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INCIDENCE OF CONGENITAL TOXOPLASMOSIS IN SOUTHERN BRAZIL: A PROSPECTIVE STUDY

Liége MOZZATTO(1) & Renato SOIBELMANN PROCIANOY(2)

SUMMARY

The study aimed to determine the incidence of congenital infection by *Toxoplasma gondii* and to describe neonatal and maternal characteristics regarding newborn infants treated at a teaching hospital in the town of Passo Fundo, State of Rio Grande do Sul, Brazil. Cord blood samples collected from 1,250 live newborns were analyzed. The laboratory diagnosis was established by the detection of *Toxoplasma gondii* IgM using an enzyme linked fluorescent assay. Gestational age, intrauterine growth, anthropometric measures, and prenatal characteristics were assessed. The incidence of congenital toxoplasmosis at birth was 8/10,000 (95%CI 0.2-44.5). Mean birthweight was 3,080 ± 215.56 grams and mean gestational age was 38.43 ± 1.88 weeks. With regard to prenatal care, 58% of the pregnant patients visited their doctors five times or more and 38.9% were serologically tested for toxoplasmosis in the first trimester of pregnancy. The incidence of congenital toxoplasmosis was similar to that found in most studies conducted in our country and abroad. Our study sample is representative of the town of Passo Fundo and therefore it is possible to consider the frequency observed as the prevalence of the disease in this town during the study period.

KEYWORDS: Congenital infection, Toxoplasmosis, Neonatal screening.

INTRODUCTION

Toxoplasmosis is a ubiquitous zoonosis caused by *Toxoplasma gondii*. The disease was discovered by Splendore, in Brazil, and Nicolle and Manceaux, in Tunisia, in 1908. In 1923, Janku described the congenital form of the disease in an infant with hydrocephalus and microphthalmia[25]. Individuals usually get infected consuming contaminated water and food containing oocysts, or by ingesting uncooked or undercooked meat containing tissue cysts. Other forms of transmission include blood transfusions, organ transplantation and laboratory accidents. Fetal transmission occurs hematogenously through the placenta[9,25].

Fetal involvement results from acute infection in the mother; however, those mothers with chronic infection can transmit the disease by reactivation, which is caused by an immunological dysfunction[4,25]. The rate of fetal transmission during primoinfection is 25%, 54% and 65%, in the first, second and third trimesters, respectively[9]. The treatment of pregnant women is believed to prevent serious sequelae in newborn infants, but does not prevent fetal transmission; therefore, the outcome is certainly better the earlier the treatment[23].

'The severity of the disease is inversely proportional to gestational age. Fetal involvement is more severe when mothers get infected at the beginning of pregnancy. If transmission occurs after the 30th week up to term, the forms of the disease are milder[9]. The clinical status varies enormously. Approximately two thirds of newborn infants are asymptomatic at birth, but can develop sequelae, such as microcephalia, hydrocephalus, chorioretinitis, uveitis, deafness and delayed psychomotor development, at a later time[28]. The classical tetrad of hydrocephalus, chorioretinitis, intracranial calcifications and mental retardation is not necessarily present in all cases. REMINGTON *et al*.[25] have classified the disease into four forms: subclinical infection, neonatal disease, disease in the first months of life, and sequelae.

Serological tests, especially immunoenzyme assays, are the routine exams for the diagnosis of the disease. The detection of IgM and/or IgA and/or IgE anti-toxoplasma antibodies in cord blood or in the newborn's blood establishes the diagnosis[9,16,28]. Also, it is possible to detect the parasite in body fluids by isolating it in tissue culture or by inoculation into mice, by the detection of parasitic antigens and toxoplasma DNA using the polymerase chain reaction (PCR)[9,28].

The treatment of congenitally infected infants with pyrimethamine, sulfadiazine and folinic acid, implemented within the first month of life and extended for the first year of life, improves the prognosis, reducing the frequency and severity of later sequelae. Late diagnosis and delayed treatment implementation contribute to remarkable disabilities[13].

