

Short Communication

Prenatal Diagnosis of Toxoplasma Gondii Infection During Pregnancy Using Toxoplasma Gondii Igg/Igm, Igg Avidity Index Serology and Polymerase-Chain-Reaction (PCR): A Lagos Prenatal Diagnosis and Therapy Centre Experience

G.O. Ajayi*

Department of Obstetrics and Gynecology, University of Lagos, Nigeria

*Corresponding author

G. O. Ajayi, Department of Obstetrics and Gynecology, University of Lagos, Professor / Consultant, College of Medicine, P. M. B. 12003, Idi-Araba, Lagos, Nigeria. Tel: 234-1-295 5853; Email: goicajayi@yahoo.com

Submitted: 31 May 2017

Accepted: 30 August 2017

Published: 31 August 2017

ISSN: 2373-9282

Copyright

© 2017 Ajayi

OPEN ACCESS

Keywords

- Prenatal diagnosis
- Toxoplasma gondii
- Amniocentesis
- Cordocentesis
- IgG/IgM
- Serology
- Polymerase-chain-reaction (PCR)

Abstract

Background: Maternal Toxoplasma gondii infection during pregnancy can cause significant morbidity and mortality in the developing fetuses. But there is a little consensus about screening during pregnancy and the test used to establish a Toxoplasmosis diagnosis are complex.

Objective: The objective is to determine the seropositivity rate of Toxoplasma gondii IgG/ IgM in maternal and fetal compartment and compare the fetal Toxoplasma gondii IgM result with polymerase-chain-reaction test (PCR) result.

Setting: The outpatient of Prenatal Diagnosis and Therapy Centre laboratory of a University Tertiary Care Centre in Lagos.

Design: A retrospective study.

Method and Material: Prenatal Diagnosis Test including ultrasonography, maternal blood screening for Toxoplasma gondii IgG/IgM, amniocentesis and fetal blood sampling were performed in n =398 patients referred to rule out present or past Toxoplasma gondii infection. Amniocentesis was performed in 70 (78.65%) cases including a set of twin and cordocentesis in 19(21.35%) cases. The results obtained from a Toxoplasma gondii IgM positivity in Amniotic fluid and Cord blood were compared with the result of the PCR test on the 17 Toxoplasma gondii IgM positive in amniotic fluid and 5 Toxoplasma gondii IgM positive in cord blood. Congenital infection was found in 22 (24.72%) cases out of the 89 patients with IgG/IgM Toxoplasma gondii sero positivity. The PCR was positive in 20 (90.90%) out of 22 cases.

Conclusion: Prenatal diagnosis using ultrasound, amniocentesis, cordocentesis, serology testing and PCR is relatively safe, reliable and accurate. It must be done by experienced personnel.

INTRODUCTION

Acute primary maternal Toxoplasma gondii infection during pregnancy can cause significant morbidity and mortality in the developing fetuses (Singh 2003 [1], Thiebaut et al, 2007 [2]). The modes of transmission are from mother to fetus through food, or water contaminants with cat faeces or by eating undercooked meat of infected animals (Bahra – Oliveira et al. 2003 [3], Singh 2003 [1]). Congenital infection of the fetus in women infected before conception is rare and even during the first few weeks of pregnancy. The maternal fetal transmission rate is low (Emna et al, 2006 [4], Press et al, 2005 [5]). It is therefore very important to check the time of infection as precisely as possible to properly manage the link to the fetus of a maternal infection. A

positive IgM test result in a single serum specimen may reflect an acute infection, but low levels of Toxoplasma gondii-specific IgM antibodies may be found for up to several years, and thus lead to wrong interpretation.

The combination of a sensitive test for Toxoplasma gondii – specific IgM antibodies and measurement of the avidity of IgG antibodies for Toxoplasma gondii had the highest predictive value with regard to the time of infection (Petersen et al, 2005 [6]).

Prenatal diagnosis of congenital toxoplasmosis is based on ultrasonography, amniocentesis and fetal blood sampling (Ajayi 2009 [7], 2011 [8], 2015 [9], Hohlfield et al, 1991 [10], 1994 [11], Desmont et al, 1985 [12], Daffos et al, 1985 [13]).

Since 1992, Prenatal Diagnosis and Therapy Centre was established with College of Medicine but amniocentesis as procedure started 1988 (Ajayi 2011 [8]).

No report of prenatal diagnosis of toxoplasmosis from Nigeria exist, therefore the present study was undertaken to detect an ongoing or recent *Toxoplasma gondii* infection before and during pregnancy using Enzyme Immunoassay for *Toxoplasma gondii* – specific IgG / IgM, IgG –Avidity Index test and polymerase-chain-reaction test (PCR).

