Original Research Article

Difficulties in Interpreting Toxoplasmosis Serology during Pregnancy: Experience of the Department of Parasitology-Mycology Department of the HMA

H. Saffour[*], L Darfaoui, M. Sbaii, A. El Hakounni, E. El Mezouari, R. Moutaj

Parasitology-Mycology Department, Avicenne Military Hospital, Marrakech, Morocco

*Corresponding author: H. Saffour

Abstract

Toxoplasmosis acquired in an immunocompetent individual will usually be asymptomatic. However, the problem arises in the case of a primary infection in a pregnant woman, which may cause congenital infection of the fetus. Through maternal-fetal contamination, this infection poses two main risks to the fetus and the child, one neurological (hydrocephalus and intracranial calcifications) and the other ophthalmic (chorioretinitis that can affect the visual prognosis), which makes preventive actions necessary, through compulsory monthly serological surveillance of women who are not immune to toxoplasmosis. The biologist is often confronted with difficulties in interpreting the results, particularly during the monitoring of pregnant women. However, this is an essential step in the prevention of congenital toxoplasmosis because the results of the serological analysis are the basis for subsequent prenatal and postnatal treatment serodiagnosis is based on the detection of specific IgG and IgM. Although serological techniques have improved in recent years, problems of interpretation of serology persist, particularly in the presence of IgM antibodies. Results should be interpreted with caution. The mistake not to be made is to conclude immediately that the patient is primitively infected on the basis of the presence of IgM or IgG antibodies associated with IgM antibodies alone. The knowledge of the evolution of antibodies during the infection and the practice of complementary tests in particular the measurement of the avidity of antitoxoplasmic IgG allow in the majority of the cases a correct interpretation of the results and a dating of the infection.

Keywords: Toxoplasmosis serology , primary infection , immune status , specific IgM antibodies , IgG avidity.

INTRODUCTION

Congenital toxoplasmosis is a disease that threatens the pregnant woman, which can cause congenital infection of the fetus, making preventive actions necessary.

Considerable progress has been made in serological techniques to better quantify the specific antibodies synthesized by the organism against toxoplasma antigens and to confirm a diagnosis of toxoplasmosis.

Hence the interest of our study, which will show the difficulties, of interpretation of toxoplasmosis serology in pregnant women.

MATERIALS AND METHODS

This is a retrospective descriptive and analytical study, which extends from January 1, 2016 to December 31, 2020, a period of 5 years. Analyzing 4150 toxoplasmic serological tests realized in pregnant women, within the service of Parasitology-Mycology of the military hospital Avicenne in Marrakech.

The tests analyzed were essentially the quantitative determination of IgG, IgM and IgA antibodies directed against Toxoplasma gondii in serum or plasma, as well as the demonstration of the avidity of IgG. And this by automated immunoenzymatic tests of indirect ELISA type, Evolis Twin Plus automate, and by semi-automated method. The tests are performed on serum or plasma samples collected on anticoagulant, sodium heparin or sodium citrate.

Our study allowed the collection of different epidemiological data in order to compare our results with those of the literature and to evaluate the degree of knowledge and determine the seroprevalence of pregnant women followed in our training. A questionnaire was developed for this purpose, including the parameters necessary for our work: demographic, socioeconomic, cultural data, knowledge of toxoplasmosis and immune status.

RESULTS

During this study period of 5 years of data, we analyzed 4150 serologies (IgG, IgM) of pregnant women. It was found that 75.58% of the pregnant women were between 18 and 30 years of age while the age range between 31 and 50 years was only 24.42%.

709 women (17.1%) were in their first trimester of pregnancy, 1578 (38.03%) of women in their second trimester and 1862 (44.87%) in the last trimester.

2481 of the women (59.82%) had already had multiple pregnancies and 1667 women were primigravida (40.18%). No woman had a stillbirth. 3334 women lived in urban areas (80.34%), and 816 women in rural areas (19.66%).

Pregnant women's knowledge about toxoplasmosis was evaluated, 2837 (68.37%) women had not heard of toxoplasmosis, 1312 (31.62%) had information about it, the source of information was mainly the internet (60%).

The vast majority of the women recruited in our study (74%) had received only one serology test, two serologies were performed in 15% of the women, while almost 11% of the pregnant women had three serologies.

The rate of toxoplasmic infection in this study was 3.5% with IgG and IgM positive at baseline. 21.5% of our pregnant women were seropositive (IgG+, IgM-) while 78.5% were seronegative; of these seropositive women, 8.6% had IgG > 100 IU/ml.

An appearance of IgM was reported in 0.8% of these cases in the following months, reflecting seroconversion. The difficulties in interpreting the toxoplasmic serology of our collected cases are not obvious. They are either a non-significant increase in IgG levels, always in the absence of IGMs and in which the performance of an avidity test (in the absence of the ISAGA technique) was in favor of an old infection, or stabilization or a decrease in these high levels.

Cases of difficult interpretation showing IgM type antibodies in the absence of IgG, in 6 of our patients, i.e. 0.5% of the cases, translating the presence of non-specific IgM (in the absence of evidence of specific IgG in subsequent serological follow-ups) or delayed response for IgG antibodies, with detection of specific IgM justifying an urgent start of spiramycin treatment.

