

Difficulties observed in a reference center in the diagnosis and management of pregnant women with toxoplasmosis

Dificuldades observadas em um centro de referência no diagnóstico e manejo de gestantes com toxoplasmose

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ABSTRACT

Aims: To evaluate the difficulties met in the care of pregnant women with toxoplasmosis diagnosis in antenatal care services. **Methods:** Longitudinal prospective study with 262 pregnant women referred to the Toxoplasmosis Clinic at Instituto de Pesquisa Clínica Evandro Chagas-Fundação Oswaldo Cruz, between January 2005 and July 2009. **Results:** Most women (91.2%) were in the second and third trimesters of pregnancy, and 81.3% were referred by public health services. The average delay was 113.4 days in the collection of the first sample for serological tests in antenatal care, 52.1 days for referral and 160.6 days in starting treatment. Younger women ($P=0.03$) and those from the public health system were referred later ($P<0.05$). Treatment was initiated at the origin for only 16% of the pregnant women, and 5% of these did not receive the recommended dose of spiramycin. At the Reference Center there was a low rate of confirmation of the serological tests performed in the health services of origin. It was found that 12.6% of pregnant women with an initial diagnosis of acute toxoplasmosis were susceptible to infection by *Toxoplasma gondii*. These tests were considered false positives. **Conclusions:** This study highlights the difficulties met in the management of pregnant women with toxoplasmosis in the antenatal care, including the quality of diagnostic tests and the need for greater emphasis on continuing education of health professionals.

Keywords: TOXOPLASMOSIS, CONGENITAL; TOXOPLASMOSIS/diagnosis; PRENATAL DIAGNOSIS; PRENATAL CARE; PREGNANCY COMPLICATIONS, INFECTIOUS; REFERENCE CENTERS; LONGITUDINAL STUDIES; PROSPECTIVE STUDIES; FEMALE; PREGNANCY.

RESUMO

Objetivos: avaliar as dificuldades encontradas no atendimento de gestantes com diagnóstico de toxoplasmose por parte de serviços de atendimento pré-natal. **Métodos:** estudo longitudinal, prospectivo, com 262 gestantes encaminhadas ao Ambulatório de Toxoplasmose do Instituto de Pesquisa Clínica Evandro Chagas-Fundação Oswaldo Cruz, entre janeiro de 2005 e julho de 2009. **Resultados:** a maioria das gestantes foram encaminhadas no segundo ou terceiro trimestre de gestação (91,2%) e por serviços públicos de saúde (81,3%). O tempo médio de demora na coleta de sangue para os testes sorológicos no pré-natal foi de 113,4 dias. Houve demora média de 52,1 dias para o encaminhamento e 160,6 dias para o início do tratamento. Mulheres mais jovens ($P=0,03$) e aquelas provenientes do sistema público de saúde ($P<0,000$) foram encaminhadas mais tardiamente. O tratamento foi iniciado na origem em apenas 16% das gestantes, e 5% destas não receberam a dose preconizada de espiramicina. No Centro de Referência houve baixa confirmação dos testes sorológicos realizados nos serviços de saúde de origem. Constatou-se que 12,6% das gestantes com diagnóstico inicial de toxoplasmose aguda eram suscetíveis à infecção por *Toxoplasma gondii*, sendo os testes considerados falso-positivos. **Conclusões:** este estudo destaca dificuldades observadas no manejo de gestantes com toxoplasmose por parte do atendimento pré-natal da rede básica de saúde, incluindo a atenção quanto à qualidade dos testes diagnósticos e a necessidade de maior ênfase na educação continuada dos profissionais de saúde.

Descritores: TOXOPLASMOSE; TOXOPLASMOSE CONGÊNITA/diagnóstico; DIAGNÓSTICO PRÉ-NATAL; CUIDADO PRÉ-NATAL; COMPLICAÇÕES INFECCIOSAS NA GRAVIDEZ; CENTROS DE REFERÊNCIA; ESTUDOS LONGITUDINAIS; ESTUDOS PROSPECTIVOS; FEMININO; GRAVIDEZ.

