Antenatal Risk Factors Associated with Preterm Prelabour Rupture of the Membranes

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The objective of this study was to test the hypothesis that infections by diverse organisms causing different diseases during pregnancy increase the risk for preterm prelabour rupture of the membranes (P-PROM) independently of the other risk factors. A case-control design was used to compare women who had P-PROM and full-term delivery. Even after adjusting for concomitant risk factors, women with P-PROM were more likely than controls to have had intraamniotic ($p = 0.04$), cervical *C. trachomatis* ($p = 0.02$), *E. coli* ($p = 0.04$) and *S. aureus* ($p = 0.04$) infection.

Key words: preterm prelabour rupture of the membranes, preterm delivery, intraamniotic infection, cervical microflora, risks

INTRODUCTION

The role of infection in preterm labour (PTL), preterm prelaboure rupture of membranes (P-PROM), and prematurity has received much attention in recent years (1–3). Several observations support the hypotesis that maternal genital tract infection may frequently play an etiologic role in preterm labor and P-PROM. Cultures of amniotic fluid obtained by transabdominal amniocentesis consistently have demonstrated cervical and vaginal bacteria in women having either P-PROM or PTL with intact membranes (4–7). The recovery of various organisms from the cervix during pregnancy has also been associated with increased risk for P-PROM. A number of clinical studies have indicated that asymptomatic genital tract infection may play a significant role, with several studies showing significant associations between carriage of *C. trachomatis* (8, 9), *N. gonorrhoeae* (8), group B streptococci (8, 10), bacterial vaginosis microorganisms or enteropharyngeal bacteria (9–11) and either premature delivery or P-PROM.

Despite accumulated evidence relating infection with P-PROM and preterm delivery, it is still difficult to interpret the significance and magnitude of such an association, because most studies have not been controlled for known risk factors such as exposure to cigarette smoke, vaginal bleeding during pregnancy, and previous reproductive histories. We tested the hypotesis that infections by diverse organisms causing different diseases during pregnancy increase the risk of P-PROM independently of the other risk factors.

MATERIALS AND METHODS

The study was performed at the Department of Obstetrics and Gynaecology of Kaunas University of Medicine, a tertiary-care perinatal referral centre. Three study groups, because of ethical problems of receiving amniotic fluid (AF) from healthy pregnant women, were defined to evaluate the prevalence of infectious risk factors in early and late third trimester and to assess the risk factors associated with P-PROM.

- I study group (cases) – patients with a singleton pregnancy between 22 and 36 weeks of gestation complicated by P-PROM, without evidence of labour and without signs of clinical chorioamnionitis were considered candidates for this study.
- II study group (controls I) – healthy pregnant women in early third trimester, screened by transabdominal amniocentesis (TA) for serologic conflict

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at the Kaunas Perinatal Centre because of ABO group incompatibility or Rh sensibilisation.

• III study group (controls II) – women delivering by elective term cesarean section (C/S).

Women with major congenital anomalies, serologic conflict, history of antibiotic treatment within one week, medical conditions necessitating delivery or unwilling to sign the informed consent form were excluded.

After exclusion there remained 87 women in study group I (cases), 52 in study group II (controls I) and 45 in study group III (controls II).

A standardized questionnaire was used to interview cases and controls. The same interviewer (D. V.) obtained information about the mother's obstetric and gynaecologic history, course of the pregnancy, lifestyle, behaviour and sociodemographic information.

Gestational age was determined by last menstrual period, early clinical examination and the results of any ultrasonographic examinations before 24 weeks' gestation. Membrane rupture was confirmed by the visualising AF leaking from the cervical os on sterile speculum examination or by a fern test. Swabs from the endocervical canal for aerobic culture, *C. trachomatis* detection and Gram stain were taken at the initial examination. Swabs were inoculated on 5% human blood agar, chocolate agar, Endo agar and yolk salt agar. The cultures were incubated for 18–48 h. Presence of *Chlamydia trachomatis* was studied on wet smears with MicroTrac®, using fluorescein-labelled monoclonal antibodies.

