Aerobic Microbiological Study in Term Pregnant Women with Premature Rupture of the Membranes : A Case-Control Study

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Abstract

To determine the aerobic microorganisms related to premature rupture of the membranes (PROM) in term pregnant women, a case-controlled study was performed on pregnant women delivered at Rajavithi Hospital between November 1, 1996 and July 30, 1997. Two hundred and twenty pregnant women with PROM and 220 pregnant women without PROM were recruited by simple random sampling. The diagnosis of rupture of the membrane was made by history and by positive microscopic ferning and pH testing performed during speculum examination. The demographic characteristics were not statistically significantly different between both groups. We could not isolate any organisms (35.9% in the study group and 49.5% in the control group). *Candida albicans* and *Klebsiella pneumoniae* were the only two significant differences demonstrated between the study and control group ($p<0.05$). *Candida albicans*, the most prevalent organism in the study group, demonstrated significant difference between the study and control group (14.5% and 7.7% respectively) ($p<0.05$). *Klebsiella pneumoniae* demonstrated significant difference between the study and control group (7.30% and 4.10% respectively) ($p<0.05$). *Gardnerella vaginalis*, the most prevalent organism in the control group, showed no significant difference between the control and study group (16.40% and 14.10% respectively) ($p=0.547$).

Key word : Premature Rupture of the Membranes, Microbiological Study

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The etiology of spontaneous premature rupture of the membranes (PROM) is unknown (1). Infections were suggested to be one of the causes of PROM by weakening the membranes (2, 3). Microbiologic flora of the cervix and vagina during pregnancy and puerperium have been previously studied(4, 5). Minkoff et al(6) reported some vaginal pathogens in early pregnancy were

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associated with the subsequent development of PROM or preterm labor. In this case-controlled study we sought to determine the aerobic microorganisms comparing term pregnant women with and without PROM.

MATERIAL AND METHOD

Our research was done in the labor room (LR), Rajavithi Hospital from November 1, 1996, to July 30, 1997. Term pregnant women were enrolled as study cases from patients admitted to the LR with a proved diagnosis of rupture of the membranes. The diagnosis of rupture of the membranes was made by history and by positive microscopic ferning and pH testing performed during speculum examination. Control cases were enrolled from patients admitted to the LR just after the study cases with the same condition but no PROM. We excluded patients with medical or obstetric complications, recent antibiotic treatment, previous pelvic examination before proof of PROM. During the process of proof of PROM, we performed cervical swab culture from the endocervix with a cotton wool swab which was then put in Stuart's medium and transported to the microbiology laboratory within 2 hours Culture were processed immediately upon receipt in the laboratory. Primary plating media consisted of chocolate agar and Mac Conkey agar. We could perform only aerobic culture in our study. Plates incubated aerobically were examined at 24, 48 and 72 hours.

Definition

1. Premature rupture of the membranes is defined as the rupture of the amniotic sac before the onset of labor, regardless of gestational age[7].

Statistics

The qualitative data was analysed using Chi-square test (X^2) and Fisher exact test when the frequency in each cell was less than 5. The quantitative data was analysed using arithmatic mean, standard deviation and unpaired t-test. Statistical significance was refined as $P<0.05$. All data was collected and analysed by using computer SPSS for Window Version 6.13.

RESULTS

Four hundred and forty cases were enrolled in our study. Both groups had an equal number of 220 term pregnant women. There was non significant difference of the study and control cases in demographic characteristics (Table 1). We could not isolate the organism in 79 (35.90%) and 109 (49.50%) patients of study and control groups respectively. The details of isolated microorganisms are demonstrated in Table 2.

DISCUSSION

Our study had rather good randomization because there was no significant difference of all demographic characteristics between the study and control groups. Until now there is no concensus about the problem of whether infection causes PROM or PROM causes infection. Infection may instigate chorioamnionitis that then leads to PROM. Conversely, a cervicovaginal infection may be a primary factor instigating PROM, and subsequently a secondary cause of

Table 1. Demographic characteristics.