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INTRODUCTION

Congenital toxoplasmosis occurs secondary to transplacental transmission of *Toxoplasma gondii* (*T. gondii*) during the period of maternal acute infection.¹⁻³ It is characterized by a broad spectrum of clinical manifestations, with neurological, ocular and systemic involvement. Although most newborns with congenital infection show no manifestations of infection at birth, 85% of these will develop visual impairment in the first two years, and 55% will present neurological disorders.⁴⁻⁶ The frequency of vertical transmission is low in the first trimester (3-9%) increasing at the end of pregnancy to 60-81%. The severity of fetal damage is higher in the beginning of pregnancy (34-85%) and lower at the end of pregnancy (4-17%).⁷ Serological tests such as the detection of anti-*T. gondii* IgG and IgM, associated with the IgG-avidity test⁸⁻¹¹ have been the main means of diagnosis used in clinical practice. Seroconversion of IgG, a significant increase in IgG titers in paired tests and the appearance of IgM, are considered indicators of acute infection. Since toxoplasmosis is usually subclinical, its diagnosis depends on the detection of serological markers during pregnancy. Serological diagnosis is a challenge for professionals involved in management of pregnant women. False-positive IgM or residual IgM persistence are confounding factors in the serological diagnosis of toxoplasmosis.^{7,12} Testing of IgG avidity is a time counter, and the diagnosis of recent infection based on the low avidity of IgG is particularly useful when performed in the first trimester of pregnancy. Despite the absence of clinical trials, there is consensus that the use of antiparasitic drug may reduce the risk of transmission or the severity of fetal injury, especially if given early in pregnancy.¹³⁻¹⁶

The Toxoplasmosis Outpatient Clinic at Instituto de Pesquisa Clínica Evandro Chagas (IPEC)/Fundação Oswaldo Cruz (Fiocruz) is a reference in monitoring cases of toxoplasmosis, receiving pregnant women from various health units in Rio de Janeiro. In order to describe some difficulties in the care of pregnant women referred with the diagnosis of toxoplasmosis, the authors studied the cases of pregnant women seen at the clinic between January 2005 and July 2009, referred with positive IgM for toxoplasmosis, regarding: (a) the referral source and referral of patients with prescription; (b) delay in the performance of serological tests, in referral to the reference service and in beginning of treatment; and (c) existence of tests with discordant results or false-reactive, performed in the services of origin.

METHODS

A prospective longitudinal study was conducted at the Toxoplasmosis Outpatient Clinic at the IPEC/Fiocruz, Rio de Janeiro. This study included the totality of women with suspicion of toxoplasmosis acquired during pregnancy (positive anti-*T. gondii* IgM) referred to the clinic between January 2005 and July 2009. The study was approved by the Committee on Ethics in Research of IPEC/Fiocruz.

The patients were seen at Toxoplasmosis Outpatient Clinic at IPEC/Fiocruz, Reference Center for Toxoplasmosis (RC), according to the same clinical-epidemiological protocol. Serological tests for toxoplasmosis were made in the RC using an enzyme-linked immunosorbent assay (ELISA capture Biokit, Barcelona, Spain) and an enzyme-linked fluorescent assay (ELFA) (VIDAS, bioMérieux S.A., Lyon, France). In case of positive IgM samples, testing was performed for IgG avidity to *T. gondii* (VIDAS Toxo-IgG Avidity, bioMérieux, Marcy-l'Etoile, France).

Patients were clustered according to age group (<20, 20-29, and ≥30 years), origin (health care system, public or private, that referred them to the RC), and trimester of pregnancy at first visit in the RC (first gestational trimester: <12 weeks, second gestational trimester: 12 to 24 weeks, third gestational trimester: > 24 weeks). Variables were created to estimate changes in time (in days), such as: delay in serological tests (difference between the beginning of pregnancy, identified by date of last menstrual period, and date of collection of first sample for antenatal serological tests); delay in treatment (difference between the date of last menstrual period and the beginning of the treatment for toxoplasmosis); and delay in referral (time between the date of collection of blood for serological tests in antenatal care and the date of the first visit at the RC).

Data were compiled in the statistical program SPSS version 11 (SPSS Inc., Chicago, USA). Data analysis was performed with Stata software version 8.2, 2005 (Stata Corp., College Station, USA). Statistical analysis estimated confidence intervals (CI) of 95% for means and time differences. Chi-square test was used in bivariate distributions, with the criterion of statistical significance being P value less than 0.05.

RESULTS

The study included 262 pregnant women, aged between 12.9 and 45.5 years (25.4±6.7). Most of them

(81.3) were referred by public health services, and 91.2% were in second and third trimesters of pregnancy when referred (Table 1). Twenty two patients presented low IgG avidity. Treatment was initiated at the origin in 42 women (16%), and 13 (5%) of these did not receive the recommended dose of spiramycin. No patient was prescribed with sulfadiazine, pyrimethamine and folinic acid.