METHOD AND MATERIAL

The study was performed at Prenatal Diagnosis and Therapy Centre, College of Medicine, University of Lagos, Lagos between January 1997 and December 2015. A total of n = 398 patients were referred including in patients and were screened to rule out *Toxoplasma gondii* IgG / IgM positivity using ELISA kit (Dia-Pro Diagnostic, Bio probes Srl, Milano, Italy, Euroimmun, Diagnostik, Luebeck / Germany, Diagnostic Automation, INC. Calabasas, CA 91302, USA) according to the manufacturers' instructions after informed consent obtained.

If *Toxoplasma gondii* IgG and IgM is positive, the maternal serum is further screened for *Toxoplasma gondii* IgG Avidity Index test using ELISA kit (Euroimmun, Diagnostik, Luebeck / Germany) according to the manufacturers' instructions and amniocentesis or cordocentesis is being offered to rule out fetal infections. The technique amniocentesis and cordocentesis used in our program has previously been reported (Ajayi 2009 / 2011 [7,8]). We used Siemens Vidoson /Sonoline(Siemens AG, Erlangen/Germany), Kretz Technik Combison 350S / Austria and Picker International LS 2700 Ultrasound machines for this purpose. A 20 or 22 gauge spinal needle was used. After separation of the amniotic fluid or the buffy coat from the fetal blood, DNA was extracted. The PCR was performed on 10µl of the supernatant from amniotic fluid and that of cordocentesis. The target of application Suant instrument, Britain was the 35- fold repetitive B1 gene of *Toxoplasma gondii*. The application products were analysed after electrophoresis on an 8% acrylamide gel and staining with ethidiumBromide.

The procedures were done after genetic counseling and TORCH antibodies screening (Ajayi 2003[14]).

RESULT

(Table 1) and (Figure 1) show our results.

89 out of 238 (37.39%) *Toxoplasma gondii* IgG seropositive were found to be IgG / IgM *Toxoplasma gondii* sero-positive (Table 1).

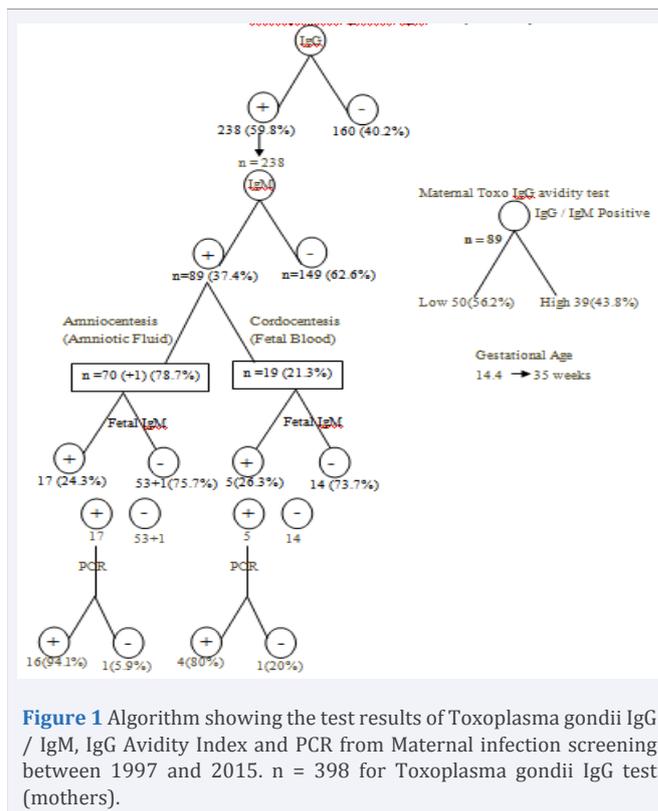
Out of 70 patients including one set of twin carrier, who had amniocentesis done 17 (24.29%) were found to be *Toxoplasma gondii* IgM positive in amniotic fluid.

Out of 19 IgG / IgM *Toxoplasma gondii* seropositive patients who had cordocentesis done, 5 (26.32%) cord blood tested *Toxoplasma gondii* IgM positive.

A total of 22 (24.72 %) patients using both amniocentesis and cordocentesis procedures were Fetal IgM *Toxoplasma gondii* positive.

Table 1: Result for *Toxoplasma gondii* IgG / IgM, in maternal blood, amniotic fluid, cord blood and PCR test in amniotic fluid and cord blood.

