Journal of Clinical Virology 58 (2013) 559–563

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Preterm prelabor rupture of membranes (PPROM) is not associated with presence of viral genomes in the amniotic fluid

Shubhada Bopegamage[a,*], Marian Kacerovsky[b,c], Vojtech Tambor[c], Ivana Musilova[b,d], Sona Sarmirova[a], Eveline Snelders[e], Arjan S. de Jong[e], Sandor G. Vari[f], Willem J.G. Melchers[e], Jochem M.D. Galama[e]

[a] Enterovirus Laboratory, Medical Faculty, Slovak Medical University, Limbova 12, 83303 Bratislava, Slovak Republic
[b] Department of Obstetrics and Gynecology, Charles University in Prague, Faculty of Medicine in Hradec Kralove, Czech Republic
[c] Biomedical Research Center, University Hospital Hradec Kralove, Czech Republic
[d] Department of Obstetrics and Gynecology, University Hospital Pardubice, Czech Republic
[e] Department of Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[f] International Research and Innovation Management Program, Cedars-Sinai Medical Center, Los Angeles, CA 90048-5502, United States

ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form 28 August 2013
Accepted 12 September 2013

Keywords:
Pregnancy
Premature rupture of membranes
Amniotic fluid
Viral genomes
Pregnancy outcome

ABSTRACT

Background: The role of viral infections in preterm prelabor rupture of the membranes (PPROM) is not established. Studies on the presence of viral genomes in the amniotic fluid (AF) collected in pregnancies complicated by PPROM show contradictory outcomes.
Objectives: To investigate AF samples of PPROM pregnancies for the presence of viral genomes.
Study design: AF samples from patients with PPROM were collected during a 4-year (2008–2012) observational study. 174 women were included with selection criteria of singleton pregnancy, PPROM, and maternal age of 18 years and above. PCR was used for detection of human cytomegalovirus (HCMV), herpes simplex virus (HSV), parvovirus B19, human adenoviruses (HAdV), enteroviruses (EV) and human parechovirus (HPeV). The selection of these viral targets was based on literature regarding screening of AF for presence of viral genomes.
Results: Only a single sample was positive out of the 174 tested AFs, HCMV DNA was detected.
Conclusions: PPROM is not associated with active viral infections.

1. Background

Preterm prelabor rupture of the membranes (PPROM) is defined as rupture of the fetal membranes with leakage of amniotic fluid (AF) at less than 37 gestational weeks, preceding the onset of uterine activity [1]. PPROM occurs in 2–4% of all pregnancies and represents 30–40% of preterm deliveries, which may have serious consequences for pregnancy outcome, particularly when occurring early in pregnancy [1–4].

Intrauterine infections are a well-known cause of preterm birth [3], but microbial invasion of the amniotic cavity (MIAC) is detected in only 20–50% of PPROM pregnancies, depending on the type of detection techniques applied [5–8]. Intrauterine inflammation is also an important feature of PPROM. Inflammation is reflected by elevated cytokines (interleukin (IL)-6, IL-8 and others) in the AF as well as by presence of neutrophils and other immunoactive cells in the uterine wall, placenta and fetal membranes [9]. The diffuse infiltration of the placenta and fetal membranes is termed histological chorioamnionitis (HCA) [10,11]. Although MIAC correlates in most cases with the presence of HCA, many more cases of HCA (approximately 50%) occur without MIAC being detectable [12]. Non-infectious stimuli, e.g. cell- and tissue damage, may inflict HCA [13], but fastidious infections, which are difficult to detect, may still be involved [5]. Viruses, for example, have been investigated to a lesser extent than bacteria in PPROM pregnancies.

The information about viral invasion of the amniotic cavity and subsequent development of HCA is rather conflicting. Some studies reported absence of viral genomes in second trimester AF samples from low-risk populations [14]. In subsequent studies up to 27% of low-risk second trimester AF samples were positive for adeno-associated viruses (AAV) [15] and from zero to 7% for human adenovirus (HAdV) [14–22]. The consequences of detecting viral genomes in AF for pregnancy outcome are also contradictory. Associations with fetal anomalies/malformations and/or preterm birth were reported by some investigators but were not reproduced by others [16–22]. Remarkably, most studies investigated besides the established fetal and/or perinatal viral pathogens parvovirus B19, human cytomegalovirus (HCMV) and herpes simplex virus (HSV),

* Corresponding author. Tel.: +421 2 593 707 77; fax: +421 2 593706 83.
E-mail address: shubhada.bopegamage@szu.sk (S. Bopegamage).

1386-6532/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jcv.2013.09.013

also viruses for which a relationship with the pregnancy outcome is uncertain like human papilloma viruses (HPV), respiratory syncytial virus (RSV), influenza virus, Epstein–Barr virus (EBV), human herpes virus-6 (HHV-6), HAdV, AAV and enteroviruses (EV). The significance of detecting genomes of the latter viruses in AF is unclear and remains a subject of debate warranting further investigation [15–22].

A potential role of viruses in PPROM pregnancies has not extensively been investigated. Their presence could provide an explanation for cases where so far no MIAC was detected. The aim of the present investigation was to study well-defined AF samples from PPROM pregnancies for the presence of viral genomes of HCMV, HSV, parvovirus B19, HAdV, EV, HPeV. The selection of most targets was based on existing literature regarding screening of AF for viral genomes. HPeV was added because of its relatedness to EV.

2. Study design

2.1. Patients and samples

Two hundred twenty two pregnant women at gestational ages between 24+0 and 36+6 weeks with PPROM admitted to the Department of Obstetrics and Gynecology, University Hospital Hradec Kralove, Czech Republic between May 2008 and May 2012 have been considered for this study. Selection criteria were singleton pregnancy, PPROM, and maternal age ≥18 years. PPROM was defined as the leakage of AF prior to the onset of labor, which was diagnosed as described before [7,8,12].

