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ORIGINAL ARTICLE

Placental histological inflammation and reproductive tract infections in a low risk pregnant population in Latvia

DACE REZEBERGA[1], GUNTA LAZDANE[1], JUTA KROICA[2], LUDMILA SOKOLOVA[3] & GILBERT G.G. DONDERS[4]

[1]*Department of Obstetrics and Gynecology,* [2]*Department of Microbiology,* [3]*Department of Pathology, Riga Stradins University, Latvia, and* [4]*Departments of Obstetrics and Gynecology of the General Hospital Heilig Hart Tienen and the University Hospital Gasthuisberg, Leuven, Belgium*

Abstract
Background. To investigate the correlation of reproductive tract infections (RTI) and endogenous vaginal flora at first antenatal consultation with placental histological inflammation. *Methods*. In a follow-up study, 154 low risk women with no miscarriage risk factors were examined for the presence of *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis*, *Streptococcus agalactiae* (GBS), *Staphylococcus aureus*, *Enterococcus faecalis* (GDS) and bacterial vaginosis (BV). At delivery, outcome data were collected and the histology of the placenta was studied. *Results*. Some 85 (56.3%) of all pregnant women had RTI or endogenous vaginal flora. Placental histological inflammation correlated with genital tract colonisation with *G. vaginalis* ($p=0.013$), BV ($p=0.031$), *S. aureus* ($p=0.04$) and aerobic vaginitis ($p=0.017$). BV and BV-related *G. vaginalis* correlated with the presence of parietal and placental chorioamnionitis in 53.8 and 43.5% of cases. Genital tract colonisation with GDS and other aerobic flora in combination with inflammatory vaginitis correlated with the presence of funisitis in 33.3 and 40.0% of cases. Mycoplasmas increased the risk for intrauterine infection only when present in combination with other RTIs ($p=0.023$). *Conclusion*. Histological placental inflammation is associated with both BV and genital tract colonisation with aerobic bacteria, while funisitis is associated with colonisation of aerobic bacteria at first prenatal visit before the 17th gestational week.

Key words: *Reproductive tract infections, endogenous vaginal flora, aerobic vaginitis, bacterial vaginosis, chorioamnionitis, placenta*

Abbreviations: *AV: aerobic vaginitis, BV: bacterial vaginosis, CA: chorioamnionitis, GBS:* Streptococcus agalactiae, *GDS: Enterococcus faecalis, IL-1: interleukin 1, RTI: reproductive tract infections, STI: sexually transmitted infections, PROM: premature rupture of membranes, PPROM: preterm premature rupture of membranes*

Introduction

Placental histological inflammation is one of the most frequent findings in placental histology, especially if it is performed in cases of preterm birth prior to 30 weeks' gestation. Clinical diagnosis of intrauterine infection is chorioamnionitis (CA). Even though in most situations CA is a subclinical condition and can be diagnosed only by histological examination of placenta (1), placental inflammation is an important type of pathological process, leading to fetal morbidity and mortality caused by microorganisms or caused by host immune response to non-replicating antigens (2). The microorganisms found in fetal membranes in cases of CA are the same as those found in the lower genital tract of pregnant women.

About 4% of infant morbidity in Latvia is due to perinatal infections, and the incidence is still high (3). Perinatal infection rate was 18.5 per 1,000 live mature newborns in 2004, but in preterm newborns

Correspondence: Dace Rezeberga, Department of Obstetrics and Gynecology, Riga Stradins University, Dzirciema Street 16, Latvia. E-mail: dace.rezeberga@ginasoc.lv

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it was as high as 62.9 per 1,000 babies. In 2005, the respective figures were 16.8 and 42.1 per 1,000 (3).

The aim of this study was to investigate the presence of reproductive tract infections (RTIs) (according to the WHO classification of RTIs including sexually transmitted infections (STIs) and overgrowth of endogenous vaginal flora in cases of bacterial vaginosis (BV) or aerobic vaginitis (AV)) and endogenous vaginal flora (4) and their association with placental histological inflammation. Pregnancy and delivery outcome data in relation to inflammatory changes in the placenta were studied.

Materials and methods

A prospective study on the incidence of RTI and endogenous vaginal microbial colonisation and their influence on pregnancy outcome in Latvia was conducted from 1997 to 2002. In total, there were 386 pregnant women included in the study during their first antenatal visit in one of the 5 outpatient clinics in Riga. Patients were consecutively included if they attended the antenatal care clinic on one particular day of the week, depending on the availability of organised transportation. No woman had undergone previous recurrent miscarriages or preterm deliveries. Histological examination of placentas was part of the study protocol. Placentas for investigation were obtained in 154 cases – from all study patients who delivered in Riga Maternity hospital in 1998–2002. The mean gestational age at the first antenatal visit varied from 6 to 12 weeks in 146 cases, and from 13–17 weeks in 8 cases. The research protocol was reviewed and approved by the Ethics Committee of the Riga Stradins University. Before entry into the study, each patient signed an informed consent and completed a specially designed and approved study questionnaire.

