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STUDY ON SPECIFIC IgG AVIDITY AS A TOOL FOR RECENT PRIMARY *TOXOPLASMA GONDII* INFECTION DIAGNOSIS

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Abstract. *Toxoplasma gondii*, protozoan with worldwide distribution, can cause life-threatening disease for the unborn child when maternal infection is acquired during pregnancy. The accurate dating of maternal toxoplasmosis is essential in order to assess the risk of subsequent congenital infection. Specific *Toxoplasma* IgG of low avidity is considered to be a good mean for recent primary *Toxoplasma* infection diagnostic made on a single serum. During a period of 18 months a group of 1441 pregnant women residents in northeastern Romania were screened for *Toxoplasma* antibodies. 33 of them, identified as being at risk for recent primary infection, were tested for specific IgG avidity. Only one third of the women with positive specific IgM had recent primary toxoplasmosis confirmed by the low level of the specific IgG avidity. For another group of 17 patients diagnosed with lymphadenopathy caused by recent primary toxoplasmosis the low or borderline values of IgG avidity confirmed the diagnosis.
Key words: pregnant women, toxoplasmosis, IgG avidity

Rezumat. *Toxoplasma gondii*, protozoar cu largă răspândire la nivel mondial, reprezintă un deosebit risc pentru produsul de concepţie atunci când mama contactează primoinfecţia pe parcursul sarcinii. Pentru aprecierea cât mai exactă a riscului transmiterii congenitale a infecţiei de la mamă la făt, stabilirea corectă a momentului primoinfecţiei materne este esenţială. Prezenţa anticorpilor specifici anti-parazitari de tip IgG cu aviditate scăzută este considerată un indicator deosebit de fidel a primoinfecţiei toxoplasmice recente, diagnostic ce se poate realiza pe un singur ser. Pe o perioadă de 18 luni, un număr de 1441 femei gravide rezidente în zona de nord-est a României au fost testate pentru prezenţa anticorpilor specifici anti-*T.gondii*. 33 dintre ele au fost identificate ca prezentând riscul unei infecţii acute toxoplasmice recentă şi testată aviditatea IgG specifice anti-parazitare. La numai o treime dintre gravidele purtătoare de IgM specifice anti-parazitare, infecţia acută recentă a fost confirmată prin prezenţa IgG cu aviditate scăzută. De asemenea, la un al doilea grup de 17 subiecţi cu limfadenopatie la care toxoplasmoza acută a fost incriminată drept cauză, aviditatea scăzută sau la limită a IgG a confirmat diagnosticul.
Cuvinte cheie: gravide, toxoplasmoză, aviditatea IgG

INTRODUCTION

The protozoan *Toxoplasma gondii* has a worldwide distribution, more than 10^9 people having chronic infection (1). Toxoplasma infection of immuno-competent individual is usually an asymptomatic or self-limiting illness, but becomes a life-threatening disease for the unborn child when maternal infection is acquired during pregnancy or for the immunosupressed individual. Accurate dating of maternal toxoplasmosis is essential in order to assess the risk of

subsequent congenital infection and the corresponding therapy (2).

Toxoplasmosis diagnosis is based mainly on serological methods and consists in detection of the specific *Toxoplasma* IgG, IgM, IgA and follow up their kinetics. The presence of detectable IgM or IgA in mother's blood is associated with recently acquired infection, but their persistence is variable and can be prolonged over one year. The specific IgG reaches its peak within 2-3 months followed by a slow decline over many years (3).

Taking into account these limitations, improved serological assays were required for infection better dating. It is well known that the strength of the bond between antibody and individual epitopes increases with the duration of the infection (4). An assay measuring the avidity of the antibody-antigen interaction, first applied for viral infections, is now more and more used to define *Toxoplasma* infection (5,6). On a single serum specimen, the test separate the low-avidity antibodies produced in the early stage of the infection (first 3-4 months) from those with higher binding avidity reflecting past immunity (7-9). IgG avidity results less then 30% is considered low; 30-40%, borderline; and over 40% high.

In this study we present our experience on toxoplasmosis serodiagnostic based on IgG avidity and illustrates its ability to identify primary *Toxoplasma* infection acquired during pregnancy.

MATERIALS AND METHODS

Patients and sera

Starting from the year 2001, during a period of 18 months, a group of 1441 pregnant women residents in northeastern counties of Romania were screened for *Toxoplasma* antibodies in our laboratory. A number of 33 women were identified as being at risk for acute acquired infection during pregnancy or nearby the conception moment. The criteria to include them in the risk group were the presence of detectable specific *Toxoplasma* IgM and / or elevated level of specific IgG. The patients were clinical evaluated and serial serum sample were tested. *Toxoplasma* specific IgG avidity was also investigated using 6M urea as dissociating agent.

