Prolonged Maternal Shedding and Maternal-fetal Transmission of Measles Virus

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Key words: measles, placenta, pregnancy, congenital infection

Running title: measles in pregnant women and newborns

Word count: 1535

Key points: Measles in pregnant women is associated with prolonged detection of viral RNA in saliva and blood. Asymptomatic maternal-fetal transmission and congenital infection can occur.

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Abstract

Prolonged measles virus detection in maternal saliva and blood was evidenced in 6 pregnant women. Maternal-fetal transmission was evidenced in 2/4 infants who were asymptomatic at birth, 21-24 weeks after maternal infection. Whereas peripartum congenital measles is severe, asymptomatic measles virus vertical transmission can occur earlier in pregnancy.

Introduction

Measles is re-emerging, despite the availability of a safe and effective vaccine. This is due to the highly contagious nature of measles virus, requiring vaccine coverage > 93% to achieve herd immunity. Suboptimal vaccination has led to a massive resurgence and non-immune adults now account for up to 50% of cases. Measles, that was exceptionally rare in pregnant women, is increasingly reported [1-3]. Available data in the pregnant population mainly focus on measles severity [4, 5]. 10-40% of infected pregnant women develop pneumonia [2, 6]. Mortality (3-15%) is at least twice higher than in non-pregnant patients [3, 5, 6], depending on access to supportive care and comorbidities like HIV infection [7, 8]. Fetal losses or premature deliveries are also reported in 20-60% [4, 5, 9], and maternal viremia within ten days before delivery may lead to congenital measles, a severe condition that warrants administration of immunoglobulins [10]. Data on viral shedding in pregnant women and maternal-fetal transmission at earlier stages are lacking. We retrospectively studied pregnant women and their infants with measles which had been confirmed by serological and molecular methods, to study the duration of viral shedding and maternal-fetal transmission.

Patients and methods

Data collection – Measles is a reportable disease in France. We contacted each physician that reported a case of measles during pregnancy from 05/2012 to 04/2015 to Santé Publique France. Patients with available information were included after informed consent. Physicians at the study site were interviewed to collect medical and follow-up data. Mothers were re-contacted ≥3 years after onset to evaluate infants' outcome. Given its retrospective nature, this study did not require Institutional Review Board approval according to French law, as assessed by our ethical committee (Necker-Enfants Malades Hospital Ethical Committee).

Case definition – Cases were defined as pregnant women with measles, confirmed by measles IgM and IgG in saliva, and/or significant rise in measles serum IgG between acute and convalescent titers, and/or viral RNA detection by reverse transcription polymerase chain reaction (RT-PCR) in saliva.

Characterization of viral isolates – Available saliva, whole blood, cord blood, serum and/or placenta samples from cases and infants were sent to the French National Reference Centre (NRC) for measles. RT-PCR were performed: total RNAs were extracted (EZ1 Advanced XL Qiagen EZ1 DSP Virus kit 48), followed by two rounds of RT-PCR and semi-nested PCR [11]. A highly conserved region located on the N gene of MV genome, coding the carboxy-terminal 150 amino acids of the nucleoprotein, was amplified. The test included primers for RNAse P housekeeping gene. Sequences were analyzed on Sequencing Analysis6 software (Applied Biosystems, USA). Genotyping could be carried out when the cycle threshold (Ct) was < 30Ct. Phylogenetic trees were generated based on Kimura 2-parameter model, the Neighbor-Joining algorithm and 450 nucleotides of the measles virus nucleoprotein using MEGA6 software, using the most recent reference sequences and all named strains of genotype D4 (MN922136-MN922137).

Immunological assays – Specific IgM and IgG antibodies detection in saliva and/or blood samples were performed by capture enzyme immunoassay (Microimmune Clin-Tech kits).

Results

Study population – Six patients were included from 1,017 notified cases, including 218 in women of childbearing age (15-45 years) (features are detailed in **Table 1**). One patient had received a single-dose measles vaccination. Others (5/6) were unvaccinated.

Clinical features – Measles occurred at a median of 19 weeks of gestation (WG) (range, 6-33). All mothers reported rash and fever (median temperature 39°C). Lower respiratory tract involvement (bronchiolitis or interstitial pneumonia) was reported in three (3/6). Four patients required hospitalization in the obstetrical (3/6) or infectious diseases ward (1/6). One patient reported fetal loss attributed to measles at 6 WG (case#6). Others experienced uneventful pregnancy and delivery. All infants were reported healthy at birth, except for one born at 36 WG (16 weeks after maternal infection) with mild uncomplicated jaundice (case #2). As all mothers experienced measles weeks before delivery, no infant was prescribed immunoglobulins. Follow-up data at 3-6-year were available for four children: all exhibited normal growth without reported neurodevelopmental defect, except for one with unexplained tooth enamel damage (case #1).