Microorganism screened for	Toxoplasmosis
Total Patients referred	n = 398
Maternal IgG positive	n = 238 (59.79%)
Maternal IgG/IgM positive	n = 89 (37.39%)
Amniotic Fluid samples	n = 70x – 1 sample twin
Fetal Toxo IgM Positive	n = 17 (24.29%)
Fetal Toxo IgM Negative	n = 53 (75.71%)
Cord Blood samples	n = 19
Fetal Toxo IgM Positive	n = 5 (26.32%)
Fetal Toxo IgM Negative	n = 14 (73.68%)
PCR TEST	n = 22
Amniotic Fluid samples	n = 17
PCR Positive	n = 16 (94.12%)
PCR Negative	n = 1 (5.88%)
Fetal Cord Blood samples	n = 5
PCR Positive	n = 4 (80%)
PCR Negative	n = 1 (20%)



Out of 89 IgG / IgM *Toxoplasma gondii* positive who had *Toxoplasma* IgG – Avidity test done 39 (43.8%) had high – avidity antibodies suggesting that the infection was acquired before gestation. Only 50 (56.2%) had low avidity IgG antibodies suggesting a recent *Toxoplasma gondii* infection in these patients.

Using PCR method, out of 17 amniotic fluid *Toxoplasma gondii* IgM positive samples, 16 (94.12%) were positive and 1 (5.88%) negative. In the 5 fetal blood samples with *Toxoplasma*

gondii positive IgM, 4 (80%) samples were PCR positive and 1 (20%) negative (Table 1) and (Figure 1).

DISCUSSION

The diagnosis of primary Toxoplasmosis in pregnancy is of utmost importance in order to offer early treatment or other intervention to prevent congenital infection of the fetuses (Reis et al, 2006 [15], Singh 2003 [1]). Based on this, it is useful to be informed about the Toxoplasma-specific antibody status of a woman before or during pregnancy. Our results show that Toxoplasma gondii IgG Avidity Index test when used as a confirmation test along with the Toxoplasma gondii IgG / IgM test in women during pregnancy, was highly useful in distinguishing a recently acquired infection from a chronic infection (Figure 1).

Routine serological diagnosis of toxoplasmosis shows high sensitivity, but specificity varies depending on the test used. In our study, 89 (37.4%) out of 238 Toxoplasma gondii IgG positive out of a total of 368 screened were Toxoplasma gondii IgG / IgM positive, suggesting an acute infection necessitating appropriate treatment measures (Table 1). Detection of anti-Toxoplasma gondii-specific IgM antibodies is a sensitive indicator of an ongoing infection. Recently, Emna et al, 2006 [4] reported false – positive IgM antibody result in cases like this, the diagnosis can be improved on by running Toxoplasma gondii IgG Avidity Index test, which has the ability to discriminate between recent and previous infections. In our result, 50 (56.2%) of the IgG / IgM positive women had low-Avidity IgG antibodies suggesting a recent Toxoplasma gondii infection in these patients and 39 (43.8%) of the IgG / IgM positive women had high – Avidity IgG antibodies suggesting that the infection was acquired before pregnancy (Figure 1). This discrepancy in detecting infection status by IgM serology and IgG Avidity Index tests may be due to the fact that IgM antibodies may stay for months or years after the acute infection phase in some individuals, thus the presence of IgM antibodies is not always an indication of a recent infection (Gras et al, 2004 [16], Montoya et al, 2002 [17]). The presence of Toxoplasma gondii IgM antibodies in the chronic stage of an infection as observed in 39 (43.8%) of the Toxoplasma gondii IgM – positive cases may have resulted in unwarranted concern and a misdiagnosis particularly in women at early gestation.

The prenatal diagnosis of Congenital Toxoplasmosis is important to prevent unnecessary termination of pregnancy (Hohlfeld et al, 1994 [11]). Several studies have shown the results with the polymerase-chain-reaction test (PCR) in the diagnosis of Toxoplasmosis with the use of P30 (Dupouy – Camet – et al, 1992 [18], Savva et al, 1990 [19] and B1 gene targets (Grover et al, 1990 [20]). Van de Ven et al, 1991 [21] or a segment of the 18S ribosomal DNA (Cazenave et al, 1992 [22], Guay et al, 1993 [23]). In our study, the PCR test based on the B1- gene target in amniotic fluid with higher sensitivity than conventional methods was used (Van de Ven et al, 1991 [20]).