In study groups I and II, AF for Gram stain and culture was harvested by transabdominal amniocentesis performed under ultrasonographic guidance with a 20-gauge needle. In the group of women delivering by cesarean section (study group III) amniotic fluid was obtained by uterine wall punction with a 20-gauge needle during operation (Figure). The samples of AF were plated within 60 min of collection on blood agar, MacConkey's agar, chocolate agar, Sabouraud dextrose agar and sheep blood agar (36 °C in 8% carbon dioxide) for aerobic bacteria by previously described microbiological techniques (12). Gram stain on unspun AF was performed with standard reagents (crystal violet, safranin and Gram's iodine) and examined for bacteria and leukocytes under standard conditions. The remaining 5 ml of AF were transferred to a polypropylene tube, capped and stored at –70 °C until assayed for *C. trachomatis* by cell culture, enzyme immunoassay (EIA) and direct immunofluorescence tests. For *C. trachomatis* antigen detection, AF samples were aliquoted in two 1 ml tubes and 30 min. centrifuged at 12,000 × g. One sediment was reconstituted in 1 ml of Specimen Dilution Buffer (MicroTrac® *Chlamydia trachomatis* EIA, Behring Diagnostica, San Jose, CA, USA). Detection of two chlamydial elementary bodies served as a cut-off value for a positive antigen detection test. Samples were considered positive if positive by cell culture or/and at least in two of the different chlamydia detection assays used.

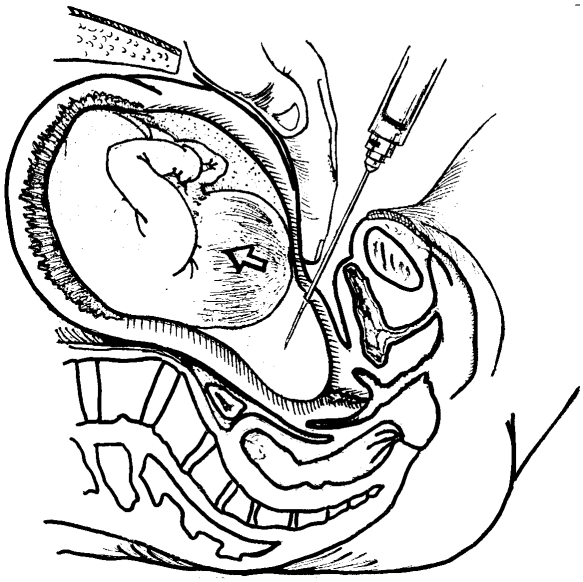

Figure. Technique of uterine wall puncture during cesarean section

Intraamniotic infection (IAI) was defined as the presence of an AF culture or Gram stain testing positive for microorganisms or positive tests for *C. trachomatis*.

Urinary tract infection was documented by urine culture of ≥100 000 colony forming units per ml (cfu/ml) of a single species of organisms anytime during the pregnancy. History of lower respiratory infections (pneumonia and bronchitis) during this pregnancy was included because of the potential for associated viremia or bacteremia during such infections.

After the univariate analysis of all variables showing no significant difference between study groups II and III (controls I and controls II) these groups were merged and drawn into multivariate logistic regression analysis as one control group of the "women delivered at term".

Data were analyzed using the χ^2 test with Yate's correction for small numbers or Fisher's exact test for nonparametric data and the Student's t test for normally distributed parametric data. Odds ratios (OR) and 95% exact confidence intervals (CI) were also calculated using EPI-INFO software, version 6.0 (Centers for Disease Control and Prevention, Atlanta, Georgia USA).

