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Amniotic Fluid Microflora in Asymptomatic Women At Mid-Gestation

REET MÄNDAR[1], KRISTA LÕIVUKENE[2], AIVAR EHRENBERG[3], IMBI SMIDT[1], ELVE RAUKAS[4], VIRVE KASK[3] and MARIKA MIKELSAAR[1]

From the [1]Department of Microbiology, University of Tartu, Tartu, Estonia, [2]United Laboratory, Tartu University Clinicum, Tartu, Estonia, [3]Tartu University Women's Clinic, Tartu, Estonia and [4]Institute of Molecular and Cellular Biology, University of Tartu, Tartu, Estonia

The presence and composition of amniotic fluid (AF) microflora, as well as AF glucose concentration and white blood cell (WBC) count, were investigated in 22 consecutive asymptomatic women with intact membranes at mid-gestation. AF was retrieved by trans-abdominal amniocentesis. Three of the 22 women (13.6%) had microorganisms in their AF: Chlamydia trachomatis in 2 and both Corynebacterium group absolute nonfermenter (ANF) group and Propionibacterium spp. in 1. No differences were found in clinical characteristics, glucose concentration or WBC count in patients with and without microorganisms in their AF.

R. Mändar, MD, Department of Microbiology, University of Tartu, 19 Ravila Street, EE-50411 Tartu, Estonia

INTRODUCTION

In general, amniotic fluid (AF) has normally been considered sterile. AF contains several humoral and cellular antimicrobial factors and its anti-bacterial activity increases during pregnancy. The presence of microorganisms in AF has usually been associated with infection, which may result in spontaneous abortion, pre-term delivery, fetal and neonatal infections or postpartum endometritis (1, 2). However, asymptomatic women with intact membranes may also sometimes (incidence 1.4–24%) have microorganisms in their AF, as shown by several studies (3–5). Recent data suggest that this value is even higher when modern methods, e.g. polymerase chain reaction (PCR), are used to detect microorganisms (6–8). It has been demonstrated that some microbes, e.g. group B streptococci (GBS), Candida spp., Staphylococcus epidermidis and Ureaplasma urealyticum are able to cross intact membranes. Thus, a simple positive smear or culture of AF may indicate not the presence of infection but the translocation of microorganisms from vaginal or cervical microflora. To solve the problem of whether microorganisms found in AF are associated with inflammation several tests for AF samples have been proposed, such as a decrease in glucose concentration and increases in white blood cell (WBC) count and cytokines, etc. (9–11). Most previous investigators have studied intra-amniotic infections during labor (1, 9, 10); less information is available regarding the first half of pregnancy.

The purpose of this investigation was to determine the presence and composition of AF microflora in asymptomatic women with intact membranes at mid-gestation and to compare them with indicators of intra-amniotic infection such as AF glucose concentration and WBC count.

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MATERIALS AND METHODS

Study population

Twenty-two consecutive pregnant women admitted to Tartu University Women's Clinic over a 2-month period for trans-abdominal amniocentesis for genetic studies underwent assessment of the microbiologic status of the amniotic cavity. The mean age of the women was 34.3 y (range 20–42 y) and the mean gestation period 17.3 weeks (range 15–21 weeks). None of the women had received antibacterial treatment during the previous month. All women gave consent for additional microbiological studies of AF.

Retrieval of AF

AF was retrieved by trans-abdominal amniocentesis under antiseptic conditions monitored with ultrasound. Material was collected into a sterile syringe and the needle was then sealed with a sterile rubber stopper. AF was transferred to the laboratory and processed within 2 h.

Laboratory techniques

AF was cultured quantitatively for aerobic, micro-aerophilic and anaerobic bacteria using freshly prepared blood agar, MRS agar (LAB M, Bury, England), Gardnerella vaginalis selective agar (Oxoid, Unipath, Basingstoke, UK) and Fastidious Anaerobe Agar (LAB M). Microorganisms were identified by morphologic criteria, biochemical tests and Analytical Profile Index (API) Coryne (BioMerieux, Marcy l'Etoile, Paris, France). For mycoplasmas and ureaplasmas, the Mycoplasma Identification and Susceptibility Test (IST) (BioMerieux) was used. In order to detect Chlamydia trachomatis, AF was investigated with the PCR method using previously published (12) primers T1 and T2 (produced by GenSet, Paris, France) derived from sequences of an endogenous plasmid present in all known C. trachomatis serovars.

WBC counts were performed in a standard hemocytometer chamber. Glucose concentrations were determined using a Hitachi 912 biochemistry analyzer.