Demographic characteristics	Case (N=220)	Control (N=220)	P-value
Age (yr) (X ± SD)	29.97 ± 6.80	25.00 ± 6.76	0.14
Height (m) (X ± SD)	1.53 ± 0.91	1.54 ± 0.90	0.76
Gestational age (wk) X ± SD at delivery	39.27 ± 1.40	39.16 ± 1.42	0.70
Gravidity	1.88 ± 0.86	1.73 ± 0.83	0.05
Parity	0.59 ± 0.69	0.53 ± 0.31	0.84

Case = Pregnant women with proved diagnosis of rupture of the membranes
Control = Pregnant women without proved diagnosis of rupture of the membranes

Table 2. Result of cervical aerobic culture.

Microorganism	Case (N=220)	%	Control (N=220)	%	P-value
1. β-Streptococcus group B	2	0.90	0	0	0.180
2. *Klebsella pneumoniae*	16	7.30	9	4.10	0.038
3. Non-hemolytic streptococci	2	0.90	1	0.50	0.575
4. *Gardnerella vaginalis*	31	14.10	36	16.40	0.547
5. *Candida albicans*	32	14.50	17	7.70	0.004
6. α-hemolytic streptococci	13	5.90	7	3.20	0.050
7. Lactobacilli	5	2.30	6	2.70	1.000
8. *Escherichia coli*	10	4.50	11	9.00	0.624
9. Yeast (except *candida albicans*)	30	13.70	24	10.90	0.079

Case = Pregnant women with proved diagnosis of rupture of the membranes
Control = Pregnant women without proved diagnosis of rupture of the membranes

chorioamnionitis from an ascending infection[3]. Goplerud et al[4] reported the most prevalent flora in pregnant women during 34-40 weeks of gestational age were aerobic gram positive rods and cocci (Lactobacilli 97.4%) and anaerobic gram positive cocci (peptococcus asaccharolyticus and peptococcus magnus 42.1% for both).

Lactobacilli Sp was also reported to be the most prevalent organism of the cervix and vagina isolated from white and black pregnant women (93.3% and 84.4% respectively)[5]. In our study, we examined only aerobic cultures of the cervix because of some difficulty in the technical process in anaerobic cultures. We could not cultivate any organism in about 35.9 per cent and 49.5 per cent of the study and control group. *Candida albicans*, the most prevalent organism in this study group, showed significant difference between the study and control group (14.5% and 7.7% respectively) ($p<0.05$). The most prevalent organism in the control group was *Gardnerella vaginalis* (16.40%) compared with 14.10% in the study group (p=0.547). When we combined the prevalence of *Candida albicans* and yeast (except *Candida albicans*), we found that these organisms were the most common organisms in the study and control groups (28.20% and 18.60%). Walsh et al[5] reported a low prevalence of *Candida albicans* of the cervix and vagina in white and black pregnant women of 12.5 per cent and 22.9 per cent respectively. *Klebsiella pneumoniae* was the other significant organism isolated in the study group (7.30%) more than in the control group (4.10%) (p=0.038). Goplerud et al[4] reported a low prevalence of cultures positive for *Klebsiella pneumoniae* 2.6 per cent in 34-40 weeks of gestation in the normal pregnancy group. However, we could not compare our study with Goplerud's study because they examined only normal pregnant women and in various gestational ages from 34-40 weeks.

We found a low prevalence of Lactobacilli sp in the study and control groups (2.30%) and 2.70 per cent respectively) (p=1.000). Bacterial vaginosis is characterized by the presence of increased variety and number of cervical and vaginal micro-organims, including, *Gardnerella vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Mobiluncus* species and various other anaerobes, most prominently *Bacteroides bivius* [8]. McGregor et al[8] reported that bacterial vaginosis can be strongly (risk ratios up to 7.3, CI 95% = 1.8-29.4) associated with preterm premature rupture of the membranes as well as preterm labor and birth. But in our study, we demonstrated no significant difference in the result of positive culture for *Gardnerella vaginalis* in both groups (p=0.547). We assume that one of the possible reasons might come from the term gestational age of our patients.

