The role of mycoplasmas, ureaplasmas and chlamydiae in the genital tract of women presenting in spontaneous early preterm labour

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Summary. The genital carriage of *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Chlamydia trachomatis* was assessed in 72 women admitted to hospital in spontaneous preterm labour and in 26 women requiring preterm delivery for other reasons who formed a control group. Women in preterm labour significantly more often carried ureaplasmas, had large numbers of *M. hominis* and subsequently developed chorioamnionitis than women in the control group. *M. hominis*, in particular, occurred more frequently and in large numbers in women who had chorioamnionitis associated with ruptured membranes. Genital carriage of the various micro-organisms appeared not to be associated with fetal growth retardation, although subsequent isolation of ureaplasmas from infants was common. It is suggested that mid-second-trimester vaginal specimens should be cultured on a research basis to establish whether these various micro-organisms identify women at risk of labouring preterm.

Introduction

The aetiology of preterm labour is multifactorial (Turnbull and Anderson, 1978) and infection is thought to be one of the precipitating factors (Minkoff, 1983). Genital mycoplasmas have been associated with non-specific vaginitis (bacterial vaginosis) and are considered to be one of the causes of pelvic inflammatory disease (PID) and post-abortion and post-partum fever (Taylor-Robinson and McCormack, 1980a). Furthermore, there is increasing interest in the role of genital mycoplasmas in disorders of reproduction, namely infertility, habitual abortion and stillbirth, low birth weight and prematurity (Taylor-Robinson and McCormack, 1980b). Genital immunotypes of *Chlamydia trachomatis* are a cause of cervicitis and PID, with its associated infertility, and are candidates for causal roles in the aforementioned reproductive problems (Taylor-Robinson and Thomas, 1980). To assess the importance of *Ureaplasma urealyticum*, *Mycoplasma hominis* and chlamydiae in perinatal disease, we have conducted

a prospective study of the occurrence of these micro-organisms in the lower genital tract of women presenting in spontaneous early preterm labour compared to their occurrence in a group of pregnant women not in labour but at the same gestational stage.

Subjects studied and methods

Subjects

Ninety-eight women were investigated and informed consent was obtained from all of them. They consisted of a study group of 72 patients and a control group of 26 patients. The study group comprised women who presented in labour between 26 weeks and 33 completed weeks of pregnancy. The control group comprised women who were not in labour but who required abdominal delivery before 34 weeks of gestation because of some fetal or maternal complication of pregnancy.

There were no differences between the study and control groups with respect to age, race, social class, or mean gestational age (table I), but more of the patients in the control group were primigravidae and had smaller babies, and whereas only 28 (39%) of the patients in the study group had intact membranes at the time of investigation, all those in the control group had intact membranes.

Neonatal infection was diagnosed if there was a

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Table I. Comparison of characteristics of patients comprising the study and control groups

Characteristic	Number (%) of patients (or value) in indicated category	
	Study group (72)	Control group (26)
Mean age (SD)	26.6 (6)	27.3 (5)
Race		
Caucasian	49 (68%)	20 (78%)
Asian	8 (11%)	5 (19%)
Negro	16 (22%)	1 (4%)
Social class		
I or II	15 (21%)	6 (23%)
III or IV	45 (62%)	17 (65%)
V or unclassified	13 (18%)	3 (12%)
Parity		
Primigravidae	16 (22%)	13 (50%)*
Primiparae	31 (43%)	16 (62%)
Multiparae	41 (57%)	10 (38%)
Mean gestational age (SD) (days)		
On entry to trial	211 (16)	222 (15)
At delivery	215 (18)	223 (15)
Mean birthweight (SD) (g)	1590 (510)	1383 (363)*

* $p < 0.02$.

positive bacterial culture of neonatal blood, or if there were signs of infection (respiratory distress, early jaundice, apnoea, neutropenia or elevated immature white cell count) in the first 48 h of life sufficient for the infant to receive a 5-day course of antibiotics.

