Case of fatal congenital toxoplasmosis associated with I/III recombinant genotype

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Abstract. We report a case of fatal congenital toxoplasmosis case in Tunis (North of Tunisia) associated with I/III recombinant genotype. The Toxoplasma gondii strain was isolated from placenta and characterized molecularly by a multilocus typing (3'SAG2, 5'SAG2, SAG3, AK69, APICO, and UPRT1) and a sulfadiazine resistance mutation analysis. The isolate was shown to be a wild drug sensitive I/III recombinant strain.

INTRODUCTION

Congenital toxoplasmosis is caused by transplacental contamination of the fetus with Toxoplasma gondii following maternal primary infection during pregnancy. The contamination can cause various degrees of damage ranging from subclinical forms to fetal death. The clinical manifestation depends mainly on the stage of pregnancy at the time of infection. Fetal disease is more severe when contamination occurs early in pregnancy and less severe in the later trimester (Cook et al., 2000).

Disease severity can also be influenced by the parasite strain. Most T. gondii strains belong to three clonal types i.e. I, II and III (Howe & Sibley, 1995). However, some mixed/recombinant types are isolated from African continent (Boughattas et al., 2010) and few atypical genotypes are isolated from exotic areas (Dubey & Su, 2009). These latter strains are reported to induce severe and fatal toxoplasmosis forms (Delhaes et al., 2010). In addition, drug resistance of the parasite could increase the severity of clinical forms (Aspinall et al., 2002). We report in this study the identification and the molecular characterization of recombinant toxoplasma strain isolated after the death of an infected newborn.

Case report

A 31-year-old female living in Tunis (North of Tunisia) pregnant with her second baby was tested for T. gondii infection status. The diagnosis of the first sample at 16 weeks of amenorrhea (WA) was performed by ELISA Kits for IgG and IgM antibodies (Platelia toxo IgG®; Platelia toxo IgM® -Biorad, France). Negative results were obtained showing a seronegative toxoplasma status of the patient and so, the need of her follow-up until delivery. Six weeks later, presence of IgG and IgM antibodies against T. gondii was revealed indicating a seroconversion of the mother at her 2nd trimester of pregnancy. Spiramycin treatment was then initiated. Fetal contamination was investigated by ultrasoundography and by amniotic fluid analysis using real time quantitative (qPCR) and mouse inoculation. Normal fetal ultrasound was obtained. Investigation by molecular method using qPCR, targeting repetitive genes Rep529 and B1, and by classical method using mouse isolation also failed to detect the parasite. At delivery, placenta of the mother was treated with trypsin and...
subjected to qPCR and mouse bioassay. Direct detection was negative; however positive reaction was obtained by the mouse culture. A preterm female baby was born, and a confirmation of the congenital toxoplasmosis case was performed by biological and clinical processes. Western Blot (LDBIO Diagnostics- France) analysis demonstrated specific IgM antibodies synthesis by the newborn (Figure 1) and ophthalmologic finding revealed a chorioretinitis with pigmentary changes. Cardiopathy was also diagnosed leading to the baby’s death at day 30 before any treatment was given. No autopsy was investigated after the death. The different biological and clinical data are summarized in Table 1.

Toxoplasms gondii was isolated from the placenta from the mouse culture. The strain, called PL03/06, was virulent for mice at the time of inoculation: two of the five inoculated mice died 10 days post inoculation (D10pi). The three remaining mice were euthanized at the 4th week after inoculation by cervical dislocation. Anti-toxoplasma antibodies in mice sera samples was positive by indirect immune-fluorescent (IIF) serology, and presence of Toxoplasma cysts in mice brains was observed by microscopy. Molecular method allowed the detection of the parasitic DNA in mouse extracts by PCR amplification. Multilocus genotypic characterization of the strain was performed using multiplex PCR using the following markers 3’SAG2, 5’SAG2, SAG3, Apico (Dubey & Su, 2009), AK69, UPRT1 (Khan et al., 2005) and DHPS (Aspinall et al., 2002). For the last marker, the amplified region encoding amino acid residue 407 was previously described as critical for sulfonamide resistance in T. gondii. Except for the UPRT1 gene, the polymorphism within each locus was analyzed by RFLP patterns to distinguish each strain type. Isolate type were determined by comparison of its profiles to the profiles of the three clonal reference strains. Confirmation by sequencing, using Applied Biosystems 3130 Genetic Analyzer, was investigated for DHPS gene, the polymorphic UPRT1 intron and whenever there were unclear enzyme digestion results. All sequences were deposited in GenBank.

The genetic characterization of our patient’s isolate yielded a combination of type I and III alleles. No digestion was observed in the both extremities 3’ and 5’ of SAG2 gene indicating type I allele. The same allele type I was revealed after double digestion of the Apico PCR product. The product of restriction of the AK69 marker indicated type III allele. The profile obtained after RFLP of SAG3 product showed concomitant presence of the two

Figure 1. Mother-baby comparative immunologic profiles at day 12 after birth by Western blot IgG, IgM. Arrows indicate baby’s new synthesized bands.

