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Polymerase chain reaction analysis of amniotic fluid for diagnosis of fetal toxoplasmosis

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Summary

Purpose: To assess the efficacy of the polymerase chain reaction (PCR) analysis of the amniotic fluid to diagnose fetal toxoplasmosis. *Material and Methods:* The PCR method was used to test amniotic fluid via the B1 primer for detecting *T. gondii* in pregnant women whose serology was positive for IgM. To validate the method, 84 pregnant women underwent amniocentesis and were followed-up for a period of six years. All the newborns were assessed using serology (IgM), transfontanellar ultrasound, and examination of the fundus. *Results:* The positive PCR rate for the etiologic agent of toxoplasmosis was 17.9% (15 patients) and the rate of newborns in contact with this agent was 16.7% (14 infants). Of these 14 infants, five manifested the disease, while nine only had contact with *T. gondii* with no signs of toxoplasmosis until hospital discharge. Of the five newborns with the disease, three were born to women who had a negative pre-natal PCR. The PCR method had a sensitivity of 78.6%, specificity of 94.3%, positive predictive value of 73.3%, and negative predictive value of 95.6%. *Conclusion:* The PCR method is effective in detecting congenital toxoplasmosis and leads us to question the efficacy of maternal serological status as a diagnostic marker.

Key words: Polymerase chain reaction; Amniotic fluid; Toxoplasmosis.

Introduction

Even today, the diagnosis of fetal toxoplasmosis infection presents a serious challenge to obstetricians, because diagnosis implies invading the fetal environment to obtain fluid. The decision to perform amniocentesis is based on the analysis of maternal serology, which is not always simple to interpret due to the different methodologies used in clinical pathological examinations and serial repetition of serological tests in different laboratories when in doubt [1].

There are many studies that have sought a method to diagnose fetal infection as early as possible. Diagnosis has evolved over time from amniocentesis and inoculation of the amniotic fluid in mice to cordocentesis, with non-specific and specific assessment of the fetal blood, and more recently to the use of the polymerase chain reaction (PCR) method applied to fetal fluids, especially amniotic fluid [2, 3].

This evolution has led to the fulfilment of the true role of the specialist in maternal-fetal medicine: not only to assess the natural course of the disease through ultrasound, but also to take action to preserve the fetus.

Given the aspects presented above, and the fact that an infected fetus is a potentially high-risk patient and deserves differentiated postnatal care, the authors sought to assess the PCR to diagnose fetal toxoplasmosis infection in the amniotic fluid.

Materials and Methods

A retrospective longitudinal study was conducted by analysing the medical records of pregnant women in the Department of Obstetrics as well as the newborns in the nursery at the Neonatal Paediatrics Division of the Federal University of São Paulo (UNIFESP). This study was approved by the UNIFESP Research Ethics Committee.

Inclusion criteria were as follows: pregnant women of any age, parity and gestational age with specific positive serology (IgM) for toxoplasmosis, either reactive or with values above the cut-off level, with indications for amniocentesis and PCR of amniotic fluid, along with the possibility of post-natal follow-up. Exclusion criteria were as follows: withdrawal of the couple during interviews or examinations prior to amniocentesis, multiple gestation, missing the post-natal appointment, fetal death unrelated to toxoplasmosis, termination of pregnancy without formal indication, and patients using spiramycin prior to amniocentesis.

Data about serology (IgM), transfontanellar ultrasound, and examination of the fundus were taken from the medical records of newborns whose mothers had undergone amniocentesis to perform PCR on the amniotic fluid, confirming or dismissing congenital infection (whether manifesting or not) during hospitalization.

Interviews were conducted with all the pregnant women with specific IgM indicating recent and/or current maternal toxoplasmosis in their charts. After the interview, the details and risks of the procedure were explained, and ultrasound was performed to confirm gestational age of >14 weeks and absence of alterations in the amniotic membrane and placental site. Next, amniocentesis was performed. The material was immediately taken to the lab-

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oratory, and the patient was directed to rest for 24 hours and begin treatment with spiramycin (1,000 mg every eight hours), continuing this treatment until returning for the results. Subjects with negative PCR continued spiramycin until the end of pregnancy, and underwent serial ultrasound every three weeks. Positive cases initiated triple therapy with sulfadiazine, pyrimethamine, and folinic acid, alternating every three weeks with spiramycin. The ultrasound was also repeated at three-week intervals. The newborn was considered to have had positive contact with the etiologic agent if changes were seen in the transfontanellar ultrasound, fundus or specific serology (IgM) for acute toxoplasmosis.

Statistical analysis was performed with descriptive determination of sensitivity, specificity, and positive and negative predictive values. Statistical significance was defined by Fischer's exact test, with error fixed at < 0.05 to reject the null hypothesis. The Epi Info statistics program was used to calculate the tests.

Results

The mean gestational age at amniocentesis was 20.61 ± 4.41 weeks. The mean gestational age at amniocentesis was 20.61 weeks, with no statistically significant difference between the results of the punctures performed before or after the 20th week. Of the 84 newborns who underwent transfontanellar ultrasound, changes were only observed in four cases (4.8%).

The positive PCR rate for the etiologic agent of toxoplasmosis was 17.9% (15 patients) and the rate of newborns in contact with this agent was 16.7% (14 infants). Of these 14 infants, five manifested the disease, while nine of them only had contact with *T. gondii* with no signs of toxoplasmosis until the time of hospital discharge.