Exclusion criteria were clinical chorioamnionitis, diabetes mellitus, hypertension, preeclampsia, signs of fetal growth restriction, the presence of either congenital or chromosomal fetal abnormalities, signs of fetal hypoxia, or significant vaginal bleeding. Moreover, women with ultrasound markers of subclinical infections (intraamniotic and/or fetal inflammatory response) were excluded but not women with potential signs of infection such as small fetal thymus or pulsatile flow pattern in fetal splenic vein. In all pregnancies, the gestational age was established based on first trimester ultrasound evaluation.

Forty-eight women had incomplete data or inadequate samples for histopathology and/or PCR analysis: the remaining 174 women were included into the study.

In the Czech Republic, women with PPROM at less than 34 weeks of gestation are treated with corticosteroids for the induction of lung maturation, tocolytics for 48 h, and antibiotics, whereas no treatment except antibiotics is initiated to delay delivery after 34 weeks. Management of PPROM women in the Czech Republic differs substantially from most clinical guidelines. Details have been described previously and can be found in a National Guideline [7,12,23].

AF sampling, offered to women with PPROM as a part of our local standard protocol was carried out as described previously [7,8,12]. Ultrasound-guided trans-abdominal amniocentesis was performed on admission prior to the administration of corticosteroids, antibiotics, or tocolytics, and approximately 5 ml of AF was aspirated. Upon collection, AF samples were immediately processed as described earlier [7,8,12]. MIAC was determined by PCR for *Ureaplasma* species, *Mycoplasma hominis*, *Chlamydia trachomatis*, or growth of any bacteria in the AF except for coagulase-negative *Staphylococcus epidermidis*, a skin contaminant. HCA was diagnosed by presence of neutrophil infiltration in the chorion-decidua (Grades 3 and 4), the chorionic plate (Grades 3 and 4), the umbilical cord (Grades 1–4), and/or the amnion (Grades 1–4). Funisitis was diagnosed based on the presence of neutrophil infiltration in the umbilical cord (Grades 1–4) [11]. Histopathological samples were reviewed by a single perinatal pathologist (HH), blinded to the clinical status of the women.

Written informed consent was obtained from all subjects. Ninety-three samples from the present study group had been examined in one of our previous reports on the intraamniotic inflammatory response in a subgroup of women with PPROM [12].

2.2. Detection of targeted viral genomes

The viruses selected for real time PCR testing were HCMV, HSV1, HSV2, Parvovirus B19, HAdV, EV and HPeV. Total RNA/DNA was purified from 174 selected AF samples, each spiked with 5 μl of the isolation control Equine Arthritis virus (EAV) and Phocine Herpes virus (PhHV), which served as internal controls. Total nucleic acid (NA) isolation was performed on the MagNA Pure 96 System, using the *MagNA Pure 96 DNA and Viral NA Small Volume Kit* (Roche Diagnostics) and the *Viral NA Plasma SV protocol*. The input and elution volumes were set at 200 μl and 50 μl respectively. A negative control sample (195 μl PBS) was included in each run. RNAs were reverse transcribed by TaqMan Reverse Transcription Reagents kit (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands) in a 50 μl reaction mix containing 20 μl of NA isolate and random hexamers as primers, as per manufacturer's instructions. All reverse transcription (RT) reactions included a negative RT control (PCR grade H_2O instead of template RNA) and a positive RT control (EAV RNA). Real-time PCR mixes (50 μl) consisted of 25 μl of 2× LightCycler®480 Probes Master (Roche), 0.5 μM of each primer and 0.1 μM of each probe, and 5 μl of cDNA or extracted DNA. Real-time PCR was performed on the Roche LightCycler®480 system, with following conditions for all viral targets: 10 min denaturing and hot-start at 95 °C, followed by 50 cycles of 15 s at 95 °C and 15 s at 55 °C, and 20 s at 72 °C. Each real-time reaction included one negative PCR control sample (5 μl of PCR grade H_2O), and one positive control sample (purified plasmid preparations of the respective PCR products).

3. Results

Table 1 shows the demographics and characteristics of the 174 women with PPROM. PPROM pregnancies were divided in categories with HCA and without HCA. Based on histology of placenta and membranes, 109/174 (63%) of the cases had a diagnosis of HCA (data derived from previous studies) [7,8,12].

In only 44/109 (40%) cases with HCA, microbes could be detected in the AF (MIAC) providing an explanation for the intrauterine inflammation. MIAC was detected by culture for aerobic and anaerobic bacteria and PCR for genital mycoplasmas and *Chlamydia trachomatis* as reported previously [7]. The 65 HCA-negative cases could be further subdivided in cases with (n = 19), or without evidence for MIAC (n = 46). Overall there were 111/174 (64%) patients without MIAC: 65/111 (59%) had the signs of HCA; the remaining 46/111 (41%) were negative for both HCA and MIAC (Table 2).

All 174 AF samples have been tested by a sensitive real-time PCR for presence of HCMV, HSV, parvovirus B19, HAdV, EV and HPeV. Only one sample was positive: CMV-DNA was detected with a load of 5 copies/ml.

4. Discussion

Association between spontaneous preterm delivery and infections has been mainly focused on indigenous bacteria normally present in the vagina, or present due to bacterial vaginosis, and furthermore on genital mycoplasma and/or *Chlamydia trachomatis* infections. Asymptomatic bacteremia has been considered another route of MIAC and evidence therefore was sought in the