Laboratory methods

Specimens were collected in a similar manner from subjects in the study and control groups by one of the investigators (RFL). Specimens from the study patients were taken at the first vaginal examination after admission in preterm labour and those from control patients in the 24 h before elective delivery. A high vaginal specimen to be examined for ureaplasmas and *M. hominis* was taken with a cotton-wool swab which was expressed in 1.5 ml of mycoplasmal transport medium (Taylor-Robinson and Furr, 1981); this was deemed to be a ten-fold dilution. Polypropylene swabs to obtain surface epithelial cells were used to take specimens from the endocervix for chlamydial culture and were expressed in sucrose-phosphate transport medium (2SP) (Thomas *et al.*, 1977). All specimens in transport medium were stored in liquid nitrogen and examined as a group at the end of the study. The genital mycoplasmas were isolated and identified with media and procedures described previously (Taylor-Robinson and Furr, 1981) and the numbers of these organisms (titres) are expressed as colour-changing units (ccu)/ml of transport medium.

Chlamydiae were sought by inoculation of specimens into cycloheximide-treated McCoy cell cultures, as described previously (Thomas *et al.*, 1977).

Specimens for histological examination were taken as soon as possible after delivery. Umbilical cord (about 1 cm in length), villous plate (about 2 cm³) and membranes (a 3 cm × 0.5 cm strip from the edge of the membrane rupture site to the placental edge) were obtained and placed in formal saline. Smaller samples were taken from these fixed specimens for histological processing and staining with haematoxylin and eosin. At the time of examination, neither the histopathologist nor the microbiologist knew whether the tissues had been obtained from a member of the study group or of the control group. Chorioamnionitis was diagnosed if there was histological evidence of polymorphonuclear leukocyte infiltration into any of six areas of the cord, membranes or placenta. These areas were the cord vessels, the extraplacental membranes, the chorionic plate, the subchorial lake of the villous plate, the villi and the intervillous space. Histological data were available for 58 patients.

The statistical significance of the data was calculated by either Students *t* test or the χ^2 test with Yates's correction for small numbers. Only those probability values which reach statistical significance ($p < 0.05$) are quoted.

Results

Isolation of micro-organisms from patients and controls

Ureaplasmas were isolated from 62 (86%) of the patients in the study group and from 12 (46%) of those in the control group, a difference which is significant ($p < 0.01$). *M. hominis* was isolated from 17 (24%) of the study patients and from 2 (8%) of the control patients, a difference which is not significant. However, these organisms in large numbers ($\geq 10^5$ ccu) were detected in 13 (18%) of the study patients but in none of the control patients ($p < 0.05$).

Chlamydiae were isolated from 5 (8%) of 63 patients in the study group whose specimens could be validly tested, but from none of 21 in the control group. Specimens from 9 of the patients in the study group could not be validly tested because they destroyed the McCoy-cell monolayers.

Relation between carriage of micro-organisms and interval since coitus

The carriage of ureaplasmas, *M. hominis* or chlamydiae in patients in the study group who gave a history of coitus < 48 h or < 7 days, respectively, before study entry was similar to that in those in whom coitus did not take place during these times.

Table II. Isolation of micro-organisms from study patients in relation to the state of the membranes

Micro-organism	Intact membranes			Ruptured membranes		
	Number (%) of patients positive	Number of organisms isolated (ccu/ml)		Number (%) of patients positive	Number of organisms isolated (ccu/ml)	
		range	geometric mean		range	geometric mean
<i>U. urealyticum</i>	22 (79%)	10 ¹ –10 ⁶	10 ^{4.2}	36 (81%)	10 ¹ –10 ⁷	10 ^{4.4}
<i>M. hominis</i>	7 (25%)	10 ² –10 ⁷	10 ^{4.8}	10 (23%)	10 ³ –10 ⁷	10 ^{5.2}
<i>C. trachomatis</i>	1 (4%)	...	...	4 (10%)	...	...

Relation between carriage of micro-organisms and membrane rupture

As shown in table II, the proportions of study patients with and without ruptured membranes who were colonised by ureaplasmas, *M. hominis* or chlamydiae were not different, nor were the numbers (geometric mean ccu/ml) of the genital mycoplasmas in women with and without ruptured membranes.