Multivariate logistic regression analysis of infectious risk factors for P-PROM was performed using

the Microsoft EXCEL statistical software programme, version 7.0, to allow for confounding demographic and obstetric variables. A model using all factors and all organism groups thought to be associated with P-PROM with univariate analysis ($p < 0.05$) was used in the regression analysis. The odds ratio (OR) and 95% confidence intervals (CI) in logistic regression analysis are derived from beta-coefficients.

RESULTS

The demographic characteristics of cases and controls are shown in Table 1. There was no difference in maternal age, gravidity, parity, education. However, cases were significantly less likely to be married than controls I (75.9% *versus* 96.1%, OR 7.95, 95% CI 1.79–72.33, $p = 0.004$) and controls II (75.9% *versus* 93.3%, OR 4.45, 95% CI 1.21–24.52, $p = 0.03$). Gestational age at amniocentesis in cases and controls I, like gestational age at delivery in two controls, was not significantly different ($p > 0.05$). Statistically significant difference was noted in neonatal sex. There were more male neonates in cases than in controls I (47.1% *versus* 23.1%, OR 2.97, 95% CI 1.29–6.92 $p = 0.008$) and controls II (47.1% *versus* 20.0%, OR 3.57, 95% CI 1.43–9.07, $p = 0.004$).

Table 1. **Distribution of sociodemographic variables for cases with P-PROM, controls I and controls II**

Variables	P-PROM (n = 87)	Term delivery (n = 52)	Elective term C/S (n = 45)	P value
	cases	controls I	controls II	
Age, years	27.7 ± 6.1[a]	27.5 ± 6.4	27.4 ± 5.1	0.96
Gestational age at amniocentesis, weeks	32.0 ± 2.8	32.3 ± 0.9	39.0 ± 1.1	0.000
Gestational age at delivery, weeks	32.5 ± 2.3	38.6 ± 1.1	39.0 ± 1.1	0.000
Birth weight, g	2043 ± 489	3221 ± 613	3450 ± 373	0.000
Gravidity	2.7 ± 1.7	2.1 ± 1.5	2.5 ± 1.4	0.10
Parity:				
nulliparity	38 (43.7)[b]	16 (30.8)	17 (37.8)	0.59
parity ≥ 1	49 (56.3)	36 (69.2)	28 (62.2)	0.59
Education:				
less than secondary school	8 (9.2)	4 (7.7)	4 (8.9)	0.95
secondary school	37 (42.5)	17 (32.7)	24 (53.3)	0.12
higher than secondary school	42 (48.3)	31 (59.6)	17 (37.8)	0.10
Marital status:				
unmarried	21 (24.1)	2 (3.9)	3 (6.7)	0.001
married	66 (75.9)	50 (96.1)	42 (93.3)	0.001
Neonatal sex:				
female	46 (52.9)	40 (76.9)	36 (80.0)	0.001
male	41 (47.1)	12 (23.1)	9 (20.0)	0.001

[a] Mean values ± SD. [b] Number with percentages in parentheses.

Table 2. **Behavioral characteristics of the women in the study**

	P-PROM (n = 87)	Term delivery (n = 52)	Elective term C/S (n = 45)	P value
	cases	controls I	controls II	
Cigarette smoking	4 (4.6)	2 (3.8)	2 (4.4)	0.98
Exposure to toxic chemicals	9 (10.3)	5 (9.6)	3 (6.7)	0.78
Expose to vibration	10 (11.5)	6 (11.5)	6 (13.3)	0.95
Hard physical work	28 (32.2)	14 (26.9)	13 (28.9)	0.80
Experienced great emotional stress	6 (6.9)	6 (11.5)	2 (4.4)	0.40

Figures in parentheses indicate percentages.