Women were tested for BV, *Neisseria gonorrhoeae (N. gonorrhoeae)*, *Trichomonas vaginalis (T. vaginalis)*, as included in the routine antenatal screening program, and additionally for *Chlamydia trachomatis (C. trachomatis)*, *Ureaplasma urealyticum (U. urealyticum)*, *Mycoplasma hominis (M. hominis)*, *Gardnerella vaginalis (G. vaginalis)*, *Streptococcus agalactiae* (GBS), *Staphylococcus aureus (S. aureus)* and *Enterococcus faecalis* (GDS). The samples were obtained from the upper part of the vagina and/or cervix. The results were unknown to the attending gynecologist and pregnant women received treatment only in case of symptomatic BV, presence of N. gonorrhoeae or T. vaginalis as foreseen in the latvian antenatal screening program.

Aerobes were cultivated on Columbia agar with 5% sheep blood. For identification of *S. aureus*, Mannitol salt/Chapman's medium, the catalase test and the Slidex Staph-Kit were used. GBS and GDS were identified by Slidex Strepto-Kit method and Bio Merieux media. Mycoplasma Lyo was used for the cultivation of *M. hominis* and *U. urealyticum*. For examination of *G. vaginalis*, *T. vaginalis* and *N. gonorrhoeae* microscopy method was used with confirmation by DNA hybridisation test for *N. gonorrhoeae* (5). Vaginal fluid collected with a spatula from the posterior fornix was investigated with methylene blue dye and Gram's method. *C. trachomatis* was examined by a DNA hybridisation test.

For diagnosis of BV, Amsel's criteria were used (6). For diagnosis of AV, the following criteria were used: positive culture for aerobic flora, patient's complaint of the presence of abnormal yellow discharge, clinical signs of inflammation in vagina or exocervix, and the presence of leucocytes >10 per high power field (7).

Placental morphology was studied using conventional histological methods. Infiltration of any part of placenta with poly-morpho-nuclear leucocytes was recognised as placental histological inflammation.

Results were analysed using software of statistical analysis SPSS (SPSS Inc., Chicago, IL, USA). Chi-square and Fishers exact test were used and a significance level of 0.05 was required for all tests.

Results

Due to its hematogenous origin, mostly caused by viral infection, three cases of isolated villitis in placental histology were excluded from the final analysis. As expected, RTI was not present in these cases.

In 46/151 (30.5%) women, placental histological inflammation was detected. The screening data at first prenatal visit of these 46 patients (study group) were compared to those of the remaining 105 patients without histological signs of inflammation in the placenta (control group).

In patients with histologically proven signs of placental inflammation, the preterm delivery rate was 4%. The same preterm delivery rate of 4% was observed in the larger study group of 386 patients. Duration of pregnancy at delivery, obstetrical complications and placental inflammatory changes are shown in Table I. The preterm delivery rate was higher in the study group compared to the control group, but the difference was not statistically significant. There were no cases of very preterm birth at gestational age <28 weeks. Antibiotic use during pregnancy, labour or delivery was similar between study patients and controls. Symptomatic patients

Table I. Pregnancy and delivery complications and histological placental inflammation.

	Histological placental inflammation ($n=46$) n (%)	No histological placental inflammation detected ($n=105$) n (%)	p Value
Premature delivery	4 (8.6)	2 (1.9)	0.08
PROM	13 (28.2)	23 (21.9)	0.51
Duration of ruptured membranes (h)			
<12	39 (84.8)	101 (96.2)	0.62
> =12	7 (15.2)	4 (3.8)	0.04
Systemic antibacterial treatment during pregnancy, labour or delivery	13 (28.3)	16 (15.2)	0.13

with BV (25%) all received metronidazole (2 in the study group and 2 in the control group). Clinical signs of CA – maternal fever, maternal and fetal tachycardia, maternal leukocytosis – were only present in 1 case (2.2%) with placental histological inflammation.

The inflammatory changes in the placenta in relation to the genital infections found at the first prenatal visit are shown in Table II. Inflammatory changes in the placenta were more frequent in women with genital tract colonisation with *G. vaginalis* ($p=0.013$), *S. aureus* ($p=0.04$) or the presence of AV ($p=0.023$) at first prenatal visit, as well as in women having clinical BV at that time ($p=0.031$).