The incidence of congenital toxoplasmosis in Passo Fundo is still unknown. Dwellers of this town ingest large amounts of meat, with rates similar to the those of the neighboring town of Erechim, which is known

The present study was carried out at Hospital Universitário São Vicente de Paulo, Teaching Hospital affiliated with the School of Medicine of Universidade de Passo Fundo.
(1) Pediatrician, Head of the Residency Program in Pediatrics, Hospital São Vicente de Paula, Passo Fundo, State of Rio Grande do Sul, Brasil
(2) Professor of Pediatrics, Universidade Federal do Rio Grande do Sul (UFRGS), Head of the Neonatology Division of Hospital de Clínicas de Porto Alegre, Rio Grande do Sul, Brasil.
Correspondence to: Liége Mozzatto, Rua Teixeira Soares 777/608, 99010-081 Passo Fundo, RS, Brasil. E-mail: ped.liege@razaoinfo.com.br

for the high frequency of congenital toxoplasmosis[26]. In 2001, 45% of pregnant women living in Passo Fundo did not have prenatal care or received it in an inadequate manner. This hinders the identification of pregnant women at risk for toxoplasmosis.

The aim of the present study was to determine the incidence of congenital toxoplasmosis at birth in the town of Passo Fundo and to describe neonatal and maternal variables.

MATERIAL AND METHODS

This incidence study was carried out at Hospital São Vicente de Paula, in Passo Fundo, state of Rio Grande do Sul. The analysis of cord blood was conducted at the Lavoisier-Labcenter Laboratory, located in the same town. The screening for *Toxoplasma gondii* DNA in blood and cerebrospinal fluid (CSF) was performed at the DNA Reference laboratory, in Porto Alegre, the state capital. The study was approved by the Scientific and Ethics Committees of the Graduate Research Group of Hospital de Clínicas de Porto Alegre and of Hospital São Vicente de Paula in Passo Fundo.

After consent by the assisting physician and written consent by the patient and her family at admission to the maternity ward, all live newborns were included in the study. After delivery and after birth, 2 ml of cord blood were collected. The samples were centrifuged for 5 minutes at 3,500 rpm, separated, quantitated and stored at -20 °C until later analysis. The serum obtained was screened for IgM anti-toxoplasma antibodies using the Enzyme Linked Fluorescent Assay (ELFA) in the mini VIDAS system (BioMérieux). ELFA is an IgM capture test with a final detection in fluorescence, with the following indices: $i < 0.55$, negative; $i \geq 0.65$, positive and $0.55 \leq i < 0.65$, undetermined. A positive and a negative control were used for recalibration of the device and for the confirmation that reagents were unaltered whenever a new MINIVIDAS TOXO IgM kit was opened.

Next, a questionnaire for the mother/newborn characteristics was filled out, with the following material data: age, socioeconomic status, years spent in school, marital status, number of prenatal consultations, serological tests for toxoplasmosis every three months, type of serological test used, treatment against toxoplasmosis during pregnancy, ingestion of uncooked or undercooked meat, ingestion of unpasteurized milk, uncooked eggs, and presence of cats at home or in the workplace. The data collected for the newborn were the following: weight, gestational age, intrauterine growth, length and head and chest circumference at birth. Aside from the interview, information contained in the prenatal records was used. The socioeconomic status was determined by the Economic Classification of the Brazilian Population[1]. The anthropometric measures were obtained according to the recommended techniques, gestational age was determined by the method of CAPURRO *et al.*[6], and intrauterine growth was assessed by the curve of BATTAGLIA & LUBCHENCO[3].

The protocol included the collection of peripheral venous blood (2 ml in a vacutainer with EDTA) for every newborn infant who tested positive for IgM. In addition, 30 drops of CSF were collected for the investigation of toxoplasma DNA by PCR, for follow-up of IgM titers during neonatal treatment and for the collection of blood from the mother for the quantitation of anti-toxoplasma IgM (ELFA). The salting-out and GFXTM Genomic Blood DNA Purification methods were used for the extraction of DNA from blood and cerebrospinal fluid, respectively. Nested DNA amplification was also employed.

The sample size was calculated as 1,195 newborns considering an incidence of approximately five cases/1,000 live births and a 95% CI. The population rate was estimated by the 95% CI based upon binomial distribution. Neonatal and maternal variables were categorized by simple frequency tables. The mean and standard deviation were calculated for some variables.

RESULTS

Between July 2001 and February 2002, 1,250 cord blood samples were collected. One newborn infant tested positive for IgM at birth (i = 10.13), showed subclinical infection. On the examination of the ocular fundus under mydriasis, the newborn showed bilateral cicatricial chorioretinitis in the temporal region, and the CSF analysis revealed 65 mm³ cells, 24% neutrophils, 76% monocytes, 0% eosinophils, 23 mg% glucose, 109 mg% proteins, and ++ globulins. The incidence of congenital toxoplasmosis was 8/10,000 (95% CI 0.2-44.5). The serological test was repeated in infected newborns on the 5th, 30th, 60th, 90th and 120th days, with respective results of: 10.10, 8.22, 1.8, 0.95 and 0.43. PCR in venous peripheral blood and in CSF was negative. The quantitation of IgM of seropositive mothers was 4.55.