Table 3. **Distribution of obstetric and gynecologic variables**

Variables	P-PROM (n = 87)	Term delivery (n = 52)	Elective term C/S (n = 45)	P value
	cases	controls I	controls II	
Previous preterm delivery (>22 weeks)	13 (14.9)	4 (7.7)	2 (4.4)	0.13
Previous midtrimester miscarriage	5 (5.7)	5 (9.6)	2 (4.4)	0.54
Previous elective abortions	32 (36.8)	11 (21.2)	5 (11.1)	0.004
Conception with intrauterine device	2 (2.3)	–	1 (2.2)	0.56
Uterine malformation	2 (2.3)	–	1 (2.2)	0.56
Polyhydramniosis (ultrasound confirmed)	7 (8.1)	5 (9.6)	5 (11.1)	0.84
First trimester bleeding	16 (18.4)	6 (11.5)	6 (13.3)	0.51
Treatment for preterm labour	21 (24.1)	9 (17.3)	11 (24.4)	0.60
Cervical circlage in this pregnancy	6 (6.9)	2 (3.8)	1 (2.2)	0.46

Figures in parentheses indicate percentages.

The behaviour of women during current pregnancy was not different among cases and controls (Table 2).

In univariate analysis, only a significant difference in gynecologic and obstetric history was found between cases and controls II in the incidence of previous elective abortions (Table 3). Cases with P-PROM were nearly five times more likely to experience elective abortions than were patients delivering by elective term C/S (36.8% *versus* 11.1%, OR 4.65, 95% CI 1.59–16.48, p = 0.004). The corresponding figures between cases and controls I didn't a reach statistically significant difference (36.8% *versus* 21.2%, OR 2.17, 95% CI 0.92–5.20, p = 0.08).

Analysis of individual infections during current pregnancy indicated that positive amniotic fluid culture and Gram stain, nonyeast vaginitis and lower respiratory infections in this pregnancy were associated with preterm PROM delivery (Table 4). There were no differences in the incidence of individual infections between two control groups.

Univariate analysis indicated that women with preterm PROM delivery were 2.3 times more likely to have positive amniotic fluid culture than women normally delivering at term have had in early third trimester of pregnancy (95% CI: 1.08–5.04) and 4.3 times than women delivering at term by S/C (95% CI: 1.77–10.66). Corresponding figures for positive amniotic fluid Gram stain were 3.4 (95% CI: 1.53–7.56) and 5.2 (95% CI: 2.22–12.19). The odds for P-PROM delivery increased threefold that for term deliveries (95% CI: 1.30–6.56) and term S/C (95% CI: 1.26–6.97) among women treated for nonyeast vaginitis in this pregnancy, and about nine-tenfold that for women treated for lower respiratory infections (OR 10.6 and 9.2).

Table 5 lists the microorganisms of endocervical flora from the study women. No difference was demonstrated between carriage of endocervical microorganisms in early third trimester in women that delivered at term and endocervical flora at delivery in women that delivered by term C/S. In the uni-

Table 4. **Incidence of individual infections during current pregnancy**

Infections during current pregnancy	P-PROM (n = 87)	Term delivery (n = 52)	Elective term C/S (n = 45)	P value
	cases	controls I	controls II	
Positive amniotic fluid culture	48 (55.2)	18 (34.6)	10 (22.2)	0.001
Positive amniotic fluid Gram stain	66 (75.9)	25 (48.1)	17 (37.8)	0.00003
Nonyeast vaginitis in this pregnancy	45 (51.7)	14 (26.9)	12 (26.7)	0.002
Urinary tract infections in this pregnancy	8 (9.2)	8 (15.4)	6 (13.3)	0.52
Lower respiratory infections	15 (17.2)	1 (1.9)	1 (2.2)	0.002

Figures in parentheses indicate percentages.

variate analysis, significant associations were demonstrated between the positive endocervical culture, positive endocervical culture for two or more organisms, endocervical carriage of some microorganisms (*E. coli, Staphylococcus aureus* and *C. trachomatis*) and preterm PROM (Table 5).