We also noted a serological profile with an equivocal IgG level in 7 patients, of whom only 2 repeated the serology in our training, 3 months later with the same result.

DISCUSSION

A good control of the kinetics of antibodies by the biologist and the clinician is an essential step for a targeted research of its antibodies by using appropriate techniques, in order to be able to date the seroconversion. This ensures adequate and rapid management and also conditions the realization of other biological tests such as amniocentesis. Such a decision requires close collaboration between clinician and biologist with other health professionals.

Natural evolution of antibodies during acquired toxoplasmosis

Classically, IgM antibodies appear first, at the end of the first week following contamination, and are detected 7 to 12 months on average with current techniques; the presence of IgM class antibodies does not allow for the confirmation of progressive toxoplasmosis. IgG antibodies usually appear from the 8th day of life (their delay of appearance can sometimes reach 5 to 6 weeks) and rise progressively to reach a plateau from the 2nd or 3rd month. Titers then slowly decline. IgG persists throughout life at a residual level (Figure 1). Knowledge of this kinetics allows interpretation of the serologic status. Diagnosis of progressive toxoplasmosis requires the study of two serums 15 to 20 days apart, demonstrating either seroconversion (first serum negative, second serum positive) or the presence of IgM associated with a significant increase in IgG titer between the first and second serum titrated in parallel. To be significant, an increase in IgG titer implies at least two dilutions (of reason 2) of difference with the dilution methods (IFI, for example); in ELISA, the increase to be considered as significant varies according to the kits but a doubling of the titer is the minimum to be considered.
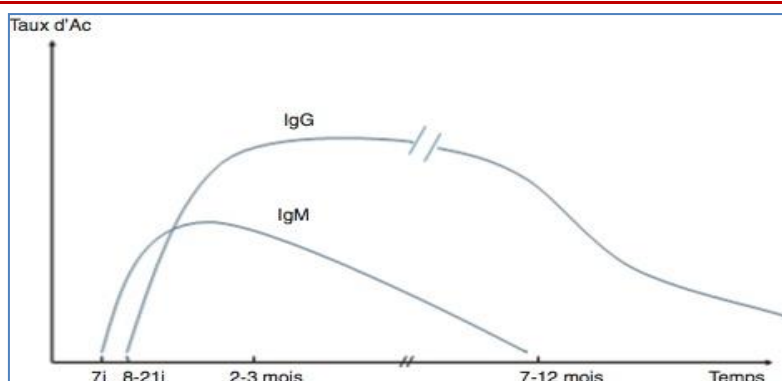
Fig-1: Kinetics of IgM and IgG antibodies during primary toxoplasma infection

Course of action according to IgG, IgM and avidity test results

Situation 1: absence of IgG and IgM detection

The conclusion is that the subject has no specific antibodies. In the case of a pregnant woman, monthly serological monitoring should be continued until delivery and one month afterwards, and strict adherence to hygienic and dietary measures should be recommended. In the case of a woman of childbearing age, it is useful to plan a serological check-up when contraception is prescribed and when it is stopped if the first serology is negative.

Situation 2: absence of IgG detection but with IgM detection

It is then necessary to carry out a second technique of detection of IgM of different principle. Two situations may then arise:

If the confirmatory technique is negative and it is a first serum, the presence of IgM with a single technique may correspond to non-specific natural IgM detecting ubiquitous antigens or to interference. However, the performance of IgM detection techniques is variable, especially in terms of early detection. The onset of seroconversion cannot be totally excluded and serology should be checked on a second serum spaced 1 to 2 weeks apart. If the results of the second serum are identical to the first, the first hypothesis of natural IgM or interference tends to be confirmed. This hypothesis will be all the more easily confirmed if the delay between the 2 samples is important.

For a pregnant woman, monthly serological surveillance should be continued until delivery and one month after delivery, and follow-up of hygienic and dietary measures should be recommended.

If the confirmatory technique is positive and it is a first serum, a recent infection is very likely. However, the presence of positive IgM, even with 2 techniques, does not definitively exclude the hypothesis of the presence of non-specific natural IgM or of interference. Indeed, both techniques can theoretically present the same specificity defects. It is therefore recommended that the complementary confirmatory technique be of a totally different principle. If a recent infection is suspected, a serological control within 1 to 2 weeks, followed by a close follow-up, should be performed until the seroconversion is confirmed or not. Toxoplasma seroconversion can only be confirmed by the appearance of specific IgG antibodies, which occurs in less than one month in most cases, although this period may vary according to the techniques used and the possible introduction of treatment. In the case of a pregnant woman, diagnostic and therapeutic measures for congenital toxoplasmosis, adapted to the gestational age, should be implemented after discussion with the clinician. If the results of the second serum are identical to those of the first (negative IgG and positive IgM by 2 different techniques), this is a case of non-specific natural IgM or interference and serological monitoring should be continued. On the other hand, if, in addition to IgM, an appearance of IgG is observed at the time of this control, it is then a confirmed seroconversion and in a pregnant woman, medical management adapted to the gestational age must be instituted as soon as the diagnosis is confirmed.

