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ORIGINAL ARTICLE

Evaluation of the western blotting method for the diagnosis of congenital toxoplasmosis☆,☆☆

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KEYWORDS
Congenital Toxoplasmosis;
Western Blotting;
Diagnosis;
Serology

Abstract
Objective: To evaluate the Western blotting method for the detection of IgG anti-*Toxoplasma gondii* (*T. gondii*) (IgG-WB) in the serum of children with suspected congenital toxoplasmosis.
Methods: We accompanied 47 mothers with acquired toxoplasmosis in pregnancy and their children, between June of 2011 and June of 2014. The IgG-WB was done in house and the test was considered positive if the child had antibodies that recognized at least one band on IgG blots different from the mother's or with greater intensity than the corresponding maternal band, during the first three months of life.
Results: 15 children (15.1%) met the criteria for congenital toxoplasmosis and 32 (32.3%) had the diagnosis excluded. The symptoms were observed in 12 (80.0%) children and the most frequent were cerebral calcification in 9 (60.0%), chorioretinitis in 8 (53.3%), and hydrocephalus in 4 (26.6%). IgM antibodies anti-*T. gondii* detected by chemiluminescence (CL) were found in 6 (40.0%) children and the polymerase chain reaction (PCR) for detection of *T. gondii* DNA was

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positive in 5 of 7 performed (71.4%). The sensitivity of IgG-WB was of 60.0% [95% confidence interval (CI) 32.3–83.7%] and specificity 43.7% (95% CI 26.7–62.3%). The sensitivity of IgG-WB increased to 76.0 and 89.1% when associated to the research of IgM anti-*T. gondii* or PCR, respectively.
Conclusions: The IgG-WB showed greater sensitivity than the detection of IgM anti-*T. gondii*; therefore, it can be used for the diagnosis of congenital toxoplasmosis in association with other congenital infection markers.

PALAVRAS-CHAVE
Toxoplasmose Congênita;
Western blotting;
Diagnóstico;
Sorologia

Avaliação do método Western Blotting para diagnóstico de toxoplasmose congênita

Resumo
Objetivo: Avaliar o método Western Blotting para detecção de IgG anti-*Toxoplasma gondii* (*T. gondii*) (IgG-WB) no soro de crianças com suspeita de toxoplasmose congênita.
Métodos: Acompanhamos 47 mães com toxoplasmose adquirida na gravidez e seus filhos, entre junho de 2011 e junho de 2014. O IgG-WB foi feito internamente e o teste foi considerado positivo quando a criança apresentava anticorpos que reconheciam pelo menos uma banda nas manchas de IgG diferente das bandas da mãe ou com maior intensidade que a banda materna correspondente, durante os primeiros 3 meses de vida.
Resultados: 15 crianças (15,1%) atenderam aos critérios para diagnóstico de toxoplasmose congênita e 32 (32,3%) tiveram o diagnóstico excluído. Os sintomas foram observados em 12 crianças (80,0%) e os mais frequentes foram calcificação cerebral em nove (60,0%), coriorretinite em oito (53,3%) e hidrocefalia em quatro (26,6%). Os anticorpos IgM anti-*T. gondii* detectados por quimiluminescência (QL) foram encontrados em seis crianças (40,0%) e a reação em cadeia da polimerase (RCP) para detecção do DNA de *T. gondii* foi positiva em cinco de sete reações realizadas (71,4%). A sensibilidade do IgG-WB foi 60,0% [intervalo de confiança (IC) de 95%, 32,3 a 83,7%] e a especificidade foi 43,7% (IC de 95%, 26,7 a 62,3%). A sensibilidade do IgG-WB aumentou para 76,0 e 89,1% quando relacionada à pesquisa de IgM anti-*T. gondii* ou à RCP, respectivamente.
Conclusões: O IgG-WB mostrou maior sensibilidade que a detecção de IgM anti-*T. gondii*; portanto ele pode ser utilizado para o diagnóstico de toxoplasmose congênita em associação com outros marcadores de infecção congênita.

Introduction

Most children with congenital toxoplasmosis (CT) show no signs or symptoms at birth, yet there is a risk of developing late sequelae, particularly ocular and neurological impairment.[1]

All children are considered suspect whose mothers had acute toxoplasmosis in the course of pregnancy; therefore, these children must be subjected to serological investigation with antibody detection for anti-*Toxoplasma gondii* (*T. gondii*).[1,2]

However, a confirmed serological diagnosis of *T. gondii* infection through the detection of specific IgM and/or IgA antibodies against the parasite does not occur in all newborns.[1,3–5] Therefore, IgG antibodies against *T. gondii* in serial serum samples must be analyzed and the child remains under outpatient follow-up, which can take months until the definitive diagnosis.[1,6]

In an infected fetus, IgG and IgM antibodies produced against the antigenic determinants of *T. gondii* may differ from those anti-*T. gondii* IgG and IgM antibodies detected in the maternal serum, suggesting a neosynthesis of specific antibodies. Thus, children with CT with non-reactivity in conventional tests for IgM detection have been diagnosed in the first months of life through the western blot method (WB).[7–9]

There is a need for early and rapid diagnosis using a low complexity method that allows the reference laboratories for toxoplasmosis to differentiate the dubious results obtained using the routine conventional serological methods, such as indirect immunofluorescence (IIF), enzyme-linked immunoassay (ELISA), and immunoassay of microparticles using chemiluminescence (CL). The objective of this study was to evaluate the WB method for the detection of IgG antibodies against *T. gondii* (IgG-WB) in the serum of the child and his/her mother with acquired