Of the five newborns with the disease, three were born to women who had a negative pre-natal PCR. There was a rate of 83.3% false positive maternal serology. The PCR had a sensitivity of 78.6%, specificity of 94.3%, positive predictive value of 73.3%, and negative predictive value of 95.6% (Tables 1 and 2).

Table 1. — *Polymerase chain reaction (PCR) positivity in the amniotic fluid and serological examination (IgM) of 84 infants born to pregnant women with positive serology for T. gondii.*

PCR in AF	Newborn serology		Total
	Positive	Negative	
Positive	11	4	15
Negative	3	66	69
Total	14	70	84

PCR: polymerase chain reaction; AF: amniotic fluid. $p < 0.05$.

Table 2. — *Polymerase chain reaction (PCR) results in the amniotic fluid of 14 women with newborns who had contact with T. gondii and manifestation of the disease in this group.*

PCR in the AF	Disease manifestation in the NB who had contact with *T. gondii*		Total
	Yes	No	
Positive	2	9	11
Negative	3	0	3
Total	5	9	14

PCR: polymerase chain reaction; AF: amniotic fluid; NB: newborn. $p < 0.05$.

Discussion

Toxoplasmosis is caused by *T. gondii* and becomes more important when the affected host is a pregnant woman. Infected pregnant women are asymptomatic in most cases, but the disease can cause irreversible harm to the fetus such as microphthalmia, cerebral calcifications, microcephaly, hydrocephalus, mental retardation, hepatosplenomegaly, chorioretinitis, prematurity, growth restriction, and even foetal death [4].

The first choice for maternal treatment is spiramycin; in addition to this agent, materno-fetal treatment uses sulfadiazine, folinic acid, and pyrimethamine, thereby avoiding vertical transmission and fetal sequelae in around 50% of cases [5].

In this study, the patients with positive serology for toxoplasmosis (IgM) were submitted to amniocentesis at different gestational ages, with an average of 20.61 weeks. Among the PCR tests performed before the 20th week, 16.6% (8/48) were positive. Among those performed in the 20th week or later, 19.4% (7/36) were positive, showing that there was no significant statistical difference between the two groups ($p = 0.74$). Thus, it became clear that the gestational age at the time of the puncture did not influence the results, which led the authors to question if vertical transmission truly increased with gestational age.

Treating the fetuses with positive PCR using the alternate scheme was seen to modify neonatal outcome, preventing greater disease involvement in the treated infants compared to the untreated infants. When the authors evaluated the four cases of newborns who had no contact with the causative agent, but presented positive PCR (false positive) in the prenatal examination, they questioned whether the false positives were the result of laboratory contamination of the amniotic fluid sample or whether maternal and fetal treatment had been effective.

The fact that only 16.7% of newborns had evidence of contact with the causative agent, regardless of whether the disease was expressed or not, and that 83.3% of newborns with no evidence of contact with *T. gondii* were born to seropositive mothers, i.e. false positives, led the authors to consider two possibilities: maternal serological false-positives and adequate maternal treatment preventing vertical transmission.

It can be inferred that not all fetuses who had contact with the agent presented the disease due to interference from effective maternal-fetal treatment, reducing the number of sick newborns by 66.8%.

Of the study population, when considering the newborn results (i.e. contagion with the aetiologic agent or not), the authors obtained a sensitivity result of 78.6% and a specificity result of 94.3%, with a positive predictive value of 73.3% and a negative predictive value of 95.6% for the PCR test. The results of this study were similar to those in the literature, showing PCR to be an effective method for prenatal diagnosis of fetal contact with the etiological agent, regardless of whether the newborn manifests toxoplasmosis or not [6].

Conclusion

In toxoplasmosis, time is a determining factor in fetal prognosis. When the clinical presentation does not permit correct interpretation of maternal serology in inconclusive situations, assistance can be obtained as quickly as possible through this method instead of repeating new serologic testing, which causes more confusion than clarification and results in a loss of precious time in maternal-fetal treatment.

References

[1] Gondry J., Chandenier J., Mida M., Aubert S., Boulanger J.C., Vitse M.: "Problèmes pratiques posés par la toxoplasmose en cours de grossesse". *Rev. Fr. Gynecol. Obstet.*, 1989, *84*, 635.
[2] Hohlfeld P., Daffos F., Costa J.M., Thulliez P., Forestier F., Vidaud M.: "Prenatal diagnosis of congenital toxoplasmosis with a polymerase-chain-reaction testo n amniotic fluid". *N. Engl. J. Med.*, 1994, *331*, 695.
[3] Kieffer F., Wallon M.: "Congenital toxoplasmosis". *Handb. Clin. Neurol.*, 2013, *112*, 1099.
[4] Moncada P.A., Montoya J.G.: "Toxoplasmosis in the fetus and newborn: an update on prevalence, diagnosis and treatment". *Expert Rev. Anti. Infect. Ther.*, 2012, *10*, 815.
[5] Serranti D., Buonsenso D., Valentini P.: "Congenital toxoplasmosis treatment". *Eur. Rev. Med. Pharmacol. Sci.*, 2011, *15*, 193.
[6] Saadatnia G., Golkar M.: "A review on human toxoplasmosis". *Scand. J. Infect. Dis.*, 2012, *44*, 805.

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