Table 1. Age group, origin and trimester of gestation of pregnant women referred with diagnosis of acute toxoplasmosis to the Toxoplasmosis Outpatient Clinic at Instituto de Pesquisa Clínica Evandro Chagas-Fundação Oswaldo Cruz, 2005-2009

Variables	n	%
Age group (years)		
<20	64	24.4
20-29	138	52.7
≥ 30	60	22.9
Total	262	100.0
Origin		
Public system	213	81.3
Private system	49	18.7
Total	262	100.0
Trimester of gestation		
First	23	8.8
Second	137	52.3
Third	102	38.9
Total	262	100.0

Younger women (P=0.03) and those from the public health system (P<0.000) were sent later to the RC (Table 2), and most young women were referred by public health units (P=0.03) (Table 2). Overall, the pregnant women took an average time of 113.4 days (95% CI: 106.9-118.8) to collect the serological tests in prenatal care, 52.1 days (95% CI: 47.7-56.6) to be referred to the RC and 160.6 days (95% CI: 153.6-167.6) to start treatment for toxoplasmosis. Pregnant women from the public health units took 24.8 days longer to perform serological tests for toxoplasmosis and 50.3 days longer to start the treatment, compared to those referred by private physicians (Table 3).

Of the total of 262 pregnant women with positive anti-*T. gondii* IgM during prenatal care, 136 (52%) had positive or indeterminate IgM at the RC. However, false positive results were found in 126 cases (48%) and among these, 33 women (12.6% of those initially suspected of having acute toxoplasmosis) were susceptible to toxoplasmosis (IgG and IgM negative) (Table 4).

Table 2. Gestational trimester of referral according to the health system of origin or the age group of pregnant women referred with diagnosis of acute toxoplasmosis to the Toxoplasmosis Outpatient Clinic at Instituto de Pesquisa Clínica Evandro Chagas-Fundação Oswaldo Cruz, 2005-2009

Gestational trimester	Origin			Age group			Total n (%)
	Private n (%)	Public n (%)	Total n (%)	>20 years n (%)	20-29 years n (%)	≥30 years n (%)	
First	15 (30.6)	8 (3.8)	23 (8.8)	1 (1.6)	13 (9.4)	9 (15.0)	23 (8.8)
Second	26 (53.1)	111 (52.1)	137 (52.3)	30 (46.9)	75 (54.3)	32 (53.3)	137 (52.3)
Third	8 (16.3)	94 (44.1)	102 (38.9)	33 (51.6)	50 (36.2)	19 (31.7)	102 (38.9)
Total	49 (100.0)	213 (100.0)	262 (100.0)	64 (100.0)	138 (100.0)	60 (100.0)	262 (100.0)

Pearson's chi-square (2) = 40.6474, p<0.0005 Pearson's chi-square (4) = 10.6841, p=0.030

Table 3. Average time of delay between first serological tests and beginning of treatment for toxoplasmosis in pregnant women referred with diagnosis of acute toxoplasmosis to the Toxoplasmosis Outpatient Clinic at Instituto de Pesquisa Clínica Evandro Chagas-Fundação Oswaldo Cruz, 2005-2009

Origin	Delay in serological tests (days)	95% CI*	Delay in treatment (days)	95% CI*
Public system	118.3	111.5-125.1	170.0	162.7-177.4
Private system	93.5	77.0-109.9	119.7	104.1-135.4
Difference between public and private	24.8	9.0 -40.7	50.3	33.4-67.2

* CI = confidence interval

Table 4. Serological status to *Toxoplasma gondii* infection in pregnant women referred to the Toxoplasmosis Outpatient Clinic at Instituto de Pesquisa Clínica Evandro Chagas-Fundação Oswaldo Cruz (Reference Center), according to tests performed in the laboratories of prenatal care (source) and in the the Reference Center, 2005-2009