The role of genital mycoplasma was analysed separately (Table II). Inflammatory changes in the placenta were found more often in the group of patients with RTI, but the rate of placenta infection was not different whether mycoplasma colonisation was present (34.8%) or not (36.9%). Both *M. hominis* and *U. urealyticum* did not increase the risk for intrauterine infection, unless when concurring with other RTI or abnormal vaginal flora.

The presence of aerobic bacteria was clearly linked with signs and symptoms of AV: abnormal vaginal discharge, red inflamed vagina and increased leukocytosis were all significantly more frequent in the colonised than in the non-colonised group (Table III).

The caesarean delivery rate in the whole group of patients was 10.6% (16/151). There were 7 cases of elective caesarean section – in these cases no histological placental inflammation was detected.

On looking at different sites of placenta involved in inflammation we recognised 33 cases of parietal CA (71.7%), 25 cases of placental CA (54.3%), 15 cases of funisitis (32.6%) and 8 cases of subchorial

Table II. RTI, endogenous vaginal flora and placental histological inflammation.

	Placental histological inflammation ($n=46$) n (%)	No placental histological inflammation detected ($n=105$) n (%)	p Value
Aerobic microorganisms and microflora patterns			
S. aureus	7 (15.2)	4 (3.8)	0.04
GDS	5 (10.9)	7 (6.7)	0.51
GBS	4 (8.7)	4 (3.8)	0.26
Clinical AV	9 (20)	6 (5.7)	0.023
Sexually transmitted microorganisms			
T. vaginalis	1 (2.2)	1 (1.0)	0.52
C. trachomatis	3 (6.5)	2 (1.9)	0.17
Bacterial vaginosis associated organisms or clinical patterns			
U. urealyticum	21 (45.7)	28 (26.6)	0.11
M. hominis	3 (6.5)	5 (4.8)	0.7
G. vaginalis	13 (28.3)	10 (9.5)	0.013
Clinical BV	8 (17.4)	5 (4.8)	0.031
Abnormal cervico-vaginal colonisation (any of the above)			
Without mycoplasma†	17 (36.9)	11 (10.5)	0.002
With mycoplasma	16 (34.8)	15 (14.3)	0.023
Normal cervico-vaginal flora (none of the above)			
Without mycoplasma	6 (13.0)	60 (57.1)	0.0007
With mycoplasma	7 (15.2)	19 (18.1)	0.71

†'Mycoplasma' are *U. urealyticum* and/or *M. hominis*.

Table III. Inflammatory vaginitis in correlation to aerobic vaginal flora.

	One or more aerobic bacteria detected ($n=28$) n (%)	No RTI or BV detected ($n=66$) n (%)	p Value
Patient complains for abnormal vaginal discharge	15 (53.6)	16 (24.2)	0.058
Red inflammation with mucous membrane lesions of vagina and cervix	18 (64.3)	7 (10.6)	0.0001
Abnormal yellow vaginal discharge	16 (57.1)	4 (6.1)	0.00003
Presence of >10 leucocytes in high power field	21 (75.0)	23 (34.8)	0.04

intervillitis (17.4%). Parietal and placental CA was associated with the presence of BV (7/13, 53.8%) and BV-related *G. vaginalis* (10/23, 43.5%), but funisitis was associated with the presence of GDS (4/12, 33.3%) and AV (6/15, 40.0%).

Discussion

The preterm delivery rate among 151 study patients was comparable to the preterm birth rate in Latvia during the same time period (3.9% in 2002) (3). Association between infection and spontaneous preterm labour is well established and thought to be responsible for preterm births in up to 40% of cases (8). One particular causative factor in the pathogenesis of preterm labor is ascending infection from the cervix and vagina, resulting in intrauterine infection, preterm premature rupture of membranes (PPROM) and untractable labour. In most healthy women, a long and closed cervix, thick cervical mucus, intact fetal membranes, and a competent maternal immune system act together to accomplish an effective barrier against the intrauterine migration of normal and pathogenic cervicovaginal flora. In some women, regardless of these protective factors, microorganisms can ascend in the uterine cavity and cause inflammation of the placenta, extraplacental membranes and amniotic fluid. Once passed the cervical barrier, infection may cause diminished placental function, premature rupture of membranes (PROM), preterm delivery and fetal infection, both via the direct impact of infectious agents or via organ damage by release of inflammatory cytokines (2).

