

TOXOPLASMOSIS RISK IN EASTERN ROMANIA

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Abstract. *Toxoplasma gondii*, the obligate intracellular protozoan, infects humans and any other warm-blooded animal. Toxoplasmosis is probably one of the most common human infection and becomes a lifethreatening disease in case of congenital infection or immunodepressed patient. The aim of this study was to estimate toxoplasmosis prevalence among Eastern Romania human population and to emphasize the consequences. The study was performed between 1996 and 1999 in Eastern Romania including pregnant women, newborn children, HIV infected patients, eye disease patients, and lymphadenopathy patients. The following tests were used for serodiagnosis purposes: immunofluorescence assay, 2-mercapto-ethanol agglutination test for Toxoplasma IgG detection, and immunosorbent agglutination assay for Toxoplasma IgM and IgA detection. Toxoplasmosis seroprevalence among pregnant women 26 years mean age was 41%, most of them being chronic infections. In the group of apparently healthy women 1.85% were strongly suspected of acute acquired toxoplasmosis during pregnancy. In the group of pregnant women suffering spontaneous abortion in the first period of their pregnancy 4.23% had acute acquired infection. The cord blood screening among newborns noticed 44.5% toxoplasma antibodies prevalence with one case of asymptomatic congenital toxoplasmosis detected. Toxoplasmosis seroprevalence among chorioretinitis patients was 68%. Also, 12.8% of studied lymphadenopathies could be attributed to toxoplasmosis. The results confirmed *Toxoplasma gondii* wide spread in Eastern Romania, and the risk represented by this protozoan for the unborn child and HIV infected patients. A larger serological screening should be taken into consideration in order to diminish the consequences of this infection.

Key words: *Toxoplasma gondii*, Diagnosis, Prevalence, Congenital infection, HIV

Rezumat. *Toxoplasma gondii*, protozoarul obligator intracelular, poate infecta atat omul cit si orice alt animal cu sange cald. Toxoplasmoza poate fi considerata una dintre cele mai frecvente infectii umane. Ea devine o severa problema de sanatate in cazul infectiei congenitale sau a subiectului imunodeprimat. Prezentul studiu si-a propus a estima prevalenta infectiei toxoplasmice la populatia umana din zona de est a Romaniei si de a-i evidentia consecintele. El s-a desfasurat in perioada 1996-1999 in estul Romaniei si a inclus loturi de gravide, nou-nascuti, subiecti HIV infectati, subiecti cu afectiuni oculare si cu limfadenopatie. Diagnosticul serologic s-a realizat prin urmatoarele tehnici: reactia de imunofluorescenta indirecta, reactia de aglutinare sensibilizata cu 2-mercaptoetanol pentru IgG, si ISAGA pentru IgM si IgA. Seroprevalenta toxoplasmozei la femeile gravide cu virsta media de 26 ani a fost de 41%, majoritatea fiind infectii cronice. La 1,85% dintre gravidele aparent sanatoase a fost stabilit cu mare probabilitate diagnosticul de infectia acuta cu *T. gondii*. La 4,23% femei apartinand unui lot de gravide cu avort spontan in prima perioada a sarcinii, infectia acuta toxoplasmica a putut fi incriminata drept agentul cauzal. Screening-ul serologic realizat pe sange recoltat din cordonul ombilical la un lot de nou-nascuti a relevat o sero-prevalenta a anticorpilor anti-*T.gondii* de 44,5%, cu un caz de infectie congenitala asimptomatica la

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nastere. Seroprevalenta toxoplasmozei la bolnavii cu corioretinita a fost de 68%. Studiul lotului de subiecti cu limfadenopatie a putut demonstrata implicarea infectiei acute toxoplasrice drept cauza a suferintei lor in 12,8% din cazuri. Rezultatele obtinute confirma larga raspindire a *T.gondii* in zona estica a Romaniei si riscul pe care acest protozoar il reprezinta pentru fat si subiectul HIV infectat. Se cere luat in considerare un screening serologic mai larg pentru diminuarea consecintelor pe care aceasta infectie le presupune.

Cuvinte cheie: *Toxoplasma gondii*, Diagnostic, Prevalenta, Infectie congenitala, HIV

INTRODUCTION

The obligate intracellular protozoan *Toxoplasma gondii* is ubiquitous in nature. Parasite can infect humans or any other warm-blooded animal (1). With two major routes of transmission, oral through oocysts or tissue cysts ingestion, and congenital by transplacental spread of tachyzoites, toxoplasmosis is probably one of the most common human infections. The prevalence of human toxoplasmosis ranges from 1% to 90% in different countries (2,3). The clinical pattern of human primary infection is often asymptomatic or a flu-like illness or lymphadenopathy. Toxoplasmosis becomes a lifethreatening disease in two circumstances: congenital infection of the fetus and acute or reactivated chronic toxoplasmosis in immunodepressed patient (2-6).