In the meanwhile, among patients with lymphadenopathy tested in our laboratory for possible toxoplasmic etiology, 17 subjects with primary infection were selected. Criteria to imply *Toxoplasma* infection as cause of the lymphadenopathy were the following: elevated, rising level of specific IgG and positive IgM. Specific IgG avidity was also tested in these patients.

Serodiagnostic methods

All sera were examined for total specific *Toxoplasma* antibodies detection through immunofluorescence assay test (IFAT). Specific IgG and IgM were then evaluated. IgG detection was performed through a homemade 2-mercapto-ethanol agglutination test and IgM detection through ELISA (DiaSorin Saluggia, Italy).

IgG avidity test

The IgG avidity assay was performed using a modified ELISA protocol according to Hedman et al. description (10). A standard commercial ELISA kit for IgG detection was used (DiaSorin Saluggia, Italy). Each serum was analyzed in duplicate fourfold titration rows (row A and row B), starting at a dilution of 1:201. 6 M urea was used to elute low-avidity antibodies from multivalent *Toxoplasma* antigen. After 1 h incubation at 37°C row A was washed three times, 5 minutes each wash, with 6 M urea in phosphate-buffered saline 0.05% containing Tween 20%. Row B was washed with the wash buffer provided by the kit manufacturer. The following ELISA steps, including incubation with conjugate, washing, incubation with substrate, and addition of the stop solution, were preformed according to manufacturer's recommendations. The optical density of each well was read in a microtiter plate reader (ETI-STAR) at 450 / 630 nm. IgG avidity was calculated as the ratio between the titer in row A and the titer in row B and expressed as percentage.

RESULTS

The group of 33 pregnant women group considered at risk for acquired toxoplasmosis during pregnancy was subdivided into 3 subgroups based on their serological features. The first subgroup included 22 women with positive *Toxoplasma* IgM, the second one 5 women with IgM at a borderline level and the third one with negative IgM but elevated *Toxoplasma* IgG. *Toxoplasma* specific IgG avidity in all these cases is shown in tables 1-4.

Table 1. Low IgG avidity in 7 pregnant women with positive specific IgM

Patient description (age, week of pregnancy)	Serum description (*Toxoplasma* status) IFAT	IgG (i.u./ml)	IgM	IgG Avidity (%)
CL, 25 y, 16 wk pregnancy	1/80	400	+	18.9
RL, 27 y, 8 wk pregnancy	1/40	128	+	14.3
AS, 24 y, 6 wk pregnancy	1/40	200	+	17.0
IE, 26 y, 10 wk pregnancy	1/1280	6400	+	29.3
BE, 19 y, 6 wk pregnancy	1/20	128	+	4.14
VM, 21 y, 12 wk pregnancy	1/80	800	+	22.5
ST, 28 y, 18 wk pregnancy	1/20	100	+	28.0

Table 2. High and borderline IgG avidity in 15 pregnant women with positive specific IgM

Patient description (age, week of pregnancy)	Serum description (*Toxoplasma* status) IFAT	IgG (i.u./ml)	IgM	IgG Avidity (%)
LV, 26 y, 14 wk pregnancy	1/80	400	+	39.2
ST, 22 y, 20 wk pregnancy	1/40	128	+	35.2
LM, 30 y, 24 wk pregnancy	1/40	200	+	36.2
BA, 27 y, 7 wk pregnancy	1/1280	6400	+	75.7
IM, 21 y, 26 wk pregnancy	1/20	128	+	57.4
PE, 29 y, 6 wk pregnancy	1/80	800	+	68.0
AM, 34 y, 12 wk pregnancy	1/20	100	+	57.3
GA, 19 y, 14 wk pregnancy	1/20	128	+	43.4
NF, 28y, 16 wk pregnancy	1/320	400	+	55.5
CF, 25 y, 24 wk pregnancy	1/80	200	+	32.9
IO, 26 y, 26 wk pregnancy	1/40	200	+	46.6
ME, 29 y, 28 wk pregnancy	1/80	400	+	87.0
CM, 34 y, 8 wk pregnancy	1/80	800	+	61.5
MM, 27 y, 24 wk pregnancy	1/160	1600	+	43.5
TM, 30 y, 6 wk pregnancy	1/320	3200	+	40.0

Table 3. High IgG avidity in 6 pregnant women with specific IgM at borderline level

Patient description (age, week of pregnancy)	Serum description (*Toxoplasma* status) IFAT	IgG (i.u./ml)	IgM	IgG Avidity (%)
NL, 32 y, 16 wk pregnancy	1/40	200	±	68.3
JI, 23 y, 14 wk pregnancy	1/160	1600	±	90.1
DV, 20 y, 10 wk pregnancy	1/80	800	±	32.9
IC, 27 y, 12 wk pregnancy	1/40	200	±	61.6
GA, 21 y, 12 wk pregnancy	1/160	1600	±	49.9
DP, 26 y, 8 wk pregnancy	1/40	400	±	89.7