Maternal biological features – Four maternal cases were identified by positive salivary RT-PCR (cases #1-4). Two were diagnosed by salivary measles IgM (cases #5-6). One patient (case #2) also had positive RT-PCR on whole blood collected 21 days after rash onset; all other maternal sera were negative for measles virus RNA 7, 28 and 40 days after rash onset. Four cases had sequential blood and saliva samples. All exhibited measles-specific IgM in saliva samples collected 1-28 days after onset, with IgG seroconversion before D15 in one and between D5 and 16 in another. Measles virus RNA was constantly detected in saliva samples collected from day 1-28 after rash onset, including in the 4 samples collected from D15-D28. Median time interval between rash onset and last positive saliva sample was 18 days (range 15-28**)**.

Infant biological features – Four neonates had samples at birth : saliva in three, of whom two also had cord blood samples; and cord blood and serum in one. Two infants had strong evidence for maternal-fetal viral transmission: one had viral RNA detected in saliva (21 weeks after maternal infection onset, case#4); another had anti-measles IgM detected in saliva (case #3). Another had anti-measles IgM detected in cord blood but not in serum at birth, suggesting possible contamination with maternal blood (case #2), the last had anti-measles IgM and IgG in the cord blood but no serum collected to decipher whether IgM reflected fetal infection or contamination with maternal blood (case #1). Measles RT-PCR test was positive in 2/2 placentas collected 6-8 weeks after maternal infection onset, with for case #1 a concomitant negative RT-PCR in maternal blood.

Discussion

Here, we analyzed the clinical and biological features of six pregnant women with measles. Maternal clinical presentation is in line with previous European and North American reports [3], and less severe than recently reported from Namibia, where prevalence of HIV infection is 15% [8]. Early fetal loss was reported in one case (17%); all mothers recovered, despite frequent respiratory involvement (33%) and need for hospitalization (67%).

No patient was adequately vaccinated against measles and rubella (n=6), and among multiparous women, none received the catch-up vaccination recommended in France in the post-partum period.

Viral shedding appears prolonged in pregnant women: measles RNA was constantly evidenced in maternal saliva samples collected within 15 days following rash onset, and could be evidenced up to 28 days after onset. In contrast, Riddell *et al*. who analyzed by RT-PCR in saliva samples of 74 adults with measles evidenced viral RNA in only 20% 14 days after onset, and all samples were negative ≥ 20 days after onset [12]. Prolonged viremia in pregnant women had been suggested before by Forthal *et al*., who evidenced persisting viremia in 4/6 adults 6 days after onset, of which 2 pregnant women [13]. In our series, the only patient tested had detectable viremia 21 days after onset. Although detection of viral RNA in saliva or blood does not systematically translate into prolonged contagiousness, our observations suggest that longer droplet precautions is likely required in pregnant women, who frequently seek to the obstetrical ward and may contaminate other non-immune pregnant women [6, 8].

Data regarding measles placental infection are limited to the single observation of viral particles in syncytiotrophoblasts in one placenta [14]. Here, we confirm the presence of viral RNA in 2/2 tested placentas whereas maternal blood was negative in one case, arguing for prolonged fetal-placental measles virus infection after resolution of maternal infection.

We also found evidence for maternal-fetal transmission in 2/4 cases with no symptoms in neonates, although we could not compare viral genome from mother and child. One neonate had measles IgM in the saliva, reflecting fetal/neonatal antibodies toward measles virus; another had viral RNA in the saliva 21 weeks after maternal infection. Congenital measles infection usually manifests as a severe infection occurring as a consequence of maternal viremia in the last ten days of pregnancy. This condition was associated with up to 30% mortality before the immunoglobulins' era, and is associated with an increased risk of subacute sclerosing panencephalitis below 2-years of age [15]. Here, infants with evidence for congenital infection were asymptomatic. None received immunoglobulins, and all reported uneventful evolution at the age of 3 and 6 years, except for one with enamel damages, which have not been ever reported in congenital measles, and are likely not attributable to this condition. Our data suggest that the concept of congenital measles virus vertical transmission should be broadened, and include asymptomatic infections after maternal infections occurring earlier in pregnancy (14 and 33 WG). Whether asymptomatic infants with congenital infection are at risk of long-term neurological complication is unknown. This warrants measles virus detection in saliva

and blood serological assay in neonates born form mother who experienced measles during pregnancy, and their long-term neurological follow-up. The main limitation of this study is its retrospective nature.

