SHORT COMMUNICATION

Importance of screening serological examination of umbilical blood and the blood of the mother for timely diagnosis of congenital toxoplasmosis and toxocariasis

Milos Veleminsky Jr. [1], Milos Veleminsky [2], Karel Fajfrlik [3], Libuse Kolarova [4]

1 University of South Bohemia in České Budějovice, Faculty of Health and Social Studies, Department of Clinical Branches, Czech Republic
2 University of South Bohemia in České Budějovice, Faculty of Health and Social Studies, the Dean, Czech Republic
3 Charles University in Prague, Faculty of Medicine in Plzeň, Czech Republic
4 Charles University in Prague, Third Faculty of Medicine in Prague, Czech Republic

Correspondence to: Prof. Miloš Velemínský, MD., PhD., Dr.h.c.
University of South Bohemia in České Budějovice,
Faculty of Health and Social Studies,
Jírovcova 24, 37 0 04 České Budějovice, Czech Republic.
TEL: + 420 389 037 500 – 7501; E-MAIL: mveleminsky@tbn.cz

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Abstract

OBJECTIVE: Parasitic diseases, particularly the congenital form of toxoplasmosis, can negatively affect the mortality and morbidity of newborns and infants.
METHODS: The authors examined 152 samples of umbilical blood in 152 women who had experienced premature delivery with or without PROM. The samples were examined for the titre of antibodies – the CFR, levels of immunoglobulins IgA and IGM (toxoplasmosis) and for titres of antibodies against toxocariasis.
RESULTS: No presence of IGM was demonstrated in association with the congenital form of toxoplasmosis.
The values of titres of antibodies against toxocariasis were negative. There was only one case of a titre in a newborn higher than that in the mother. There was no clinical manifestation of the disease.
CONCLUSION: In spite of the negative result, the authors point out the possibility of a timely diagnosis of these parasitic diseases.

INTRODUCTION

Parasitic diseases, particularly the congenital form of toxoplasmosis, can negatively affect the mortality and morbidity of newborns and infants. Thus, the authors evaluated the possibility of the timely diagnosis of toxoplasmosis and toxocariasis based on an umbilical blood examination (Hide *et al.* 2007; Bessièress *et al.* 2001; Signorell *et al.* 2006; Taylor *et al.* 1996).

The target of the work was an assessment of the possibility of a timely diagnosis of congenital toxoplasmosis and toxocariasis from the umbilical blood.

MATERIAL AND METHODS

The authors examined the umbilical blood in 152 women with or without PROM, with the main purpose to establish levels of cytokines. They simultaneously used the blood of the mothers and the umbilical blood for monitoring titres of antibodies against toxoplasmosis and toxocariasis.

Toxoplasmosis

The blood was sampled at the time of delivery. Thereafter, the blood samples were either immediately processed or stored at temperatures up to –20 °C. CFR, specific IgA and specific IgM, were examined to find titres of antibodies against toxoplasmosis. The method is described at Andrew; 144 pairs of sera from mothers and their children were examined. The sera were collected and stored at –20 °C until used. (Andrews 2004); Ondriska *et al.* 2003)

