PRETERM LABOUR — IS *MYCOPLASMA HOMINIS* INVOLVED?

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Objective. To assess whether *Mycoplasma hominis* is associated with preterm labour in primigravidae and multigravidae with previous midtrimester abortion or preterm labour.

Design. Cohort analytical study.

Setting. Tygerberg Hospital, a tertiary academic hospital in the Western Cape.

Methods. Gram's stains were done on smears taken from the posterior vaginal fornix, at the first antenatal visit, between 16 and 26 weeks' gestation, in primigravidae and multigravidae at risk for preterm labour. Cultures for *M. hominis* and other commonly occurring organisms were done from endocervical swabs taken at the same visit. The outcome of pregnancy in mothers with positive cultures for *M. hominis* was then compared with outcome in women with negative cultures.

Outcome measures. Prevalence of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, bacterial vaginosis and preterm delivery, birth weight and perinatal deaths.

Results. Cultures for *M. hominis* were positive in 83 patients (21%) and negative in 312 (79%). Significantly more mothers in the positive group (40%) delivered before 37 weeks' gestation than in the negative group (28%, $P = 0.0313$). Their babies weighed significantly less (2 669 g v. 2 864 g, $P = 0.0141$). The positive group was also associated with more alcohol use in pregnancy and fewer of them were married. *C. trachomatis* was found in 18% of mothers in the positive group but in 8% of the negative group ($P = 0.0082$). *U. urealyticum* was cultured in 96% of mothers in the positive group in contrast to 81% in the negative group ($P = 0.001$). Bacterial vaginosis was observed on 75% of mothers with positive cultures for *M. hominis* but in 22% with negative cultures ($P = 0.00001$, odds ratio 10.21, 95% confidence interval: 5.63 - 18.65).

Conclusion. Positive culture for *M. hominis* was associated with more preterm deliveries and also with a higher frequency of *C. trachomatis*, *U. urealyticum* and bacterial vaginosis.

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Preterm labour is the major cause of perinatal mortality at Tygerberg Hospital[1] and a common indication for expensive neonatal intensive care.[2] It is also a major cause of neonatal handicap. It has been found that 11 - 67% of newborns who weighed less than 800 g and survived the neonatal period have major neurological sequelae.[3] The prevalence of preterm delivery, according to a community-based study in the Tygerberg area, is 20.3% (Tygerberg Hospital Obstetric Database — unpublished data). This is very high indeed when compared with the 4 - 9.3% prevalence in developed countries.[4] It is therefore essential to determine possible causes of preterm labour.

METHODS

This study is an extension of a previous randomised controlled trial[5] where primigravidae and multigravidae at risk for preterm labour were screened for bacterial vaginosis (BV) and where mothers with BV were treated with either metronidazole or 100 mg vitamin C as a placebo. From May 1996 to December 1996, 426 primigravidae or mothers who had had a previous preterm labour or midtrimester miscarriage were recruited at the first antenatal visit. Duration of pregnancy at entry to the study was limited to between 16 and 26 weeks. Patients with multiple pregnancies or known cervical incompetence were excluded from the study. Patients who gave a history of taking any antibiotics within the previous 2 weeks were not immediately enrolled but were seen 2 weeks after they had completed the course, providing that they were still less than 26 weeks pregnant.

After informed consent was obtained for the study a general examination was done followed by a speculum examination. Using an Ayer's spatula a posterior fornix smear was taken for a Gram's stain and a wet mount examination/amine test. For the latter a 10% potassium hydroxide solution was used.[6] The wet mount smear was immediately evaluated for prevalence of lactobacilli, clue cells, *Candida albicans* and *Trichomonas vaginalis*. The other slide was flame-dried, Gram-stained (using carbolfuchsin as the counter stain) and immediately examined for lactobacilli, clue cells, *C. albicans* and *Mobiluncus* spp. The presence of lactobacilli and clue cells was scored as described by Spiegel *et al.*[7] to diagnose BV. Two swabs were taken from the endocervix for investigation for *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. These smears

were immediately placed in a Mastazyme-*Chlamydia* transport medium (Davies Diagnostics Pty Ltd, Table View, Cape Town) and *Mycoplasma* broth transport medium (Microbiology Department, Tygerberg Hospital), respectively.