Table 4. High IgG avidity in 5 pregnant women with negative specific IgM but elevated IgG

Patient description (age, week of pregnancy)	Serum description (*Toxoplasma* status) IFAT	IgG (i.u./ml)	IgM	IgG Avidity (%)
RL, 22 y, 20 wk pregnancy	1/320	3200	--	96.0
MI, 33 y, 8 wk pregnancy	1/160	1600	--	63.7
RV, 24 y, 12 wk pregnancy	1/80	800	--	56.6
IS, 26 y, 18 wk pregnancy	1/320	3200	--	78.4
TA, 20 y, 8 wk pregnancy	1/640	6400	--	87.1

In the group of patients with lymphadenopathy *Toxoplasma* specific IgG avidity values are shown in table 5.

Table 5. Low and borderline level of IgG avidity in 17 patients with toxoplasmic lymphadenopathy

Patient description (age, sypmtomatology)	Serum description (*Toxoplasma* status) IFAT	IgG (i.u./ml)	IgM	IgG Avidity (%)
TM, 17y, lymphadenopathy	1/320	400	+	21.1
GA, 16 y, lymphadenopathy	1/40	128	+	9.8
ZA, 23 y, lymphadenopathy	1/40	200	+	18.9
PA, 12 y, tuberculosis obs.	1/640	800	+	14.4
II, 12 y, lymphadenopathy	1/640	400	+	24.1
DG, 49 y, lymphadenopathy	1/640	400	+	23.3
CT, 15 y, lymphadenopathy	1/1280	1600	+	11.4
CL, 36 y, lymphadenopathy	1/160	32	+	25.8
RJ, 38 y, lymphadenopathy	1/80	32	+	20.2
PE, 44 y, lymphadenopathy	1/160	128	+	27.7
TM, 15 y, lymphadenopathy	1/320	400	+	28.7
RF, 20 y, lymphadenopathy	1/160	200	+	32.7
TA, 15 y, lymphadenopathy	1/160	800	+	36.3
MV, 14 y, lymphadenopathy	1/1280	640	+	29.7
DC, 18 y, lymphadenopathy	1/160	400	+	34.2
SA, 23 y, lymphadenopathy	1/1280	3200	+	42.0
PM, 11 y, lymphadenopathy	1/160	800	+	44.3

In the subgroup of 22 pregnant women with positive *Toxoplasma* IgM, the specific IgG avidity vary from low values (<30%) to high values (>40%) (tables 1,2). Specific IgG of low avidity is accepted by specialists to be an excellent indicator of recent primary *Toxoplasma* infection (11). Only one third of the above mentioned pregnant women (7 subjects) had recent primary toxoplasmosis confirmed by the low level of IgG avidity. These were subjects for whom toxoplasmosis represented a real threat and the ones that urgently required specific therapy. For the rest of 15 women, even they had positive *Toxoplasma* IgM, the danger was considered to be overtaken and specific therapy unnecessary. Further evolution of the pregnancy and serological features confirmed the diagnosis.

In the other two subgroups of pregnant women with specific IgM at the borderline level or with elevated *Toxoplasma* IgG but without IgM, the IgG avidity had high values (tables 3,4). These results led to the conclusion that *Toxoplasma* infection was in a chronical stage, with no risk for the unborn child. Further evolution of the pregnancy confirmed these data.

In the group of patients with lymphadenopathy, the low and borderline IgG avidity illustrated the recently *Toxoplasma* infection etiology. The serological repeated tests and the clinical evolution of the illness also sustained diagnosis.

DISCUSSION

The diagnosis of recently acquired *Toxoplasma* infection in pregnant women must be made as soon as possible in order to avoid delay in treatment and irreversible consequences for the fetus.

Diagnosis of recently acquired toxoplasmosis based only on specific IgM detection in a single serum sample can lead to unnecessary treatment and concerns, especially with regards to pregnant women. The use of tests with lower specificity and the possible long persistence of specific IgM antibodies in the chronical stage of infection are the causes of this mistake.

Most investigators agree that the most telling significance of the *Toxoplasma* IgM antibody is its absence. A subject with specific IgG but without IgM is extremely unlikely to have recently acquired toxoplasmosis (1). But for a subject with detectable specific IgM, confirmatory evidences are crucial for an acute infection diagnosis. In practice, these evidences are carried out possible over a period of weeks.

Specific IgG of low avidity proved to be an excellent tool for a recent primary *Toxoplasma* infection diagnostic made on a single serum. The avidity technique was able to show that an important proportion of the women presenting *Toxoplasma* IgM at the time of pregnancy are not actually recently infected.

Unnecessary anxiety and additional examinations and treatment will be avoided for these women and last but not the least, money saving.

CONCLUSION

Toxoplasma specific IgG avidity assay performed on specific IgM positive sample really improves the routine toxoplasmosis diagnosis, avoids unnecessary concerns and saves money.

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