Relation between carriage of micro-organisms and chorioamnionitis

Histological examination of tissues was undertaken in 58 patients. Patients in the control group did not have ruptured membranes and only two had chorioamnionitis. On the other hand, 44 (61%) of the study patients had ruptured membranes and chorioamnionitis was found two to three times more often in them than in study patients with intact membranes. Irrespective of membrane rupture, the micro-organisms were found about as frequently in study patients who did not have chorioamnionitis as in those who did (table III), although the number of *M. hominis* organisms in

the chorioamnionitis-positive group was larger (geometric mean 10^{5.3} ccu/ml) than in the chorioamnionitis-negative group (geometric mean 10^{3.7} ccu/ml).

In women who had ruptured membranes and who subsequently developed chorioamnionitis, ureaplasmas were present in 15 (75%) of 20, *M. hominis* in 8 (40%) of 20 and chlamydiae in 1 (6%) of 17. In those who had ruptured membranes and who did not develop chorioamnionitis, ureaplasmas were present in 7 (88%) of 8, *M. hominis* in 1 (13%) of 8 and chlamydiae in 1 (11%) of 9. Although the difference in carriage rates for *M. hominis* did not reach statistical significance, it was found three times more often in women who had chorioamnionitis than in those who did not.

Relation between carriage of micro-organisms and mean birthweight and gestational age

As shown in table IV, the mean birthweight and mean gestational age of infants born to mothers who were colonised by ureaplasmas, *M. hominis* or chlamydiae were not significantly different from those of infants born to mothers who were not

Table III. Carriage of micro-organisms in relation to occurrence of chorioamnionitis

Chorioamnionitis	Micro-organism	Indicated micro-organisms in					
		Patients in study group			Patients in control group		
		Number examined	Number positive	Percentage positive	Number examined	Number positive	Percentage positive
Present	<i>U. urealyticum</i>	22	16	73	2	2	100
	<i>M. hominis</i>	22	8	36	2	0	0
	<i>C. trachomatis</i>	17	1	6	2	0	0
Absent	<i>U. urealyticum</i>	12	10	83	19	7	37
	<i>M. hominis</i>	12	3	25	19	2	11
	<i>C. trachomatis</i>	13	1	8	16	0	0

Table IV. Birthweight and gestational age in relation to maternal colonisation

Micro-organism	Mean birthweight* (SD) when micro-organism was		Mean gestation* at birth (SD) when micro-organism was	
	present	absent	present	absent
<i>U. urealyticum</i>	1835 (761) g	1693 (634) g	225 (29) days	216 (21) days
<i>M. hominis</i>	1998 (805) g	1747 (716) g	226 (28) days	223 (29) days
<i>C. trachomatis</i>	1445 (261) g	1834 (798) g	213 (10) days	224 (30) days

* None of the differences shown is statistically significant.

colonised by these micro-organisms. Infants whose mothers were colonised by ureaplasmas had a mean birthweight of 1835 (± 761) g, whereas those born to women who were colonised by both ureaplasmas and *M. hominis* were even heavier (2021 ± 854 g).

Relation between maternal carriage of micro-organisms and perinatal infection

Specimens were obtained from 29 infants. Of 19 women carrying ureaplasmas, six of their infants were also colonised. Two infants were found to be colonised by ureaplasmas although the organisms were not recovered from the mothers. Five women were colonised by *M. hominis* but none of their infants was infected. One woman was colonised by chlamydiae but they were not isolated from her infant. There were 11 perinatal and neonatal deaths, all infants delivered to mothers in the preterm labour group. None of the mothers carried *M. hominis*, one was colonised by chlamydiae and nine were colonised by ureaplasmas.