Congenital infection of the fetus occurs if his mother acquired acute infection during pregnancy and can lead to spontaneous abortion, congenital malformation, or late additional sequelae. Ocular toxoplasmosis is an important cause of human severe visual loss, even blindness (2,7,8). Many countries consider all of these reasons just enough to introduce special health policies regarding this parasitic infection. Serologic screening of pregnant women and long-term follow up of congenital infected children are taken into

consideration (2). Special therapy with preventive and curative purpose are recommended to HIV infected patients with chronic toxoplasmosis (4-6).

Romania had a parasite wide spread. Despite of these, Romania had no systematic *Toxoplasma* serological screening for pregnant women. Toxoplasmosis diagnosis is often a problem of physician's "inspiration". The aim of the presented study was to estimate toxoplasmosis prevalence among human population in eastern Romania and to emphasize its consequences.

MATERIALS AND METHODS

2.1. Study population

The study was performed on Eastern Romanian population between 1996 and 1999 involving five target groups of patients. The first three ones considered at risk to severe evolutions of infection were investigated: pregnant women, newborns, and HIV infected patients. Secondly two groups of patients with signs and symptoms that could be caused by toxoplasmosis (eye disease, lymphadenopathy) were investigated.

- The lot of pregnant women was divided in two subgroups: 810 clinic healthy women, and 378 women with spontaneous abortion in the first period of pregnancy. The mean age of

women was 26 years living in rural and urban area too.

The first subgroup was constituted from clinical healthy pregnant women, with no signs of disease. Starting from the first trimester of their pregnancy they were clinically evaluated and serologic screened for Toxoplasma infection. Sero-positive women were tested for acute or chronic infection. Sero-negative women were considered at risk to acute acquired toxoplasmosis during pregnancy and were serological followed up till the moment of birth.

Second subgroup was constituted from hospitalized women with spontaneous abortion in the first period of pregnancy. They were clinically and laboratory investigated to find the abortion cause, and with this purpose they were serological tested for toxoplasmosis.

- A lot of 1226 newborns were cord blood screened for Toxoplasma infection in order to detect asymptomatic congenital infection. Newborns considered at risk for congenital infection had a serological test and clinically followed up till they became sero-negative or the diagnosis of congenital toxoplasmosis became certitude.
- The lot of 102 HIV infected patients consisted of 102 persons, 14.5 years mean age.
- The lot of eye disease patients had 110 persons in study: 50 chorioretinitis patients, 17 uveitis patients, and 43 patients with other ocular pathology (strabismus, cataract, microphthalmia etc).
- The lot of lymphadenopathy patients was constituted from 343 persons, of

15.8 years average age. The lymphadenopathy was the only sign of disease for the most part of these persons. All of them had a serological test for Toxoplasma infection.

2.2. Serologic diagnosis methods

The following tests were used for toxoplasmosis serologic diagnosis: immunofluorescence assay test (IFAT), 2-mercapto-ethanol agglutination test for Toxoplasma IgG, immunosorbent agglutination assay (ISAGA) for Toxoplasma IgM and IgA (8-10).

Positive diagnosis of acute acquired toxoplasmosis was considered when the following criteria were fulfilled: positive IgG, IgM, IgA, and high level IgG/significant IgG risen. Chronic toxoplasmosis was considered when only IgG was positive. If the serology was equivocal, tests were repeated after 2-3 weeks.

In the newborns group there were considered at risk for congenital toxoplasmosis the ones that fulfilled one of the following criteria: high level of IgG, positive/equivocal IgM and/or IgA. Congenital infection was disabled in case of decreasing *T.gondii* antibodies without treatment. A child that became sero-negative at the end of his first year of life was considered uninfected. A child that remained sero-positive after his first year of life, with or without signs and symptoms of disease was considered congenitally infected.

RESULTS

3.1. The pregnant women group

Toxoplasmosis prevalence among pregnant women, was estimated as 41%. There was no significant

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variation between the healthy women group and the women group suffering abortion (table 1).

Table 1. Toxoplasmosis prevalence among pregnant women

<i>T.gondii</i> serologic status	Women with no clinical signs of <i>T.gondii</i> infection (810 cases)		Women suffering of abortion in the first period of pregnancy (378 cases)	
	No	%	No	%
Sero-positive	335	41.36	155	41.0
Chronic inf	320	39.51	139	36.77
Acute acquired infection during pregnancy	15	1.85	16	4.23
Sero-negative	475	58.64	223	59.0

The serological screening of the healthy women group revealed 15 cases (1.85%) of asymptomatic acute acquired infection during pregnancy, all of them with no obvious signs of disease in the moment of diagnosis. It was considered a situation of great risk for the unborn child.

Toxoplasmosis serodiagnosis of women suffering spontaneous abortion in the first period of the pregnancy revealed 16 cases (4.23%) of acute acquired infection. With no other abortion etiology that could be demonstrated, this was attributed to an acute acquired toxoplasmosis.

3.2. The cord blood screened newborns

The toxoplasma antibodies seroprevalence among cord blood screened newborns was 44.5%. Majority of these cases (43.8%) were uninfected newborns with maternal transplacental antibodies. Nine newborns (0.72%) were considered at risk to congenital infection according with the above-mentioned criteria. They were clinically as well as serologic followed up during the first year of life. Only one infant was finally confirmed as having congenital toxoplasmosis by persistent IgG antibodies at 1 year of age (table 2).