Toxocara canis

E/S antigens in the sera were determined by the Elisa method, by using antigen prepared from the *Toxocara canis*. Ag preparation: *T. canis* excretory/secretory antigens (T E/S) were obtained from the second larvae which were cultured according to the method by de Savigny (1975). Adult females were obtained from naturally infected dogs after administration of Drontal® Plus. After dissection from uteri, the eggs were embryonated in distilled water at RT for 3 weeks. The eggs were then decoated by immersion in 0.1N H_2SO_4. The decoated eggs were washed with distilled water and finally with 0.9% (w/v) NaCl containing 100 IU/ml penicillin, 100 µg/ml streptomycin and 5.6 µg/ml fungiozine. A manual, sterile glass homogenizer was used to break the decoated eggs. The suspension with eggs was centrifuged and immersed in the RPMI (Sigma-Aldrich) and incubated at 37 °C. Spent culture medium containing T E/S was collected every week; the viability of the larvae was also controlled every week and if more than 1% of the larvae appeared non-viable, viable larvae were re-harvested. All the cultures were terminated after 3 months. The aspirated medium was centrifuged at 1,000 × g for 5 min to remove any larvae which might have been aspirated. The supernatants were pooled and stored at –20 °C until used. The spent cultures were dialyzed against 250 volumes of deionised water at 4 °C until phenol red colour disappeared and concentrated by freeze-drying. The protein concentration was determined by Bradford reagent (Sigma-Aldrich). Subsequent analysis by Western blotting showed that the concentrated T E/S contained a mixture of antigens described by Magnaval (1991).The ELISA was carried out with a modification by de Savigni (1985). The polystyrene wells (NUNC, maxisorbe) were coated with 100 µl coating buffer containing T E/S. The plates were incubated in a humidified chamber at 4 °C overnight; the solution with T E/S was then removed by washing the plates three times with 250 µl of 150 mM phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20 (PBS-T). Duplicate aliquots (100 µl) of diluted human sera (1:200) were added and incubated at 37 °C for 1 h; the plates were then washed three times with PBS-T; 100 µl of diluted peroxidase-conjugated anti-human IgG (Sigma-Aldrich) was added to each well, incubated at 37 °C for 1 h and washed three times with PBS-T. The enzyme substrate solution was then added, and the reaction was stopped with 30% H_2O_2 and read at 415 nm. (Savigni 1985; Zástěra *et al.* 1987; Watthanakulpanich *et al.* 2008, Lopez *et al.* 2005)

Tab. 1. 23 cases; the titre of complement fixating antibodies was identical in both the mother and the newborn (toxoplasmosis).

Titre	1:8	1:16	1:32	1:64	Total number of pairs
Identical titres	11	9	2	1	23

RESULTS

A: antibodies against toxoplasmosis

The titre of complement fixating antibodies was identical in both the mother and the newborn (toxoplasmosis) (Table 1).

A total of 144 pairs of sera (sera from the mother and child) were examined. The titres were positive (1:8–1:64). Thus, there was no case of antibodies produced by the newborn; they were produced by the mother (Table 2). Due to this, IgM levels are not presented.

In all the cases of the serum positivity in CFR, specific immunoglobulins IgG were also positive.

In none of the cases, there were positive specific immunoglobulins IgM.

B: Antibodies against toxocariasis

A total of 144 pairs of blood (the blood of the mother and the umbilical blood) were examined for antibodies against toxocariasis. Low titre values in the mother and intermediate values in the venous umbilical blood occurred in only one case, where the birth-weight of the newborn was of 2,200 g; the birth-length was of 45 cm. The newborn exerted no pathological signs and belonged to a group of children with low birth-weights.

Tab. 2. 18 cases; the titre of complement fixating antibodies in mothers' titres higher by 1-2 than the newborns.

Titre: mother × child	1:8 × neg.	1:16 × 1:8	1:32 × 1:8	1:32 × 1:16	1:64 × 1:32	Total number of pairs
Number	1	6	6	4	1	18

Congenital toxocariasis is usually described in association with abortion (Lee *et al.* 1976; Magnaval *et al.* 1991). In the group studied here, abortions were not examined.

Primary infection of the mother in pregnancy presents a danger of the transfer of infection through the placenta to the foetus at a rate that can achieve up to 50% in untreated primary infections. This form of toxoplasmosis in children is referred to as congenital.

The positivity of serological reactions is continuously monitored together with other examinations in the mother as well as in the child immediately after the delivery and also at a later age. This monitoring is necessary for a possible differential diagnosis. The assessment of the titres of antibodies against toxocariasis and toxoplasmosis in the umbilical blood could be a further part of the algorithm for the timely diagnosis of these diseases. (Bessières *et al.* 1992; Di Carlo *et al.* 2007; Foulon *et al.* 1999; Gołab *et al.* 2002; Remington *et al.* 2004)

CONCLUSION

The authors present results of the screening examinations of the titre of antibodies in toxoplasmosis and toxocariasis. They assume that they can be useful in the timely diagnosis of congenital forms of these diseases.

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