To evaluate the possible independent effects of the presence of different microorganisms and conditions and several maternal factors on the occurrence of P-PROM, a logistic regression model with backward elimination was constructed. Variables that approached significance ($p < 0.05$) with univariate analysis in this study were incorporated into the model. Variables tested in the model were history of elective abortions, marital status, fetal gender, antepartum treatment for lower respiratory infections (pneumonia and bronchitis), antepartum treatment for nonyeast vaginitis, recovery of one or more isolates in endocervical culture, endocervical carriage of *E. coli*, *Staphylococcus aureus* and *C. trachomatis*, subclinical IAI confirmed by positive AF culture or Gram stain. Four factors remained in the model and were significantly related to the development of P-PROM: subclinical IAI, endocervical carriage of *E. coli*, *Staphylococcus aureus* and *C. trachomatis* (Table 6).

Table 6. **Multiple logistic regression analysis of the relationship of individual infections during index pregnancy to P-PROM**

	OR	95 % CI	P value
Subclinical IAI (positive AF culture or Gram stain)	1.27 [1.18]	1.09–1.49 [1.01–1.37]	0.003 [0.04]
C. trachomatis in the cervix	1.20 [1.20]	1.02–1.41 [1.03–1.39]	0.03 [0.02]
E. coli in the cervix	1.23 [1.27]	0.97–1.56 [1.02–1.59]	0.09 [0.04]
S. aureus in the cervix	1.22 [1.30]	0.92–1.63 [1.01–1.72]	0.16 [0.04]

Figures in square brackets denote values after multiple regression analysis adjusted for marital status, neonatal sex and history of previous elective abortions.

Table 5. **Endocervical flora from the women in the study**

Microorganism	P-PROM (n = 87)	Term delivery (n = 52)	Elective term C/S (n = 45)	P value
	cases	controls I	controls II	
None	8 (9.2)	11 (21.2)	12 (26.7)	0.02
≥ 2 microorganisms	52 (59.8)	14 (26.9)	14 (31.1)	0.0001
Gram-positive rods:				
Corynebacterium spp.	8 (9.2)	4 (7.7)	2 (4.4)	0.62
Gram-negative rods:				
E. coli	17 (19.5)	2 (3.8)	1 (2.2)	0.002
Enterobacter spp.	1 (1.1)	1 (1.9)	–	0.66
Klebsiella spp.	2 (2.3)	–	2 (4.4)	0.32
Gram-positive cocci:				
Acinetobacter	–	–	1 (2.2)	0.21
Staphylococcus aureus	11 (12.6)	2 (3.8)	–	0.02
Staphylococcus epidermidis	28 (32.2)	12 (23.1)	11 (24.4)	0.43
Staphylococcus saprophyticus	7 (8.0)	5 (9.6)	4 (8.9)	0.95
β-Haemolytic streptococcus	7 (8.0)	4 (7.7)	2 (4.4)	0.73
α-Haemolytic streptococcus	2 (2.3)	3 (5.8)	2 (4.4)	0.57
Enterococci	10 (11.5)	4 (7.7)	1 (2.2)	0.18
Other streptococcal species	8 (9.2)	2 (3.8)	4 (8.9)	0.48
Yeast				
Candida spp.	7 (8.0)	9 (17.3)	8 (17.8)	0.16
STD organisms:				
Trichomonas vaginalis	1 (1.2)	–	–	0.57
C. trachomatis	41 (47.1)	12 (23.1)	6 (13.3)	0.0001

Figures in parentheses indicate percentages.

DISCUSSION

In this study we have shown that IAI and vaginal carriage of *C. trachomatis, E. coli, Staphylococcus aureus* immediatelly before delivery are associated with an increased risk of P-PROM. These associations remained valid after taking account of other obstetric and demographic variables such as marital status, neonatal sex, previous elective abortions. The presence of one or more of these conditions in late second or early third trimester may therefore be considered predictive of PROM.