The mean weight and the mean gestational age of the studied sample were 3,080 ± 215.56 grams and 38.43 ± 1.88 weeks. The intrauterine growth of most newborns (72%) was adequate for gestational age and 22.9% of them were below the 10th percentile on the growth curve. Among these, 15.3% were preterm and 7.6% full-term babies. Length, and head and chest circumference are shown in Table 1.

The age of pregnant women ranged from 13 to 46 years (mean 25.6 ± 6.9) and the mean time spent in school was 8.3 ± 2.9 years. The distribution in terms of socioeconomic status and marital status is shown in Table 2. Table 3 shows the eating habits and contact with cats.

Table 1
General characteristics of 1250 infants born in Passo Fundo (RS) between July 2001 and February 2002 regarding weight, gestational age, length and head and chest circumference

Variables	n	%
Birthweight (g)[1]	3,080 ± 215.56	
< 1,500	35	(2.8)
1,500 – 2,499	311	(24.9)
2,500 – 3,999	829	(66.3)
> 4,000	75	(6.0)
Gestational age (weeks)[1]	38.43 ± 1.88	
< 37	208	(16.7)
37 – 42	1,038	(83.0)
> 42	4	(0.3)
Length (cm)[1]	48.5 ± 1.1	
Head circumference (cm)[1]	34.2 ± 1.7	
Chest circumference (cm)[1]	32.3 ± 1.3	

[1] Values expressed as mean and standard deviation

Table 2
Characteristics of pregnant patients who gave birth to 1250 infants in Passo Fundo (RS), between July 2001 and February 2002 regarding socioeconomic status and marital status

Variables	n	%
Socioeconomic status		
A1	7	(0.6)
A2	37	(3.0)
B1	58	(4.6)
B2	91	(7.3)
C	386	(30.9)
D	530	(42.3)
E	141	(11.3)
Marital status		
Single	170	(13.6)
Married	458	(36.6)
Cohabiting	602	(48.2)
Widowed	3	(0.2)
Divorced	17	(1.4)

Table 3
Characteristics of pregnant patients who gave birth to 1250 infants in Passo Fundo (RS) between July 2001 and February 2002 regarding eating habits and contact with cats

Variables	n	%
Contact with cats	696	(55.7)
Ingestion of undercooked meat	463	(37.0)
Ingestion of uncooked meat	791	(63.3)
Ingestion of unpasteurized milk	504	(40.3)
Ingestion of uncooked eggs	960	(76.8)

Only 3.4% of pregnant patients did not receive any prenatal care, whereas 58% visited their doctors more than five times. Of 1,250 pregnant women, 1,001 were tested at least once for toxoplasmosis during the prenatal period, 187 were not submitted to any test, 62 did not know whether they had been serologically tested, and 498 pregnant patients were submitted to more than one serological test during pregnancy. The distribution of the number of pregnant women who were submitted to antitoxoplasma serology per gestational trimester was: 486 in the first, 515 in the second, and 444 in the third trimester. The following serological tests were requested: indirect immunofluorescence (61.3%), MEIA (12.6%) and ELFA (6.2%).

The incidence of toxoplasma IgG and IgM in pregnant women was 48.5% (95% CI 46-51%) and 0.6% (95% CI 0.3-8), respectively.

Six pregnant women received spiramycin and one HIV-positive pregnant patient received pyrimethamine, sulfadiazine and folinic acid during prenatal care. All the infants born to these mothers tested negative for IgM-toxoplasma and none showed any disorder on physical examination at birth.

During the study period, there were 1,735 deliveries in the four town hospitals, of which 1,257 (72.5%) occurred at Hospital São Vicente de Paula. Seven losses occurred during the study, five of which included newborn infants delivered by women who had toxoplasmosis tests during gestation, and none of the newborns showed any disorder on physical examination. The distribution of the study population according to the Economic Classification of the Brazilian Population was as follows: A1 (0.6%), A2 (3.0%), B1 (4.6%), B2 (7.3%), C (30.9%), D (42.3%), E (11.3%).

