture from laparoscopy in reproductive age women diagnosed with endometriosis, in order to examine the possibility of contamination and ascending infection as a trigger in inflammatory cascade in endometriosis cases.

METHODS

This cross-sectional study was conducted at Bunda Hospital and YPK Hospital, Central Jakarta, Indonesia. A total of 31 subjects were included and obtained by consecutive sampling. Subjects included were reproductive age women (18-40 years old), diagnosed with endometriosis based on history, physical examination, and USG, agreed to laparoscopic procedure for diagnostic/therapeutic including obtaining peritoneal fluid, agreed to vaginal examination and vaginal swab sampling, agreed to join this research by filling the informed consent forms. Women with vaginal bleeding of unknown cause, suspected with tubal and ovarium abscess from history, physical examination, and further diagnostic procedures, done vaginal douching in the later five days or routinely, and whose peritoneal fluid was contaminated with blood at laparoscopic procedure, were excluded. Subjects suspected endometriosis that could not be found during laparoscopic procedure was dropped out.

Data were analysed using SPSS 20.0 for Windows. Univariate analysis was used to describe characteristics of the subjects. Data normality test continued with bivariate analysis by Spearman test was used to find correlation between independent and dependent variable. Statistical significance defined in two methods: correlation coefficient greater than r table or p-value <0.05.

RESULTS

A total of 31 subjects were included in this study with their mean age is 34.42±5.056 years old. Table 1 described clinical and demographic characteristic of the subjects.

Table 1. Clinical Characteristics

Characteristics	n (%)	
	Yes	No
Infertility	27 (87.1)	4 (12.9)
Use of contraception/hormonal therapy	3 (9.7)	27 (90.3)
Leucorrhea	5 (16.1)	26 (83.9)
Dysmenorrhea	18 (58.1)	13 (41.9)
Chronic pelvic pain	2 (6.4)	29 (93.6)

Characteristics	n (%)	
	Yes	No
Dyspareunia	3 (9.7)	28 (90.3)
Cervical motion tenderness	0 (0)	31 (100)
Rectovaginal nodule	0 (0)	31 (100)
Parametrium pain	0 (0)	31 (100)
Cyst	26 (83.9)	5 (16.1)
Unilateral;	16 (51.6);	
Size (mm, median [range])	20 (10-85)	
Bilateral;	10 (32.2);	
Size (mm, median [range])	46 (30-80)	
Tubal patency		
Left	14 (45.2)	17 (54.8)
Right	15 (48.4)	16 (51.6)
Hb (mean [SD])		12.40 (1.443)
Leukocyte (mean [SD])		7445.5 (1783.4)
Thrombocyte (mean [SD])		322910 (86996.2)
Ca125 (median [range])		24 (7-136.4)
Endometriosis classification		
AFS 1 (mild)		8 (25.8)
AFS 2 (moderate)		5 (16.1)
AFS 3 (severe)		7 (22.6)
AFS 4 (very severe)		11 (35.5)

Based on vaginal swab culture, five samples (16.1%) were negative, while the 83.9% were positive for bacteria, with Enterococcus faecalis in 10 samples (32.3%) was the most common, followed by E.coli in 9 samples (29.1%). Other bacteria found were Staphylococcus haemolyticus, actinobacter, enterobacter, Staphylococcus epidermidis, Streptococcus agalactiae, and trichomonas. Culture of peritoneal fluid revealed 28 samples (90.4%) are negative, the rest three samples (9.6%) were contained with e.coli, enterococcus faecalis, Pseudomonas. Table 2 below showed the microorganisms obtained in culture of vaginal swab and peritoneal fluid, also with the endometriosis classification.