Discussion

In this prospective study, we attempted to determine the role of ureaplasmas, *M. hominis* and chlamydiae in spontaneous preterm labour. Ninety-eight consecutive patients were examined, there being three times more patients with preterm labour than those serving as controls. This reflects the difficulty of obtaining such control patients even in a referral centre where six times the national average of very low birthweight babies are delivered. Despite the small numbers, women admitted in preterm labour at less than 34 weeks were found to have a significantly increased prevalence of ureaplasma carriage and larger numbers of *M. hominis* organisms than patients in the control group. Isolation of chlamydiae was unique to the study group. The prevalence of genital mycoplasmas in the control group was almost identical to

that seen in normal antenatal patients of similar social class in a study at a district general hospital in the same region in which the current study was conducted—ureaplasmas, *M. hominis* and chlamydiae being present in 42%, 9% and none of the normal subjects, respectively (Ross *et al.*, 1981). This suggests that the figures presented for the prevalence of micro-organisms in the control group in the current study are real and that the difference between the control and study groups is not spurious. This difference in the prevalence of micro-organisms could reflect differences in sexual behaviour, such as promiscuity, which we were unable to determine. The findings, however, appeared to be independent of recent coitus.

The relationships between maternal carriage of the various micro-organisms, the rupture of membranes and the occurrence of chorioamnionitis are complex but may be expressed in the following way. Rupture of membranes seen only in patients in the preterm labour group was apparently not associated with the presence of the genital micro-organisms; they were distributed evenly among those who did and did not have ruptured membranes. If the micro-organisms are not a direct cause of membrane rupture, are they responsible for chorioamnionitis? Chorioamnionitis occurred more often in patients with ruptured membranes and it is reasonable to surmise that the micro-organisms could stimulate an inflammatory response once they had gained access to the membranes. The evidence points to the possibility of *M. hominis* being involved. Although the distribution of the micro-organisms between patients who did and did not have chorioamnionitis was not different, *M. hominis* organisms were present in larger numbers in those who had chorioamnionitis and were present more often than the other organisms in patients who had chorioamnionitis associated with ruptured membranes.

Evidence linking ureaplasmas and *M. hominis* with low birthweight is contradictory. Braun *et al.*

(1971) found that women who were colonised by ureaplasmas gave birth to infants with significantly lower birthweights than did women who were not colonised. Furthermore, the effect of colonisation by ureaplasmas and *M. hominis* was greater than that of ureaplasmas alone. On the other hand, other workers, including Ross *et al.* (1981), have not found such an association. The current study was not initiated with this aspect in mind and the number of patients examined may be insufficient to detect any association. However, neither ureaplasmas nor *M. hominis* was found more frequently in mothers who gave birth to low-weight babies than in those who did not. It is well known that maternal carriage of these micro-organisms leads to colonisation of the newborn and, in this study, 32% and 20% of the infants were colonised by ureaplasmas and *M. hominis*, respectively. Whether they were in any way responsible for the neonatal morbidity that was seen is, of course, debatable.

The finding of a high prevalence of the micro-organisms in women who labour preterm does not necessarily mean a causal relationship. However, a mechanism whereby abnormal genital colonisation might cause preterm labour has been postulated. Bejar *et al.* (1981) have shown that many organisms associated with intrauterine sepsis produce phospholipase A₂, an enzyme that is responsible for the release of free arachidonic acid from cell membranes. Arachidonic acid is the obligate precursor of prostaglandins and these may be responsible for the initiation of labour. Furthermore, using an in-

vitro model, Lamont *et al.* (1985) have demonstrated a rise in the concentration of prostaglandin E in amnion cells in response to bacterial products. This postulated mechanism of preterm labour stimulation assumes that the micro-organisms or their products are anatomically in such a position to bring about these effects, which is possible if they are in the high vagina or cervix. At a more practical level, the question arises as to what our findings might imply. Many scores have been devised to predict preterm labour, but have proved largely unhelpful because of the large number of false positives (Newcombe and Chalmers, 1981). Recently Minkoff *et al.* (1984) carried out a prospective study of the vaginal flora of 233 pregnant women. They found that women who were infected by ureaplasmas began preterm labour significantly more frequently than those who were not infected. This observation, together with our findings, suggests that taking high vaginal swabs in the middle of the second trimester might identify a group of patients at risk of labouring preterm, and that by treating such women with appropriate antibiotics the occurrence of subsequent preterm labour and chorioamnionitis might be reduced. The idea of establishing this point by undertaking such a study in which the existence of *M. hominis* organisms in large numbers only is used as a marker of preterm labour is attractive. This would diminish the magnitude of the laboratory work associated with such a study and at the same time define better the role of *M. hominis*.

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