However, organisms often reside in the human fetal membranes without harming the pregnancy (9,10), and in 96% of the cases with placentitis; no clinical manifestation of infection was present in another study (10). In our data, histological inflammation of the placenta mainly occurred in women without clinical signs of CA and correlated with duration of ruptured membranes, a finding also described by others (11).

Prenatal abnormal vaginal flora has long been recognised as a major risk factor for intrauterine infection (12). BV is characterised by abnormal vaginal flora (lactobacillary Grade III), with an overgrowth of anaerobic and facultative anaerobic bacteria, such as *G. vaginalis, Bacteroides* spp., *Peptostreptococci, Mobiluncus, U. urealyticum, M. hominis* and a decrease or lack of normal flora, e.g. *Lactobacillus* spp. (6,13). BV is common in normal pregnancy and has been studied as a risk factor for subclinical intrauterine infection (14). The prevalence of BV in Latvia is as low as 5.6% in pregnant women and 75% of all cases are asymptomatic (15). Many authors consider abnormal flora and BV a risk factor for preterm delivery (13,16,17); however, most women with BV deliver at term. Other studies demonstrate that in a low risk population, BV did not increase preterm birth risk (18,19), and in a large randomised, placebo, controlled trial, the treatment with metronidazole of asymptomatic BV in pregnant women did not reduce the risk of preterm delivery or adverse perinatal outcomes (20,21). However, some recent studies using clindamycin early in pregnancy (22) showed a convincing reduction of preterm birth rate. The revised Cochrane database revealed no evidence that treatment with metronidazole reduces the risk of preterm birth, and demonstrated that the only effect of treatment with clindamycin was seen in women with intermediate flora (14). Therefore, a broader spectrum antibiotic other than metronidazole, which acts not only against anaerobic bacteria, but is also active against anaerobes, like clindamycin, is warranted.

Our study confirms the association of BV, *G. vaginalis* and Mycoplasma colonisation with placental histological inflammation. In this small study group, however, patients with BV and inflammatory changes in placenta were not found to have increased rates of preterm birth or poor perinatal outcome. There is probably a subgroup of women with BV who are at particularly high risk for preterm delivery, for instance those who have vaginal colonisation with *M. hominis* (17). Unfortunately, due to the small number of women with *M. hominis* infection, it was not possible to properly evaluate the role of this microorganism on the risk of pregnancy complications in our study. The only

indications for systemic antibacterial treatment during pregnancy for our study patients were urinary tract infections, and treatment consisted of penicillin, but never clindamycin.

In Latvian low risk pregnant women, *M. hominis* and *U. urealyticum* are present in the vaginal tract in 1.5 and 28%, respectively (15). Mycoplasmata are potentially pathogenic microorganisms, responsible for an increased risk in preterm birth, PROM, CA and neonatal infection (23). Whether mycoplasmata are a co-factor of genital infections or have a crucial role in pathology is a matter of debate. We compared the correlation of placental histological inflammation in 3 patient groups: no mycoplasmata found, only *M. hominis* and/or *U. urealyticum* were present, or mycoplasmata in combination with another RTI and/or abnormal endogenous vaginal flora. We found no excess risk due to mycoplasma as a single pathogen, but if combined with RTI, their presence was associated with histological placental inflammation. This confirms the hypothesis that genital tract colonisation of mycoplasmata has to be considered only as a potential marker for an increased risk of infection-related pregnancy complications, rather than a direct cause of intrauterine infection and preterm delivery. In this series, BV increased the risk for histological placental inflammation regardless of the presence of mycoplasmata.

Colonisation with GBS, *S. aureus* or GDS was twice as frequent in parturients with placental inflammation compared to controls. GBS and other aerobic bacteria are, in some symptomatic women, involved in vaginitis with microscopic evidence of vaginal inflammation (24). The clinical and microscopic characteristics of AV differ from BV (7). Donders et al. found dramatic concentrations of pro-inflammatory cytokines, such as interleukin 1 (IL-1) and IL-6, in patients with AV and postulated that AV, when occurring during pregnancy, could be responsible for ascending intrauterine infection and preterm birth (7). The data of our study show that AV posed almost a 4-fold increased risk for inflammatory changes in the placenta, and in 40% of all AV cases funisitis was presented. Severe fetal placental lesions, such as funisitis, correlate highly with neurological impairment and cerebral palsy (25,26).

We conclude that inflammatory changes in the placenta were associated with genital tract colonisation with specific bacteria, such as *S. aureus* and *G. vaginalis*, as well as with AV and BV in early gestation. Mycoplasmas may increase the risk of placental inflammation if found in combination with other RTIs or abnormal endogenous vaginal flora.

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