Table 2. Microorganisms Obtained in Culture and Classification of Endometriosis

Vaginal Swab Culture	Peritoneal Fluid Culture	Classification of Endometriosis
Enterococcus faecalis	-	4
Staphylococcus haemoliticus	-	1
Enterococcus faecalis	-	1
Enterobacter	-	4
E. coli	-	4
Trichomonas	-	3
E. coli	-	1
E. coli	-	4
Enterococcus faecalis	-	3

Vaginal Swab Culture	Peritoneal Fluid Culture	Classification of Endometriosis
Enterococcus faecalis	Enterococcus faecalis	4
E. coli	Pseudomonas	4
Staphylococcus haemoliticus	-	3
-	-	4
Enterococcus faecalis	-	2
Enterococcus faecalis	-	1
Enterococcus faecalis	-	1
-	-	4
Streptococcus agalactiae	-	1
Enterococcus faecalis	-	4
Actinobacter	-	2
Enterococcus faecalis	-	2
E. Coli	-	2
E. Coli	-	2
Streptococcus agalactiae	-	3
Enterococcus faecalis	-	3
E. Coli	-	1
Staphyloccocus epidermidis	-	4
Enterococcus faecalis	-	1
-	-	4
E. coli	E. coli	3
-	-	3

Correlation between characteristics of the subjects and findings of vaginal swab and peritoneal fluid culture was obtained with bivariate nonparametric correlation test. Nominal data in subjects characteristic were analysed using Pearson test to obtain Phi coefficient, while ordinal or numeric data using point-biserial coefficient test.

Table 3. Correlation between Subjects Characteristics and Culture Results

Characteristics	Vaginal Swab Culture		Peritoneum Fluid Culture	
	r	p	r	p
Age	-0.019	0.916	0.069	0.702
Infertility	-0.157	0.367	0.117	0.500
Use of contraception/hormonal therapy	0.134	0.443	-0.100	0.566
Leucorrhea	0.179	0.305	0.160	0.357
Dysmenorrhea	-0.046	0.790	0.077	0.658
Chronic pelvic pain	**0.601**	**0.001**	-0.080	0.645
Dyspareunia	-0.160	0.357	-0.100	0.566
Cervical motion tenderness	-	-	-	-
Rectovaginal nodule	-	-	-	-
Parametrium pain	-	-	-	-

Characteristics	Vaginal Swab Culture		Peritoneum Fluid Culture	
	r	p	r	p
Cyst	-0.179	0.305	0.134	0.443
Tubal patency				
Left	0.123	0.478	**-0.346**	**0.047**
Right	0.097	0.576	-0.326	0.061
Hb	0.178	0.321	-0.096	0.594
Leukocyte	0.122	0.500	-0.056	0.756
Thrombocyte	-0.134	0.458	0.305	0.084
Ca125	**0.440**	**0.010**	0.116	0.520
Classification of endometriosis	-0.253	0.155	0.258	0.146

There was moderate correlation between chronic pelvic pain and positive vaginal swab culture (r=0.601; p=0.001). Weak correlation showed between Ca125 and vaginal swab culture (r=0.440; p=0.010). Peritoneal fluid culture had inverse correlation with left tubal patency (r=-0.346; p=0.047) significantly, and right tubal patency (r=-0.326; p=0.061) insignificantly. Correlation between variables in this study, vaginal swab culture and peritoneum fluid culture, were obtained by inferential analysis using Lambda coefficient.

Table 4. Correlation between Vaginal Swab Culture and Peritoneum Fluid Culture

Variables	r	p
Lambda		
Vaginal swab culture as dependent variable	0.130	0.049
Peritoneum fluid culture as dependent variable	0	0

Table 4 above showed that there was a weak correlation between vaginal swab culture and peritoneum fluid culture with correlation coefficient of 0.13.