In the context of ongoing measles epidemics [1, 16], with vaccine coverage below 90% in most European countries [17], the evidence of prolonged measles virus shedding in pregnant women and the possibility of asymptomatic congenital infections with neonatal viral shedding at birth highlight a so far unsuspected transmission risk, which should be further studied to better define its magnitude and consequences.

Conflict of interest : none.

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Table 1. Main features of the 6 patients included in the cohort. D denotes days after maternal rash onset. Ct denotes Cycle threshold. RT-PCR denotes reverse transcriptase- polymerase chain reaction.

Case identification	Demographical features Past history	Term Gestity, Parity	Clinical and virologic features[1]	Delivery	Infant features[3]
Case #1	38-yr No past medical history No prior measles vaccination	33-WG G2P1	Fever, rash, diarrhea, blurred vision, interstitial pneumonia[2]. 7-d hospitalization and amoxicillin treatment Whole blood RT-PCR negative at D7 and D193 **Saliva RT-PCR positive at D7 (Ct 33.4) and D19 (Ct 34.2)** IgG and IgM positive in the saliva at D7 and D19	Uneventful 41WG	Normal examination at birth Normal neurodevelopmental features at 6-yrs follow-up, but severe unexplained tooth enamel damage **Placenta RT-PCR positive** (Ct 26,5)[5] Cord blood and saliva RT-PCR negative **IgG and IgM positive in the cord blood** IgG avidity in the cord: 19% (29% in a paired maternal sample).
Case #2	25-yr No past medical history Single dose measles vaccination	20-WG G1P0	Fever, rash, bronchiolitis **Whole blood RT-PCR positive at D21 (Ct 36.6)** **Saliva RT-PCR positive at D1 (Ct 19.1)[4] and D15 (Ct 39.5)** IgM positive in the saliva at D1 and D15 IgG negative in the saliva at D1 and positive at D15	Uneventful 36WG	Moderate unexplained prematurity Mild neonatal jaundice attributed to prematurity Normal neurodevelopmental features at 6-yrs Cord blood RT-PCR negative IgG positive in the cord blood and l serum (avidity 52%); IgM positive in the cord blood, negative in the serum
Case #3	23-yr IgA-related vasculitis in childhood	14-WG G1P0	Fever, rash, conjunctivitis Whole blood RT-PCR negative at D28 and D54 **Saliva RT-PCR positive at D28 (Ct 37.4)** IgG and IgM positive in the saliva at D28	Uneventful 40WG	Normal examination at birth Normal neurodevelopmental features at 3-yrs **IgM positive in the saliva**
Case#4	34-yr No past medical history No prior measles vaccination	19-WG G3P1	Fever, rash, interstitial pneumonia[1] 3-d hospitalization **Saliva RT-PCR positive at D5 (Ct 22.6)[5] and D17 (Ct 24.2)** IgM positive in the saliva at D5 and D17 IgG negative in the saliva at D5 and positive at D17	Uneventful 40WG	Normal examination at birth Normal neurodevelopmental features at 6-yrs **Placenta RT-PCR positive** (Ct 37.3) **Saliva RT-PCR positive** (Ct 38.4) (cord blood negative) IgG positive in the cord blood (avidity 43%) IgM negative in the cord blood
Case #5	29-yr No past medical history No prior measles vaccination	26-WG G1P0	Fever, rash, liver cytolysis 1-d hospitalization Whole blood RT-PCR negative at D40 IgM and IgG positive in the saliva at D45	Uneventful 40WG	Normal examination at birth Normal neurodevelopmental features at 6-yrs -
Case #6	26-yr No past medical history No prior measles vaccination	6-WG G1P0	Fever, rash 3-d hospitalization IgM positive un the saliva at D1		**Early fetal loss** -

[1] The Cycle threshold (Ct) value is inversely correlated with the amount of PCR product in the reaction: the lower the Ct value, the more PCR product is present.
[2] Pneumonias were radiologically confirmed. No virologic respiratory sample was performed.

[3] Long-term follow-ups were evaluated through maternal interview that compiled the following information: grade level, past or concomitant follow-up for any disorder including attention/concentration behavioral, language disorders, anxiety, depression, motor or sensorial deficiency).

[4] MeaNS ID: 144212, WHO sequence name: MVs/Labruguiere.FRA/18.12, with an exact match to MVs/Marmande.FRA/43.11/2. Genotype D4.

[5] MeaNS ID: 144213, WHO sequence name: MVs/Eaubonne.FRA/19.12, with an exact match to MVs/Manchester.GBR/10.09. Genotype D4.