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Detection of IgA Antibody Against *Toxoplasma gondii* in Newborns in Two Hospitals in Malaysia

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Abstract

IgA and IgM antibodies were determined using commercial test kits from cord blood of 550 newborns in two medical centres in Ipoh, Malaysia. One sample was positive for IgA antibody while all were negative for IgM antibody. The single positive IgA was positive for IgG antibody (1:640). The implications of these results are discussed in the light of current guidelines in the prevention and management of toxoplasmosis in pregnancy.

Introduction

Toxoplasma gondii, an apicomplexan intracellular parasite infects many types of tissues, including muscle and intestinal epithelium. It infects many species of warm blooded animals including human (Roberts and Janovy, 2005). Primary infection during pregnancy can induce transplacental transmission of the parasite resulting in congenital toxoplasmosis (Robert-Gangneux and Darde, 2012; Carlier *et al.*, 2012) and the clinical sequelae of the foetus include chorioretinitis, cerebral calcifications and hydrocephalus (Berrebi *et al.,* 2010; Olariu *et al.*, 2011).

Objectives

The aim of this study was to determine the prevalence of IgA and IgM antibodies in cord blood samples in newborns so as to shed some light on the information of *T. gondii* infection in newborns in Malaysia.

Methodology

Two medical centres were selected: Medical Centers (MC) A and B. Both are small hospitals with delivery rates of about 800 and 120 annually, respectively. Universal sampling was used to collect cord blood samples from newborns with consent from the mother. Approximately 5 mL of cord blood were collected using disposable syringes into a tube without anticoagulant from each newborn by trained nurses at each medical centre. This collection was done together with ongoing routine tests done on cord blood for Thyroid Simulating Hormone according to the protocol of National Congenital Hypothyroidism Screening, Ministry of Health, Malaysia. Precautions were taken to avoid contamination of foetal blood by maternal blood. In vaginal deliveries, the second clamp was applied to the umbilical cord as close to the vulva as possible. Cord blood samples were then collected from the segment of cord between the first and second metal clamp. For lower segment caesarean section deliveries, a third metal clamp or artery forceps was applied to the umbilical cord just before the placenta. Cord blood samples were collected from the segment of cord between the second and third metal clamps. A total of 550 cord blood samples were collected, 425 were from MC A and 125 from MC B. Each cord blood was centrifuged at 1,500 rpm for 15 minutes, and the sera were stored at -20^{0}C until use.

Detection of IgA, IgM and IgG antibodies in the cord blood samples was conducted using a double sandwich enzyme-linked immunosorbent assay (ELISA) commercial kit (Platelia, Bio-Rad, France) according to the manufacturer's instructions. The interpretation of IgA and IgM antibodies was based on the sample ratio which was calculated using the formula: Optical Density (OD) of the sample divided by the mean of two OD readings of human serum positive for *T. gondii* IgA or IgM antibody, and the reading was considered positive when the sample ratio was ≥ 1.00. The titre of IgG antibody of the test sample at 1:21 dilution ≥9 IU/mL was considered positive.

This study was approved by the Medical Ethics Committee of Universiti Kuala Lumpur Royal College of Medicine Perak (UniKL/RCMP/PF/2012/UniKL IRPS [183]).

Results

Table 1. Prevalence of IgA and IgM antibodies in cord blood samples collected from newborns at the two Medical Centres

Medical Centre	IgA antibody		IgM antibody	
	No. tested	No. positive	No. tested	No. positive
A	425	1	425	0
B	125	0	125	0
Total	550	1	550	0

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The results of the serological testing of cord blood samples in this study are shown in Table 1. Only one out of 550 cord blood samples was positive for IgA antibody (1.8 per 1,000 live births CI 95%= 0.9/1,000 – 9/1,000), while all were negative for IgM antibody. The IgA positive sample was also positive for IgG antibody which had a titre of 1:640.

Discussion

The detection of IgA antibody response directed against P30, the major surface antigen of *T. gondii*, is useful for early diagnosis of congenital toxoplasmosis (Descoster *et al*., 1988; Descoster *et al.,* 1991; Pinon *et al*., 2001).   In congenital toxoplasmosis, anti-P30 IgA antibodies are detected more frequently than anti-P30 IgM in foetuses and newborns;   IgA antibody being detected more frequently (60%) than IgM antibody [50%] (Bessieres *et al*., 2001). A study conducted in Spain on the detection of IgM, IgA and IgG in the serologic diagnosis of congenital toxoplasmosis had also shown that IgA was the most sensitive indicator for the detection of congenital infection in neonates (Roc *et al.,* 2010). In another study by Gomez-Marin *et al*., 2011, IgA antibody was detected in cord blood samples without IgM antibody. In the progressive development of the foetal immune system, anti-P30 IgM humoral foetal response appears weaker than anti-P30 IgA, this latter being more intense and persisting (Descoster *et al.,* 1991).   This probably explains the findings of our study positive for IgA antibody but negative for IgM antibody.

Postnatal screening of neonates is an approach complementary to prenatal screening and may be an alternative measure in countries where the serologic screening of pregnant women is not implemented (Robert-Gangneux and Darde, 2012).   In view of relatively low seropositivity of 1:550 live-births recorded in this study and high cost associated with the testing, neonatal screening programme for congenital toxoplasmosis might be not recommended in this country. In Canada, owing to low prevalence of the infection and high cost associated with the testing, routine screening for *T. gondii* infection is not recommended for low risk populations (Paquet and Yudin, 2013). The same authors recommend that screening for *T. gondii* infection should be offered to women who are immunosuppressed or HIV-positive because of the risk of reactivation of latent infection. When maternal infection of *T. gondii* acquired during pregnancy is highly suspected during routine ultrasound examination of the mother which shows abnormality of foetus, an amniocentesis is performed after 16 weeks of gestation and the specimen is used for detection of parasite DNA by polymerase chain reaction. In highly suspected cases the current practice is to treat the mother with spiramycin until delivery (Robert-Gangneux and Darde, 2012).

The relatively low seroprevalence of newborns obtained in the present study might be due to selection bias as mothers recruited for the study in both these hospitals were from middle and higher socioeconomic status. Studies conducted in United States and Colombia had shown that an increased risk for toxoplasmosis was associated with lower socioeconomic status of individuals and may be related to employment in jobs with greater soil exposure (Jones *et al*., 2001; Rosso *et al*., 2008). According to case-control studies conducted in Norway and France the risk factors for *T. gondii* infection in pregnancy were due to poor hand hygiene, consumption of raw or undercooked meat, eating unwashed raw vegetables or fruits, having a pet cat and washing the kitchen knives infrequently after preparation of raw meat, prior to handling another item (Kapperud *et al*.,1996; Baril *et al*., 1999). It has been recommended that in countries without prenatal screening, hygienic measures constitute the keystone of prevention of congenital toxoplasmosis and should be largely disseminated to pregnant women (Robert-Gangneux and Darde, 2012). Since there is no serologic screening of pregnant women for toxoplasmosis in this country, it is important that pregnant women be aware of the risks of acquiring *T. gondii* infection through prenatal education in preventing congenital toxoplasmosis. Expectant mothers are advised to wash their hands after touching soils, raw meat or cats or wear gloves when handling these objects. They are also encouraged to consume properly cooked meat, washed or peeled fruits and washed vegetables. Kitchen wares should be washed with soapy water after contacted with raw meat.

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