DISCUSSION

Infertility is more common in women with endometriosis. As much as 25-50% of infertile women have endometriosis and 30-50% women having endometriosis are infertile. This study found that there are 87.1% of women having endometriosis were infertile. It can be explained by mechanisms, such as pelvic anatomy abnormality, endocrine system and ovulation abnormality, altered peritoneal function, also cell-mediated alteration in endometrial hormone and function. Pelvic or tubal adhesion obstructs released oocyte from ovarium and transport ovum because of blocked tubal patency. Women having endometriosis have more inflammatory mediators such as active macrophages, prostaglandin, IL-1, TNF, and protease in the peritoneal fluid.[9]

Lower reproductive tract infection increases the risk of endometriosis, especially inflammation of the cervix, vagina, and vulva.[10] Ascending migration of untreated infection leads to endometritis and subclinical pelvic inflammatory disease which are risk factors of endometriosis. Normal flora in the vagina is predominantly Lactobacillus. Alteration in vaginal pH and vaginal flora increases the risk of lower reproductive tract infection. Women with bacterial vaginosis and nonspecific vaginitis have increasing opportunistic pathogens such as Staphylococci, enterococci, enterobacter, candida, peptostreptococci, peptococci, and predominantly anaerobic Gram-negative bacteria.[11] The growth of bacteria, often found in small amount, indicates dysbiosis which could be symptomatic or asymptomatic. Streptococci, staphylococci and E. coli have been known to cause invasive disease. S. agalactiae is also found invasive in postpartum women and neonates.[12]

Several characteristics of the subject correlated with positive vaginal swab culture or peritoneal swab culture. Moderate correlation between chronic pelvic pain and positive vaginal swab cul-

ture consistent with the theory that chronic pelvic pain is one of the symptoms of pelvic inflammatory disease.[13] Marker Ca125 was also examined in this study. Increased Ca125 in circulation could be normal in menstruation and pregnancy or a result of inflammatory reaction that changes endothelial permeability. In this study, higher Ca125 was found in positive vaginal swab culture. The weak correlation between Ca125 and vaginal swab culture can be explained by bacterial-induced inflammation process of gynaecology organs.[14,15] This study showed that peritoneal fluid culture had significant inverse correlation with left tubal patency. This might be due to different vascularisation and pelvic anatomy, in which pathologic symptoms are also found more common in the left side.[16,17] A study explained that slower drainage of left ovarium causes higher microorganisms on the left side.[18] As a result, positive peritoneal fluid culture was found higher in left tubal obstruction.

From total 31 subjects, only two of them had similarity in the result of vaginal swab culture and peritoneal fluid culture. This finding supported bacterial contamination hypothesis, which might be explained by ascending migration of bacteria. There was a hypothesis that intestinal microbes could also have a role in the pathogenesis of endometriosis, as this microbes act as the main regulator of inflammatory process outside the intestines.[18] However, there were possibilities explaining the positive results of the culture. Those bacteria could be normal flora or contamination while taking and processing the samples. Different normal flora from the vagina can be found in peritoneal fluid, such as anaerobic bacteria. The similar culture result in 2 subjects could also not correlate or have different genetic material.[19]

In this study, PCR for bacterial DNA was not performed. As a result, the correlation between bacteria found in vaginal swab culture and peritoneal fluid culture cannot be analysed. Negative cultures because of small concentration of bacteria might be detected positive by PCR and contain high endotoxin concentration.[7,19] Unfortunately, laboratory assay for LPS detection was also not performed, so we could not prove the bacterial contamination hypothesis and LPS relationship to culture findings.

CONCLUSION

Most of bacteria found in vaginal swab culture and peritoneal fluid culture were the ones found in gastrointestinal tract. There was moderate correlation between chronic pelvic pain and vaginal swab culture, weak correlation between Ca125 and vaginal swab culture, also between left tubal occlusion with peritoneal fluid culture. There was weak correlation between microorganisms found in vaginal swab culture and peritoneal fluid culture. However, vaginal swab and peritoneal fluid culture were not sufficient to prove the hypothesis that infection has a role in the pathogenesis of endometriosis. Therefore, advance examination such as LPS, PCR DNA might be needed to be done in the future research with cohort study, to overcome the limitation of this study.

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