The role of urinary tract infections (UTI) in preterm birth remains unclear (12–14). Studies appear divided as to those that show a positive association between UTI and preterm birth and those that show no association. Univariate analysis of our findings has not found antenatal UTI to be a significant risk factor for P-PROM, possibly due in part to the small number of women having UTI in this pregnancy as well as antenatal adequate treatment given to all urine culture-positive women.

Romero and co-authors (15, 16) reported that P-PROM was significantly more likely to develop in women with positive marker of infection in amniotic fluid. These findings, in addition to our own, support the hypotesis that the incidence of subclinical IAI is higher in patients with P-PROM.

Attempts to identify and correlate the presence of specific lower and upper genital tract microorganisms or bacterial conditions with P-PROM have yielded inconclusive findings. Previous studies were not simultaneously controlled for other risk variables and have shown an inconsistent association between cervical *C. trachomatis* infection and P-PROM. In agreement with some studies (8, 9, 17) we found that *C. trachomatis* infection was statistically significantly associated with an increased risk of P-PROM, while no such association has been shown in others (18, 19). Harrison et al. (18) found an increased risk of PROM only among a subgroup of their patients who had specific IgM to *C. trachomatis.* Although serologic testing was not performed in our study, we can suppose that our patients had a more recent or primary infection rather than a reinfection.

Another significant finding is that two of enteropharyngeal organisms, *E. coli* and *S. aureus,* previously shown to be associated at the time of labour with preterm birth but not commonly sought in women at risk of P-PROM, in this study became significantly associated with P-PROM after a multiple regression analysis adjusted for marital status, previous elective abortions, and neonatal sex. Both these organisms are protease-releasing. Bacterial proteasis can clearly impair the structural integrity of fetal membranes and predispose to rupture (20–22).

Interestingly, the presence of two or more microbial isolates in the cervix in univariate analysis was significantly associated with P-PROM, suggesting that whereas an individual infection or microbial condition may not have been powerful or frequent enought to be consistently associated with P-PROM in this cohort, combinations of microbial conditions may contribute to a significant number of P-PROM.

Findings of this study suggest that P-PROM is associated independently with a wide range of common and readily identifiable genital tract infectious agents. Whether selective treatment of one or more of these infections can effectivelly reduce occurrences of P-PROM is the subject of ongoing investigations. We hope the findings from this and other studies will help physicians develop health care delivery strategies to identify and counsel patients at risk of P-PROM.

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RIZIKOS VEIKSNIAI, NULEMIANTYS PRIEŠLAIKINĮ GIMDYMĄ DĖL PRIEŠLAIKINIO VAISIAUS DANGALŲ PLYŠIMO

S a n t r a u k a

Šios studijos tikslas buvo patikrinti hipotezę, kad nėštumo metu persirgtos ligos, sukeltos įvairių mikroorganizmų, ir subklinikinė cervikalinė bei intraamniotinė infekcija padidina priešlaikinio gimdymo dėl priešlaikinio vaisiaus dangalų plyšimo riziką nepriklausomai nuo kitų rizikos veiksnių. Norint kliniškai įvertinti nepasireiškiančios gimdos kaklelio ir vaisiaus vandenų infekcijos poveikį priešlaikiniam vaisiaus dangalų plyšimui, buvo atlikta analitinė kontrolinė studija. Atsižvelgus į kitus rizikos veiksnius ir atmetus mažiausią prognozinę vertę turinčius rodiklius, *C. trachomatis* ($p = 0,02$), *E. coli* ($p = 0,04$), *Staphylococcus aureus* ($p = 0,04$) gimdos kalelyje ir vaisiaus vandenų infekcija ($p = 0,04$) išliko patikimai susiję su priešlaikinio gimdymo dėl priešlaikinio vaisiaus dangalų plyšimo rizika.

Raktažodžiai: priešlaikinis vaisiaus dangalų plyšimas, priešlaikinis gimdymas, intraamnioninė infekcija, cervikalinė mikroflora, rizikos veiksniai