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alleles I and III. Sequencing confirmed this result by revealing double pick at the polymorphic sites [GenBank: HM133637, HM133638]. The presence of superposed pick was also observed by UPRT1 gene [GenBank: HM133635, HM133636] indicating existence of more than one allele in the Toxoplasma strain. The sequence analysis of the DHPS gene concurred with RFLP result obtained after restriction by Cfr13I enzyme (Zakimi et al., 2006). In fact DNA sequence analysis of these DHPS fragment revealed that the sample was similar to the wild-type DHPS in respect to amino acid residue 407 [GenBank: HM536197].

**DISCUSSION**

Congenital toxoplasmosis is acquired mainly after the contamination of the mother during pregnancy, and rarely before or due to a reactivation of an old infection (Elbez-Rubinstein et al., 2009). The majority of T. gondii strains isolated in Europe and the United States are within the three main clonal genotypes i.e. I, II, and III with a predominance of type II. However South American isolates have been shown to be highly divergent (Dubey & Su, 2009) and African isolates frequently show mixed/recombinant genotypes (Boughattas et al., 2010). Several studies suggested a correlation between T. gondii genotype and virulence severity (Darde, 2008). Type I strains have been associated with high level of virulence in mice. Types II strains are avirulent in mice and Type III strains show an intermediate virulence. Atypical strains have been shown to be virulent because the majority of mice died 09–30 days after inoculation (Delhaes et al., 2010) instead of 6th week when they should be euthanized.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Target</th>
<th>Used techniques</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>16WA</td>
<td>Serum</td>
<td>ELISA IgG, IgM</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>22WA</td>
<td>Serum</td>
<td>ELISA IgG +: 70 IU/ml, IgM +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34WA</td>
<td>Serum</td>
<td>ELISA IgG +: ≥240 IU/ml, IgM +</td>
<td></td>
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<tr>
<td></td>
<td>35WA</td>
<td>Amniotic Fluid</td>
<td>qPCR, Mouse bioassay</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foetus</td>
<td>Ultrasonongraphies</td>
<td>Normal</td>
</tr>
<tr>
<td>Delivery</td>
<td></td>
<td>Placenta</td>
<td>qPCR, Mouse bioassay</td>
<td>+: Death 2/5 mice at D10pi</td>
</tr>
<tr>
<td>Newborn</td>
<td>Birth</td>
<td>Cord blood</td>
<td>ELISA IgG +: ≥240 IU/ml, IgM –, ISAGA IgM –, Western Blot IgG –, Western Blot IgM –</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 12</td>
<td>Serum</td>
<td>ELISA IgG +: ≥240 IU/ml, IgM –, ISAGA IgM –, Western Blot IgG –, Western Blot IgM –</td>
<td>–: No new bands +: New synthetized bands</td>
</tr>
<tr>
<td>Day 30</td>
<td></td>
<td>Eyes</td>
<td>Ophthalmologic fundi</td>
<td>Pigmentary chorioretinitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 12</td>
<td>Serum</td>
<td>ELISA IgG, IgM –, ISAGA IgM –, Western Blot IgG –, Western Blot IgM –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 30</td>
<td>CARDIOPATHY / DEATH</td>
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associated with such genotypes mainly of I/III type (Genot et al., 2007). However, with regard to congenital toxoplasmosis, only two studies have been investigated in African continent (Hameed & Hassanein 2008; Boughattas et al., 2010), and none of them explored virulence study in mice or correlation with clinical observations. Our current study is the first one in this continent which used these approaches. According to the literature, I/III genotype has never been associated with fatal congenital toxoplasmosis. Even in mice, the new combinations of alleles led to a dramatic increase in virulence, this reinforces the strong argument of a higher virulence of this recombinant/mixed strain, perhaps at the same level of virulence as the atypical strains.

We also studied the sulfadiazine resistance of our isolate. This drug is the major one administrated in confirmed congenital toxoplasmosis cases. It was reported that the alteration in codon 407 of DHPS gene confers substantial resistance to a range of related inhibitors and this is due entirely to the Asparagine - Aspartate change at position 407 (Aspinall et al., 2002). However no mutation at nucleotide sequence of the coding 407 amino acid was observed in our isolate.

In conclusion, there is increasing evidence that severe toxoplasmosis cases are often associated with atypical strains. These strains are often isolated from exotics areas, rarely from Africa. In this continent mixed/recombinant toxoplasmic strains are more frequent. The reports in the literature regarding the correlation between such strains and clinical observations from congenital cases are still not enough to make recommendations for clinical practice. Our study revealed that it may be necessary for physicians to inquire about genotype of T. gondii strains infecting patients. A special awareness is needed when these strains are isolated from congenital toxoplasmosis cases. Their damage may also be increased when a drug resistance is noted.

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REFERENCES