Swabs were then immediately sent to the laboratory for enzyme immunoassay of *Chlamydia* antigens and culture of *M. hominis* and *U. urealyticum*. Specimens for the latter cultures were stored at –6°C for later examination. Specimens were then defrosted and the swabs were vortexed in the *Mycoplasma* broth. Of the *Mycoplasma* broth 0.5 ml was subcultured into fresh *Mycoplasma* broth and 0.5 ml into *Ureaplasma* broth respectively and incubated at 37°C aerobically for 20 hours. Thereafter 0.5 ml of the *Mycoplasma* broth was subcultured into 'A broth' (for subculture of *M. hominis*) and 0.5 ml of the *Ureaplasma* broth into fresh *Ureaplasma* broth. This was incubated aerobically at 37°C for 48 hours. No change in colour indicated negative growth, a pink colour in the 'A broth' indicated positive growth for *M. hominis* and pink in the *Ureaplasma* broth positive growth for *U. urealyticum*.[8]

Ultrasound examinations were done to confirm the gestational age when the estimated gestational age was less than 24 weeks, to locate the placenta, measure the cervical length and exclude beaking of the internal cervical os. The biparietal diameter was used to determine the gestational age or, after 24 weeks, the last normal menstruation or clinical estimation of the fundal height.

After 4 weeks patients who had BV at the first examination had repeat vaginal smears to assess response to the therapy. If BV was again found, another 2-day course of the same drug, as given previously, was prescribed. Patients were followed up for the outcome of the pregnancy. Mothers who were *M. hominis*-positive were then compared with those with negative cultures regarding obstetric history, pregnancy outcome and presence of BV.

The chi-square test with Yates' correction was used to test the significance of the differences between the different groups. Continuous variables were compared with the Student's *t*-test using the Statgraphics and Epi 6 statistical software programme.

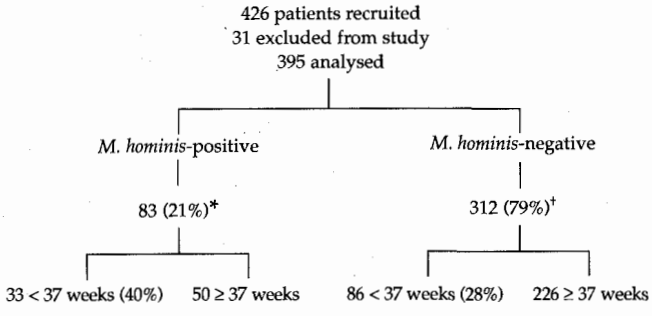
RESULTS

A total of 426 patients were invited to participate in the study. Thirty-one were excluded because laboratory results were lost, inclusion criteria were not met, information on labour was lacking and for various other reasons. Data on 395 patients were analysed, with 21% having positive cultures for *M. hominis* (Fig. 1).

The two groups were comparable regarding obstetric history except for significantly more unmarried mothers and alcohol use in the *Mycoplasma* group (Table I).

Significantly more mothers in the *Mycoplasma* group delivered before 37 weeks' gestation (40% v. 28%). Birth weights were significantly lower in the *Mycoplasma* group (Table II). Mean 5-minute Apgar scores, frequency of caesarean sections, neonatal intensive care unit (NICU) admissions, number of days in hospital and the number of neonatal deaths did not differ between the two groups (Table II).

C. trachomatis and *U. urealyticum* occurred significantly more in the *Mycoplasma* group (Table III). Bacterial vaginosis was found in 75% of mothers where *M. hominis* was cultured from the vagina in contrast to the 22% when the organism was not found (odds ratio (OR) 10.21, 95% confidence interval (CI): 5.63 - 18.65). Bacterial vaginosis was found in 71% of primigravidae with positive cultures for *M. hominis* in contrast to the 22% when cultures were negative (OR 8.95, 95% CI: 3.86 - 21.10). For multigravidae the frequency of bacterial vaginosis was 78% in the *Mycoplasma*-positive group in comparison to 23% in the *Mycoplasma*-negative group (OR 11.94, 95% CI: 4.94 - 29.65).



* One patient delivered before 22 weeks.
† Six patients delivered before 22 weeks.

Fig. 1. Study profile.