Statistical analysis

The Mann–Whitney U test (for most of the clinical characteristics, WBC count and glucose level) and Fisher's exact test (for indications of amniocentesis and urinary tract infection in anamnesis)

were used to compare the data of patients having or not having microorganisms in their AF.

RESULTS

We succeeded in detecting microorganisms in 3 out of 22 AFs (13.6%). Corynebacterium absolute nonfermenter group (ANF) ($> 10^5$ CFU/ml) was isolated from 1 patient and Propionibacterium spp. (10 CFU/ml) from another. Chlamydia trachomatis was detected in the AF of 2 patients (9.1%), 1 of whom also harbored Propionibacterium spp.

Table I describes the clinical characteristics of patients with and without microorganisms in their AF. We did not find any differences between these 2 groups. In the patient with heavy growth of Corynebacterium spp. in the AF, Down's syndrome of the fetus was diagnosed by genetic studies and this pregnancy was terminated. The other 2 women with microorganisms in their AF delivered healthy babies.

All the women investigated had similar glucose levels in their AF (27–54 mg/dl; mean 40, median 41 mg/dl). The AF WBC count ranged from 2 to 45 cells/mm^3 (median 12, mean 15.8 cells/mm^3). The women harboring microorganisms in their AF had no difference in either glucose concentration or WBC count in comparison with women having sterile AF (Table I).

DISCUSSION

We found microorganisms in the AF of 3 out of 22 patients, a finding consistent with other published reports (3–5). Corynebacterium spp., found in high concentration in 1 patient, has not been reported to be a very frequent isolate from AF cultures. However, it certainly belongs to the normal microflora of the lower genital tract, which is the probable source of AF microorganisms (13, 14). It is interesting that this microbiological finding was correlated with the presence of Down's syndrome; this may be related to altered immune status in cases of this syndrome. Propionibacterium spp. was found in very low concentration (10 CFU/ml) and can apparently originate from abdominal skin.

Controversial data exist concerning the question of whether C. trachomatis can penetrate through intact membranes or cause an intra-amniotic infection. The latest findings suggest a low (but feasible) risk of intra-uterine infection caused by C. trachomatis (15). Our data confirm that this microorganism is able to cross intact membranes.

Urinary tract infection correlates with contaminated AF; however, the difference was statistically non-significant and the number of women studied was too low to enable a final conclusion to be made. Studies have shown that causative agents of urinary tract infection may originate from the lower genital tract (16).

Previous investigators have found that patients with infected AF have significantly higher WBC count (≥ 50 cells/mm^3) and lower glucose level (≤ 16 mg/dl) in their AF than patients without AF infection (9, 10). All AFs studied by us had WBC counts < 45 cells/mm^3 and glucose concentrations > 27 mg/dl; therefore, no undiagnosed infections could be suspected in these women.

None of the women had signs or symptoms of infection or ruptured membranes; however, 3 of them harbored microorganisms in their AF. Corynebacterium spp., if originating from the lower genital tract, could act as a saprophyte not inducing inflammatory reaction. In 2 women found to have C. trachomatis in their AF by PCR we probably succeeded in detecting transitory translocation from the cervix that was suppressed or killed by the antimicrobial properties of the AF and so did not induce an inflammatory reaction.

Our data suggest that most asymptomatic women seem to have sterile AF. However, the proportion of contaminated amniotic fluids detected can be significantly higher if molecular methods are used. More studies are needed to clarify the importance of asymptomatic microbial contamination of AF for the woman and the neonate.

Table I. *Comparison of clinical characteristics, AF WBC count and glucose level between patients with and without microorganisms in their AF ($p = NS$ for all comparisons)*

Clinical characteristic	Sterile AF ($n = 19$)	Microorganisms in AF ($n = 3$)
Maternal age (y; mean ± SD)	34 ± 6.2	36 ± 2.6
Gestational age (weeks; mean ± SD)	17.4 ± 1.5	17 ± 0.0
No. of previous pregnancies (median and range)	2 (0–7)	2 (1–5)
No. of previous deliveries (median and range)	1 (0–4)	1 (0–2)
Indication for amniocentesis as age risk only (numbers and percentage)	13/19 (68.4)	3/3 (100)
Urinary tract infection in anamnesis (numbers and percentage)	2/19 (10.5)	2/3 (66.7)
AF glucose level (mg/dl; median and range)	40.5 (27–52)	49 (43–54)
AF WBC count (cells/mm^3; median and range)	13.5 (2–45)	10 (5–25)

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