Group B streptococcus has emerged as a major cause of neonatal infectious morbidity over the past several decades[9]. Serial cultures from prenatal patients have suggested that women may be intermittent carriers of group B streptococci and have demonstrated that concordance

with intrapartum culture status improves as the interval between antenatal cultures and delivery is shortened[10].

US CDC published prevention guidelines that described a screening based strategy involving collection of cultures at 35-37 week's gestation[11]. Beta hemolytic streptococci group B has been shown to give positive culture in 13.2 per cent in pregnant women whose gestational age was 34-40 weeks[4]. But we could isolate only 0.9 per cent in the study group but none in the control group. *Trichomonas vaginalis* was reported by Minkoff et al[6] to be significantly more likely to have PROM ($p<0.03$). But we could not demonstrate this organism because we had no culture media for *Trichomonas vaginalis*. Screening for and treatment of lower vaginal tract infection should be an integral part of prenatal care in communities where the prevalence of the aforementioned organisms is high[3].

We suggest an extensive prospective study, including both aerobic and anaerobic organisms be performed to determine the role of microorganisms on PROM.

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การศึกษาทางจุลชีววิทยาชนิดที่ต้องใช้ออกซิเจนในสตรีตั้งครรภ์ครบกำหนด ที่มีภาวะถุงน้ำคร่ำแตกก่อนการเจ็บครรภ์ : การศึกษาแบบ Case–control

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การศึกษาเพื่อหาความสัมพันธ์ของการเพาะเชื้อขึ้น ชนิด aerobe กับการเกิดภาวะถุงน้ำคร่ำแตกก่อนการเจ็บครรภ์ในสตรีตั้งครรภ์ครบกำหนดที่มีภาวะถุงน้ำคร่ำแตกก่อนการเจ็บครรภ์ที่คลอดบุตรที่โรงพยาบาลราชวิถี ตั้งแต่วันที่ 1 พฤศจิกายน 2539 ถึง 30 กรกฎาคม 2540 โดยมีสตรีตั้งครรภ์กลุ่มละ 220 คน โดยคัดเลือดแบบการสุ่มตัวอย่างแบบง่าย การวินิจฉัยภาวะถุงน้ำคร่ำแตกก่อนการเจ็บครรภ์อาศัยประวัติ การตรวจร่างกาย และการตรวจหา Fern และสภาพความเป็นกรดด่างของของเหลวที่ตรวจพบในช่องคลอด ในการศึกษานี้เพาะเชื้อไม่ขึ้นร้อยละ 35.9 และ 49.5 ในกลุ่มศึกษาและกลุ่มควบคุม ตามลำดับ พบว่ามีเชื้อที่เพาะขึ้น 2 ชนิด ที่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) เมื่อเทียบระหว่างกลุ่มควบคุมและกลุ่มศึกษาคือ เชื้อรา (*Candida albicans*) และเชื้อ *Klebsiella pneumoniae* เชื้อรา (*Candida albicans*) ซึ่งเป็นเชื้อที่พบมากที่สุดในกลุ่มศึกษา สามารถเพาะขึ้นร้อยละ 14.5 และ 7.7 ในกลุ่มศึกษาและกลุ่มควบคุม ตามลำดับ โดยมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ส่วนเชื้อ *Klebsiella pneumoniae* โดยพบ ร้อยละ 7.30 และ 4.10 ตามลำดับ ($p<0.05$) ส่วนเชื้อ *Gardnerella vaginalis* ซึ่งเป็นเชื้อที่พบมากที่สุดในกลุ่มควบคุม สามารถเพาะเชื้อขึ้นร้อยละ 16.40 และ 14.10 ในกลุ่มควบคุมและกลุ่มศึกษา ตามลำดับ โดยไม่พบว่ามีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p=0.547$)

คำสำคัญ : ภาวะถุงน้ำคร่ำแตกก่อนการเจ็บครรภ์, การศึกษาทางจุลชีววิทยา

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