Table 2. Toxoplasma antibodies sero-prevalence in the group of cord blood screened newborns

<i>T.gondii</i> serologic status	Cord blood screened newborns (1226 cases)	
	No	%
Sero-positive	546	44.5
Non infected newborns with maternal <i>T.gondii</i> antibodies	537	43.8
Newborns considered at risk for congenital toxoplasmosis	9	0.72
Confirmed congenital toxoplasmosis	1	0.08
Sero-negative	680	55.5

3.3. The HIV infected patients

Toxoplasmosis prevalence among HIV infected patients was 34.3%. All infections had a chronic pattern. Toxoplasma IgG serum level varied between 4 u.i./ml and 6400 u.i./ml. Eleven patients, two adults and nine children, ended in toxoplasmic encephalitis. The CD4+ level was below 100/ μ L in all these cases. Despite the chemotherapy two patients died (table 3).

3.4. Eye disease patients

Toxoplasmosis prevalence in the group of eye disease patients varied according to their eye pathology. So, chorioretinitis cases reached the highest level of Toxoplasma antibodies seroprevalence (68%), most of them were chronic infections (66%). Other eye pathology

registered a seroprevalence level almost two times lower (37.3%) than chorioretinitis. Patient and physician restraint regarding ocular fluid testing made it difficult to definitively confirm toxoplasmosis as causes for all tested chorioretinitis (table 4).

Table 3. Toxoplasmosis prevalence among HIV infected patients

<i>T.gondii</i> serologic status	HIV infected patients (102 cases)	
	No	%
Sero-positive	35	34.3
Toxoplasmic encephalitis	11	10.8
Sero-negative	67	65.7

Table 4. Toxoplasmosis prevalence among eye disease patients

<i>T.gondii</i> serologic status	Chorioretinitis (50 cases)		Uveitis (17 cases)	Other ocular lesions (43 cases)	
	No	%	No	No	%
Sero-positive	34	68	9	16	37.2
Chronic infection	33	66	9	14	32.5
Acute acquired infection	1	2	-	2	4.7
Sero-negative	16	32	8	27	62.8

3.5. Lymphadenopathy patients

Toxoplasma antibodies seroprevalence in the group of lymphadenopathy patients was 34.1%. Acute acquired infection was confirmed in 44 cases (12.8%). Any other reason for lymphadenopathy could be demonstrated in these cases, so acute toxoplasmosis was considered responsible for their pathology. The disease evolution confirmed the diagnosis (table 5).

Table 5. Toxoplasmosis prevalence among lymphadenopathy patients

<i>T.gondii</i> serologic status	Lymphadenopathy patients (343 cases)	
	No	%
Sero-positive	117	34.1
Chronic infection	73	21.3
Acute acquired infection	44	12.8
Sero-negative	226	65.9

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DISCUSSION

Toxoplasmosis seroprevalence was over 30% in all studied groups. This fact underlined that *T.gondii* is a common protozoan in eastern Romania.

The 41% toxoplasmosis seroprevalence in pregnant women with an average age of 26 years place Romania at the same level as France and Austria and in a comparable position with other central European countries (1,2). The noticed incidence of acute acquired toxoplasmosis during pregnancy among healthy pregnant women is significantly high. The 4.23% acute toxoplasmosis participation in the spontaneous abortion etiology and emphasizes the severe consequences of this infection. The newborns cord blood screening reconfirmed the noticed toxoplasmosis prevalence among healthy pregnant women. This screening also notifies for asymptomatic congenital infections missed at birth but leading to late additional sequelae. All these data prove that toxoplasmosis represent a real threat for the pregnant woman and her unborn child in Romania. A larger serological screening for pregnant women should be taken into consideration in order to diminish the severe consequences implied by congenital toxoplasmosis.

The 34% toxoplasmosis prevalence among HIV infected people reflect the potential risk represented by this well-known major opportunistic parasite for the AIDS patients in our area. This fact is also supported by the significant number of toxoplasmic encephalitis occurred in the absence of a specific chemoprophylactic regimen.

The *T.gondii* infection serological status has to be well known by the physician who cares an HIV infected patient and a preventive chemotherapy has to be taken into consideration in case of severe immunodepression.

The notified toxoplasmosis seroprevalence among chorioretinitis patients was similar with others researchers' data (2). Patients and physicians restraints regarding ocular fluid testing that made difficult to definitively implied toxoplasmosis as cause for all tested chorioretinitis, but a comparison with toxoplasmosis seroprevalence in other eye diseases may indicate *T.gondii* as an important risk factor for chorioretinitis.

The 12.8% acute toxoplasmosis participation in mild lymphadenopathy etiology is also noteworthy.

We think that toxoplasmosis requires more attention and more founds for special preventive programs addressed to pregnant women and HIV infected patients. It is also a diagnosis that always has to be considered in case of chorioretinitis or lymphadenopathy.

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