

PCR IN THE FIRST OROPHARYNX ASPIRATE OF THE NEWBORN: A POSSIBLE SOURCE FOR IDENTIFICATION OF CONGENITAL INFECTION AGENTS

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SUMMARY

We present a case of prenatal diagnosis of congenital rubella. After birth, in addition to traditional serologic and clinical examinations to confirm the infection, we could identify the virus in the "first fluid aspirated from the oropharynx of the newborn", using polimerase chain reaction (PCR). We propose that this first oropharynx fluid (collected routinely immediately after birth) could be used as a source for identification of various congenital infection agents, which may not always be easily identified by current methods.

KEYWORDS: Congenital infection; Prenatal diagnosis; Postnatal diagnosis; Polimerase chain reaction.

INTRODUCTION

Congenital rubella is now a rare disease thanks to the worldwide vaccination programs³. Nevertheless, the disease has not been extinguished and, given the possible consequences of infection during pregnancy, there is still a need for effective methods for prenatal and postnatal diagnosis.

Prenatally, the diagnosis of congenital rubella is made through analysis of both fetal blood (collected by cordocentesis) and amniotic fluid^{4,9}. The etiologic agent is identified through isolation techniques and/or by application of molecular biology techniques, such as hybridization and PCR^{1,2,3,6,10}.

Postnatal diagnosis is usually made by a combination of serologic findings and/or identification of the virus in body fluids in the newborn³.

In this case report we present our findings in a pregnancy where rubella was diagnosed prenatally and we could demonstrate the presence of the virus in the first fluid collected from the oropharynx of the newborn at birth. To our best knowledge, this is the first report, in the English literature, where this fluid is used for the identification of a congenital infection agent.

CASE REPORT

A 25-year-old primigravida woman presented at a routine prenatal clinic visit in another hospital 11 weeks after her last menstrual period (LMP) with a cutaneous rash and fever. Maternal serology at presentation showed a positive anti-rubella virus IgM and a negative IgG (ELISA, Organon, USA), proving no previous immunity for the disease. The patient was not referred to our clinic until 11 weeks later. The maternal blood sample taken at 22 weeks gestation showed inconclusive IgM and positive IgG (Enzyme Immunoassay, Sorin Biomedica, Italy).

Ultrasound examination at 26 weeks showed that fetal measurements were compatible with 21 weeks and 6 days gestation. Cordocentesis was performed, during which fetal blood and amniotic fluid were collected. Rubella virus RNA was identified in amniotic fluid and fetal blood using a reverse transcription-nested PCR assay (RT-PCR) with the E1 open reading frame¹. In addition, anti-rubella virus IgM was identified in fetal blood, using Enzyme Immunoassay (Sorin Biomedica, Italy). The fetus was trombocitopenic (132,000 platelets/mm³) and presented eritroblastosis (76EOC/100 leucocytes), as well as an increased total IgM. Chromosome analysis showed a normal male karyotype. After the diagnosis, the mother did not

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attended appointments for prenatal care for several weeks. When she finally presented at 39 wks there was oligohydramnios and she was admitted to the hospital for cardiocography and amniocentesis to check lung maturity. The amniotic fluid was still positive for the rubella virus RNA and the fetus was non-reactive after sonic stimuli during the cardiocography or even when observed by ultrasound, after the same sonic stimuli.

A small-for-gestational-age male infant was delivered by cesarean section after failure of labor induction. The infant's weight was 1,500 grams and gestation age, estimated by Capurro, was 37 weeks + 6 days. We explained to the mother that we intended to use fluid routinely aspirated from the oropharynx of the newborn, immediately after birth, to search for the virus and she gave consent for the procedure. To obtain the fluid, a mucosity sterile trap (40cc Specimen Trap, Sherwood Medical, USA), was adapted to a normal vacuum source for aspiration. We obtained 12 ml of this fluid where the rubella virus RNA was detected by PCR. Cord blood was also examined and found to be anti-rubella virus IgM positive. The placenta showed no signs of viral infection. Examination of the newborn revealed microcephaly (bellow 3rd percentile), pulmonary hypertension and deafness.

COMMENTS

Postnatal diagnosis of congenital infections, including rubella, may be difficult in cases where the newborn is asymptomatic and/or IgM is negative in neonatal sera. In these cases, given the decline of virus excretion during the neonatal period, the only alternative method to obtain evidence of infection may be monitoring the IgG levels³. Maternally derived IgG normally disappears six to twelve months after birth. Therefore, evidence of persisting high levels of IgG may provide evidence of congenital infection. Obviously, this approach delays diagnosis and eventually treatment³.

The introduction of PCR technology has improved diagnosis of congenital infections. For example, the experience from prenatal diagnosis of congenital toxoplasmosis has shown that in cases of proved infection, PCR in amniotic fluid is more sensitive than the conventional analysis of fetal blood⁵. This stimulated us to apply PCR to the first aspirate of the oropharynx of the newborn. The underlying assumption was that the first fluid collected during the usual reanimation procedures (immediately after delivery), contains mainly amniotic fluid.

This case report illustrates that the presence of an etiologic agent can be demonstrated in this aspirate. Further studies are needed to confirm the hypothesis that the analysis of this aspirate can improve the sensitivity of neonatal diagnosis (mainly in cases where IgM is negative in neonatal sera) as well as, its correlation with PCR in cord blood and in the oropharyngeal swab of the neonate. It remains also to be established whether agents other than rubella virus can be identified. If this proves to be the case, routine analysis of the first aspirate of the

neonate by PCR may replace the traditional serologic screening methods applied for identification of congenital infections such as toxoplasmosis.

RESUMO

PCR no primeiro fluido aspirado da orofaringe do recém-nascido: uma possível fonte para identificação dos agentes etiológicos nas infecções congênicas

Relatamos um caso de diagnóstico pré-natal de rubéola congênita. Após o nascimento, além da confirmação feita através do exame físico e sorológico do recém-nascido, o vírus também pode ser demonstrado no primeiro fluido aspirado da orofaringe do recém-nascido, utilizando-se a reação em cadeia da polimerase (PCR). Sugerimos que este fluido (colhido rotineiramente no momento da reanimação neonatal) possa ser utilizado na pesquisa de outros agentes infecciosos, que não são facilmente identificados por outros métodos.

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