Situation 3: presence of IgG and IgM

For the pregnant woman, it is necessary to date the infection to the beginning of the pregnancy. It is advisable to look for previous serum or results and, in the absence of previous results, it is recommended to measure the avidity of IgG if the IgG titre allows it.

If the IgG avidity is high, a recent infection can be excluded (depending on the exclusion period of the reagent used). In case of pregnancy, a confirmation check at 3 weeks is recommended. If the IgG titer is stable, an old infection can be concluded. The results should be interpreted according to the date of onset of pregnancy and medical management should be adapted to the gestational age.

If the IgG avidity is intermediate or low, these results do not allow the exclusion of a recent infection and only the kinetics of the antibodies carried out on a second sample at an interval of 3 weeks will allow dating the infection. In the presence of stable IgG, it can

be concluded that the infection is probably more than 2 or 3 months old from the date of the first serum (depending on the reagent used). If a significant increase in IgG (doubling of the titre in IU/mL) is observed, the infection is less than 2 to 3 months old. The management of the pregnant woman should be adapted according to the gestational age.

Situation 4: presence of positive IgG and absence of IgM

In the absence of previous pregnancy, serology should be checked on a second serum taken 3 weeks apart. If the IgG titer is stable, it is concluded that the infection is old. If the IgG titer increases, it is recommended to date the infection by determining the IgG avidity on the first serum (if the titer allows it). If the avidity is high, it can be concluded that there is a probable serological reactivation of an old infection. If the avidity is intermediate or low, a recent infection without IgM or with fugitive IgM cannot be excluded and medical management should be adapted to the gestational age.

Situation 5: presence of equivocal IgG and negative IgM

This serological profile associating an equivocal IgG titre (in the grey zone of the technique used) and the absence of IgM, raises the problem of the exact immune status of the patient with respect to toxoplasma, and therefore, in the particular case of a pregnant woman, of the justification for continuing or not her surveillance during her pregnancy. In practice, when faced with this serological profile, it is recommended that a second IgG detection technique of different principle be performed.

If the second technique is negative, it is concluded that there are no specific antibodies. Serological monitoring of the pregnant woman should then be continued until delivery and one month after delivery. This follow-up should be accompanied by the hygienic and dietary recommendations described. In the case of a woman of childbearing age, a serological check-up will be recommended when contraception is prescribed, as well as when it is stopped.

- If the second technique is positive, we conclude that the infection is probably old. These results should be confirmed in pregnant women on a serum taken 3 weeks apart.
- If the second technique is equivocal, it is recommended that the serum be sent to an expert laboratory for further testing.

CONCLUSION

We underline the unavoidable importance, of a serological surveillance of pregnant women which will allow to detect and follow as early as possible the non-immune women, Now, it is an essential step in the prevention of congenital toxoplasmosis because, from the conclusions of the serological analysis, derives all the subsequent conduct to be held, both prenatal and postnatal and evolutive toxoplasmosis in order to take care of the contaminated children.

REFERENCES

- Abu-Madi, M.A., Al-Molawi, N., Behnke, J.M. (2008). Séroprévalence et corrélats épidémiologiques des infections à *Toxoplasma gondii* chez les patients référés pour des tests sérologiques en milieu hospitalier à Doha, au Qatar. *Vecteurs de parasites,* 1; 39.
- Al-Hindi, A., Al-Helou, T., Al-Helou, Y. (2010). Séroprévalence de *Toxoplasma gondii*, cytomégalovirus, virus de la rubéole et *Chlamydia trachomatis* chez les femmes infertiles fréquentant un centre de fécondation in vitro, bande de Gaza, Palestine. *J Egypte Soc Parasitol,* 40; 451-458.
- Alsammani, M.A., Ahmed, S.R., Alsheeha, M.A., Saadia, Z., Khairi, S.A. (2012). Co-infection à *Toxoplasma gondii* et Clostridium perfringens chez une femme en post-partum atteinte de gangrène gazeuse utérine: à propos d'un cas. *J Obstet Gynaecol Res,* 38; 1024–1027.
- Bin Dajem, S.M., Almushait, M.A. (2012). Détection de l' ADN de *Toxoplasma gondii* par PCR dans des échantillons de sang prélevés sur des femmes saoudiennes enceintes de la région d'Aseer, en Arabie saoudite. *Ann Saudi Med,* 32; 507–512.
- Elbez-Rubinstein, A., Ajzenberg, D., Darde, M.L., Cohen, R., Dumetre, A., Year, H., Gondon, E., Janaud, J.C., Thulliez, P. (2009). Toxoplasmose congénitale et réinfection pendant la grossesse: rapport de cas, caractérisation de la souche, modèle expérimental de réinfection et revue. *J Infecter Dis,* 199; 280-285.
- Jumaïen, N.F. (2005). Séroprévalence et facteurs de risque d'infection à toxoplasme chez les femmes enceintes en Jordanie. *East Mediterr Health J,* 11; 45-51.