Table I. Obstetric history for women in the *Mycoplasma*-positive and negative groups

	Mycoplasma+	*Mycoplasma*–	*P*-value
Number of patients (%)	83 (21)	312 (79)	
Mean age (years) (SD)	24.2 (5.9)	25.3 (6.4)	0.1795
Married (%)	22 (27)	119 (38)	0.0493
Mean gestational age at test (wks) (SD)	19.9 (3.1)	20.2 (2.9)	0.4303
Gravidity (mean (SD))	2.2 (1.5)	2.3 (1.6)	0.5432
Primigravidae (%)	42 (51)	142 (46)	0.4087
Previous preterm labour (%)	30 (73)	122 (72)	0.8571
Previous abortion (%)	19 (46)	80 (47)	0.9341
Previous intra-uterine death (%)	9 (22)	64 (38)	0.0866
Previous caesarean section (%)	8 (20)	25 (15)	0.4469
Alcohol use (%)	18 (22)	27 (9)	0.00089
Cigarette smoking (%)	39 (47)	122 (39)	0.1938

Table II. Outcome of pregnancy in women in the *Mycoplasma*-positive and negative groups

	Mycoplasma+	*Mycoplasma*–	*P*-value
Abortion (< 22 weeks)	1	6	
Delivery < 37 weeks (%)	33 (40)	86 (28)	0.0313
Mean gestational age (wks) (SD)	36.9 (3.4)	37.7 (2.7)	0.0409
Mean Apgar score (SD)	9.3 (1.7)	9.5 (0.9)	0.2920
Mean birth weight (g) (SD)	2 669 (732)	2 864 (654)	0.0141
Caesarean sections (%)	11 (13)	44 (14)	0.8425
NICU admission (%)	7 (9)	14 (5)	0.1215
Mean days in hospital (SD)	4.9 (8.6)	4.1 (8.9)	0.4962
Neonatal death	2	2	0.1941

NICU = neonatal intensive care unit.

Table III. Other infections in women in the *Mycoplasma*-positive and negative groups

	Mycoplasma+	*Mycoplasma*–	*P*-value
Number of patients	83	312	
Chlamydia trachomatis (%)	15 (18)	25 (8)	0.0082
Ureaplasma urealyticum (%)	80 (96)	252 (81)	0.0010
Neisseria gonorrhoeae (%)	1	6	NS
Group B streptococcus (%)	10 (12)	27 (9)	0.3964
Bacterial vaginosis (%)	62 (75)	70 (29) OR 10.21 (95% CI: 5.63 - 18.65)	0.00001

DISCUSSION

We found that *M. hominis* infection was associated with preterm labour. This confirms the finding of two previous studies at this institution.[9,10] It also confirms the finding of Hillier *et al.*[11] that preterm labour occurred more frequently among women with BV and *M. hominis* infections. In another study McGregor *et al.*[12] also found that *M. hominis* was associated with both preterm labour and preterm birth. It also confirms the findings of an older study that this organism and *U. urealyticum* were associated with preterm labour.[13] However, there are also findings suggesting the opposite. In an earlier study McGregor *et al.*[14] could not find a significant association between bacterial vaginosis, *M. hominis* infections and preterm birth or preterm rupture of membranes. A review of 12 studies, published in 1989, could not demonstrate an association between *M. hominis* and prematurity or low-birth-weight babies.[15] These controversial findings may be partially the result of differences in study populations. The high repeat rate of preterm labour in mothers who had previous episodes of preterm labour or midtrimester miscarriage in our study may be due to the fact that we did not treat mothers with *M. hominis* infection. It is also possible that the BV is not directly involved in the causation of preterm labour but that it is only a marker of a more important underlying condition such as *M. hominis* infection, as among women with BV, the highest risk of preterm delivery was found among those with both vaginal bacteroides and *M. hominis*.[11] Until more is known about the aetiology of repeated episodes of preterm labour, care should be taken when treating BV in these patients with metronidazole as a recent large randomised trial involving women with asymptomatic bacterial vaginosis did not show that treatment with metronidazole reduced the occurrence of preterm delivery.[16] It has become necessary to assess whether treatment of *M. hominis* in pregnancy improves fetal and neonatal outcome.

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