DISCUSSION

Analysis of the results obtained showed that the incidence of congenital toxoplasmosis in the study population was similar to that reported in other studies, ranging from 0 to 100/10,000 live births, according to socioeconomic, cultural, ethnic, and climatic factors of the study population and to the type of parasite strain[9,14]. The incidence of the disease per 10,000 births was 7 in New York, 53 in Germany, 86 in Austria and 30 in Paris, where pregnant women are at a high risk for toxoplasmosis[9]. Some Brazilian studies have revealed frequencies between 2 and 20/10,000 live births[17,18,21], but a study carried out in the state of Pernambuco indicated a high frequency in newborns from mothers with primoinfection, 1,250/10,000 live births (95%CI 3.5-29%)[20]. In these studies, however, the serological methods were different, with some variability in terms of specificity, sensitivity, and positive and negative predictive values. A program for neonatal screening for the detection of IgM-toxoplasma antibodies, Enzyme Immunoassay (EIA), was implemented in Porto Alegre by the Center for Neonatal Screening and Nobel RIE Laboratory, and tested between 1995 and 1996. The approximate incidence in the State was two cases per 10,000 live births[17]. This finding may not represent the actual frequency of the disease, since not all newborns are submitted to neonatal screening for toxoplasma antibodies[19] and also because the method used has a sensitivity of 61.2% and a specificity of 88.8%[24].

The present study was conducted at a hospital that handles most deliveries in the town of Passo Fundo and attends pregnant women from all socioeconomic classes, according to the data of the Municipal Department of Health.

The distribution of the study population according to the Economic Classification of the Brazilian Population was similar to that of Passo Fundo, and is classified as: A1-0.5%, A2-4.5%, B1-8.3%, B2-13.2%, C-46%, D-22.5%, E-5%. In addition, during the study, 72.5% of births in the town took place at Hospital São Vicente de Paula. Our study sample can be considered to be representative of pregnant women in the town of Passo Fundo during the study period. Our results can therefore be considered to correspond to the prevalence of congenital toxoplasmosis in the town of Passo Fundo during the study period.

Three factors that influence primoinfection in pregnant women should be taken into consideration in order to explain the incidence rate in a given population. First, the incidence of infection in the community; secondly, the frequency of possible contacts with sources of infection; and thirdly, the number of women of childbearing age who have not had primoinfection yet[25]. In some regions of the state, the rate of toxoplasma infection was higher (86%)[15], than that found in most studies conducted in our country. Nevertheless, the highest incidence of congenital toxoplasmosis in Erechim (2.1%)[26] might result from a more intense

and chronic exposure of mothers to the parasite than elsewhere[27], in addition to the fact that type I strains are more virulent and frequent in town[14]. MELAMED *et al.*[15] have underscored the high differentiation of the seropositivity of the disease, even in nearby regions.

In our environment, neither the prevalence of toxoplasma infection in the community nor the frequency of susceptible pregnant women in the first prenatal consultation (seronegative) is known. This consultation could identify pregnant women at higher risk for fetal transmission of toxoplasmosis at the beginning of pregnancy. In this study, 702 (56.2%) pregnant patients were not serologically tested during their first trimester of pregnancy and 62 women did not know about the serological exam.

NAESSENS *et al.*[16] have assessed the sensitivity and specificity of the tests with titers of IgM and IgA in blood samples and cord blood samples collected from newborns. Sensitivity was similar and the total specificity of tests with neonatal blood was better than that of tests with cord blood: specificity of IgM and IgA in neonatal blood is 99%, whereas the specificity in cord blood is 96% and 92%, respectively. Nevertheless, we opted for cord blood due to the sample size needed and also to avoid invasive procedures.

We tested the IgM antibody since it does not usually cross the placenta at any time during pregnancy (except for placentitis); being used as the basis for the diagnosis of congenital infection[25]. Nevertheless, the screening for IgA is more sensitive[4,16,25], but its specificity is limited due to the greater possibility of passive transfer of antibodies from the mother[25]. On the other hand, the detection of segments of the parasite by PCR is regarded as diagnostic[25]. Despite the high sensitivity of this test[25], BASTIEN[2] believes that the PCR does not have sufficient sensitivity for toxoplasma. There is, however, no doubt about its benefits for diagnosis. FUENTES *et al.*[10] have detected *Toxoplasma gondii* by PCR in the blood and CSF of three out of four newborns with congenital infection.