Serology in origin	Serology in the Reference Center					Total
	IgM(+)/IgG(+)	IgM(+)/IgG(-)	IgM(I)/IgG(+)	IgM(-)/IgG(+)	IgM(-)/IgG(-)	
	n (%)	n (%)	n (%)	n (%)	n (%)	
IgM(+) and IgG(+)	110 (46.2)	3 ^s (1.2)	13 (5.5)	92 (38.7)	20 (8.4)	238 (100.0)
IgM(+) and IgG(-)	4 (21.0)	1 [†] (5.3)	1 (5.3)	0 (0.0)	13 (68.4)	19 (100.0)
IgM(+) without IgG	2 (40.0)	0 (0.0)	2 (40.0)	1 (20.0)	0 (0.0)	5 (100.0)
Total	116 (44.3)	4 (1.5)	16 (6.1)	93 (35.5)	33 (12.6)	262 (100.0)

(I) = indeterminate, (+) = positive, (-) = negative; ^s Two patients did not return to the Reference Center for subsequent visits and serological confirmation, and one patient remained IgM(+) near cut-off without raising titles in paired samples collected one month apart. [†] One patient remained IgM(+) near cut-off /IgG(-) in paired samples collected one month apart, suggesting indeterminate IgM.

DISCUSSION

Despite the high prevalence of *T. gondii* infection, control and management of prenatal transmission are not yet standardized in Brazil.^{17,18} The main strategy for the prevention of this infection with potentially tragic outcomes is still the health education, and prenatal serological screening should be initiated early. Most of the women studied (91.2%) were in second or third trimester of pregnancy. The late performance of serological tests for toxoplasmosis led to a delay in referral and care in the RC. Thus, the treatment for toxoplasmosis was initiated on average in the fifth month of pregnancy. The delay between the average time of testing and the average time of initiation of treatment points to another problem of the health system: the excessive time delay in accessing the results of serological tests, therefore influencing the delay in referral to the RC. This was especially observed among pregnant women referred by public health units. In these cases, confounding factors, such as educational level and age, may interfere with the quality of prenatal care.¹⁹ The delay observed on the initiation of prenatal care and the performance of serological tests in public health facilities in Rio de Janeiro was consistent with that seen in other regions of Brazil and Latin America.²⁰⁻²²

Spiramycin was prescribed in the services of origin in only 11% of cases. The misinformation about the management of toxoplasmosis in pregnant women by physicians who perform prenatal care has already been noted by other authors.^{23,24} Despite recent controversy regarding the effectiveness of spiramycin in preventing congenital infection, it has been shown that treatment is effective when administered early.^{14,16} Due to the absence to date of randomized controlled clinical trials, spiramycin is still the recommended

drug for the treatment of acquired toxoplasmosis during pregnancy, especially in the first trimester. Sulfadiazine, pyrimethamine and folinic acid are indicated in infections acquired later, and when there is proven fetal infection.¹⁴ It is beyond the scope of this paper reporting the clinical management in each case, however it is noteworthy the fact that no pregnant women have been given this regimen at the services of origin, even when diagnosed later.

False-positive anti-*T. gondii* IgM results are described by several authors.^{13,25} Since IgM antibodies can remain positive for many months or even years after acute infection,^{26,27} a negative result virtually remove the possibility of recently acquired infection. Once external serological tests were performed by several laboratories, accuracy of interpretation of those results was impaired. Thus, a high proportion of pregnant women who attended RC had discordant results in relation to the IgM test. Many pregnant women who were susceptible to infection by *T. gondii* were misdiagnosed as suffering from an acute infection, with potential fetal damage. Besides the emotional stress to which these women were unnecessarily subjected, there was an unnecessary expense with the drugs for treatment. The average unit cost of 500 mg of spiramycin was \$1.00, which resulted in per capita daily \$6.00 with the recommended dose of 3 g/day.¹⁴ Furthermore, we assume that a lot of patients were overtreated due to a false diagnosis of toxoplasmosis acquired during pregnancy. All patients received spiramycin at the first medical evaluation, switched to sulfadiazine, pyrimethamine and folinic acid in those with positive IgM and low IgG avidity or evidence of fetal infection.

We conclude that both health systems, public and private, had difficulties in the care of pregnant women, with late diagnosis, referral and treatment, and that it

is necessary to implement measures of early assistance in order to contribute to the prevention of congenital toxoplasmosis. Knowledge about the difficulties encountered by health professionals and health systems with regard to the approach of toxoplasmosis during the prenatal period may help in the discussion about control and management of toxoplasmosis in pregnancy. Congenital toxoplasmosis is a preventable disease and the authors emphasize the importance of early prenatal serological tests, and preventive measures when necessary, in order to avoid a dramatic fetal disease.

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