The serological method chosen in this research is due to the fact that this technique includes a capture IgM. This eliminates the interference of a specific IgG, which causes false-negative results, and the interference of the rheumatoid factor and of antinuclear antibodies, which cause false-positive results, in most cases. ELFA has high sensitivity and specificity, ranging between 93.5% and 99.3%, respectively. WILSON *et al.*[29] have assessed six commercially available tests and found a sensitivity and specificity of 100% and 98.6%, respectively, for VIDAS Toxo IgM. The rate of equivocal results was 0.9%. The most cost-effective methods are those with a better combination of sensitivity and specificity and few equivocal results[29]. PELLOUX *et al.*[22] have found a sensitivity of 100% and a specificity of 97% for this method. An assessment of four diagnostic methods for the detection of IgM-toxoplasma has shown discrepancy between tests, indicating that it is important to consider the prevalence of the disease in the population to which these tests are to be applied. ELFA was the most specific test with the highest negative predictive value, 99.3% and 98.7%, respectively[11]. Therefore, we can say that this laboratory test proved to be adequate for our key objective, since a sensitivity of 100% and a high negative predictive value are desirable when attempting to identify infected newborns[8].

As the incidence of congenital toxoplasmosis estimated in this study was low, the negative predictive value of the test used by us was high[8]. Therefore, the probability of false-negative results was minimal, whereas that of false-positives would be greater, since the test-positive newborn showed signs of the disease. This, however, did not occur in our study.

NAESSENS *et al.*[16] have stated that early-infected infants are less frequently positive for IgM and IgA at birth due to the lack of immune response of the fetus before 20-22 weeks of gestation and to the short duration of this response. Studies that include infants infected before 20 weeks can have less sensitivity to the test than studies in which infants become infected at a later time[16].

The incidence of serum positivity (IgG) in pregnant women in the population studied may be compared to the levels found in countries with very high risk of toxoplasmosis, such as France[25]. It should be underscored that mothers received prenatal care in different places and that serologic tests were carried out using distinct methods.

Some eating habits observed in the study have been reported in surveys carried out to determine risk factors for toxoplasma infection in pregnant women. According to COOK *et al.*[7], the main risk factor was the ingestion of uncooked meat (30%) or undercooked meat (63%), as well as the ingestion of unpasteurized milk. KAPPERUD *et al.*[12] have observed that the patients with infection during pregnancy had had daily contact with cats at a higher frequency than the controls (OR = 3.6). These authors suggest case-control studies for the identification of the major risk factors in different populations, with the aim of prioritizing the guidelines for the prevention of acute infection in pregnant women[12].

We believe that the screening for capture IgM (ELFA) in cord blood is an important tool for the detection of newborns probably infected with *Toxoplasma gondii*, given its relatively low cost compared to more sophisticated methods and due to its accuracy as well[5]. In a situation in which the frequency of clinical manifestations at birth is low and the serological data of mothers are not always available, it is essential to establish an early diagnosis and also to offer infected infants proper treatment.[5]

RESUMO

Incidência de toxoplasmose congênita no sul do Brasil: estudo prospectivo

O objetivo do estudo foi determinar a incidência de infecção congênita pelo *Toxoplasma gondii* e descrever características neonatais e maternas, em recém-nascidos atendidos num Hospital Universitário de Passo Fundo, RS.

Foram analisadas amostras de sangue do cordão umbilical de 1250 recém-nascidos vivos. O diagnóstico laboratorial foi realizado através da detecção de IgM-*Toxoplasma gondii* pelo método ELFA (Enzyme Linked Fluorescent Assay). Avaliou-se a idade gestacional, o crescimento intra-uterino, as medidas antropométricas e características do pré-natal.

A incidência de toxoplasmose congênita ao nascimento foi de 8/10.000 (IC 95% 0,2 - 44,5). A média do peso de nascimento e idade gestacional foi 3080 ± 215,56 gramas e 38,43 ± 1,88 semanas, respectivamente. Em relação ao pré-natal, 58% das gestantes realizaram 5 ou mais consultas e, 38,9% realizaram sorologia para toxoplasmose no 1º trimestre da gestação.

Observou-se incidência de infecção congênita ao toxoplasma similar à encontrada na maioria dos estudos realizados no país e exterior. A amostra estudada é representativa da cidade de Passo Fundo, portanto, considera-se que a frequência encontrada, seja a incidência da doença na cidade durante o período do estudo.

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