The PCR can also show false positive results, mainly through contamination with amplification products (Conto et al, 2003 [24]). Hohlfeld et al, (1991[10]) studied 339 French women, who sero-converted during pregnancy from September 1990 to December 1992, congenital infection was found through conventional methods (inoculation of amniotic fluid and / or

blood from umbilical cord in mice and / other cellular cultivation and IgM research in umbilical cord serum in 34 of 339 fetuses. PCR was positive in all 34 fetuses and three others who had negative conventional test results. Prenatal diagnosis was confirmed by post – natal serological tests or by autopsy findings in case of abortion. They used a Toxoplasma gondii B1 gene as a target for the PCR test, the sensitivity was 97.4% compared to 89.5% with conventional methods demonstrating that PCR is fast, safe and reliable for prenatal diagnosis of Congenital Toxoplasmosis. Ram Castro et al, 2001 [25] using B1-gene primer for the detection of fetal infection by Toxoplasma gondii in 37 pregnant women with acute infection found PCR sensitivity of 67% and specificity of 87% and among the 37 pregnant women, 8 presented positive results for PCR Diagnosis was confirmed only in four new born. Two other fetuses with post natal diagnosis were negative in the PCR test (false negatives). In our study, out of the 17 amniotic fluids with Fetal IgM Toxoplasmosis gondii positivity, 16 (94.12%) were positive with PCR method and in 5 cord bloods, 4 (80%) were PCR positivity confirming the efficiency of PCR method. Our results showed although sample size is small that Fetal IgM testing in amniotic fluid comes close to PCR test results (Table 1) (Figure 1). There are problems with the PCR Technique. Daffos et al. 1988 (26) indicated that false-negative results could occur due to later transmission of the parasite to the fetus, after PCR, despite treatment. Grover et al, 1990 [20] stated that the primer may not be able to amplify the gene contained in the sample, quality control is necessary so that this does not occur, by the primer in several Toxoplasmosis gondii strains.

Examination with high rate of false-positive results can be a consequence of contamination at any stage of the process (Castro et al, 2001 [25]). As PCR still has limitation in sensitivity and specificity depending on the methodology and primer used in each laboratory. It should not be the only diagnostic method (Ruit Lopes et al, 2007 [27]).

Awareness of the dangers of the disease and serological follow up during pregnancy are of great importance in prevention of congenital toxoplasmosis (Hill et al, 2002 [28]). Countries that have prevention of congenital toxoplasmosis, have a low prevalence of this disease. Like Slovenia with an incidence of 9/1000 women (Logar et al, 2004 [29]) and France, fetal toxoplasmosis rate reduced 40% to 71% (Spalding et al. 2003 [30]).

In conclusion, prenatal screening for Toxoplasmosis using ultrasonography, amniocentesis, cordocentesis, serological testing using IgG / IgM and PCR is relatively safe and reliable methods of prenatal diagnosis but it needs to be carried out by experienced personnel and usage of cordocentesis is now less popular.

ACKNOWLEDGEMENT

For the training of authors / grants: DAAD /DFG (Germany); Prof. W. Holzgreve, Prof. J. Horst, Prof. Dr. H-P. Doehring and Prof. P. Miny (Bonn, Muenster, Koeln/ Germany, Basel/Switzerland). For other help: Prof.A. Agboola, Prof.O. Sofola, Prof.T. Odugbemi, Prof. O. Abudu and Prof. F. Giwa – Osagie.

In memory of the late Prof. O. Coker, Prof. O. Akinrimisi, Prof.

A. Akinkugbe, my late parents (Chief (Dr) Mr & Mrs D.M. Ajayi) and Late Mr. and Mrs. Consul. G. Nordmann, Hamburg / Germany.

REFERENCES

- Singh S. Mother-to-child transmission and diagnosis of *Toxoplasma gondii* infection during pregnancy.
- Indian J med microbial. 2003; 21: 69-76.
- Thiebaut R, Leprorist S, Chene G, Gilbert R. Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. *Lancet*. 2007; 369: 115-112.
- Bahra - Oliveira LM, Jones JL, Azevedo-Silva J, Alves CC, Orefice F, Addis, D.G. Highly endemic, waterbone toxoplasmosis in north Rio de Janeiro state, Brazil. *Emerg infect Dis*. 2003; 9: 55- 62
- Emna S, Karim A, Mohammed K, Aida B. Difficulty in dating primary infections by *Toxoplasma gondii* in pregnant women in Tunisia. *Tunis Med*. 2006; 84: 85-87.
- Press C, Montoya JG, Remington JS. Use of a single serum sample for diagnosis of acute toxoplasmosis in pregnant women and other adults. *J Clin Microbiol*. 2005; 43: 3481-3483.
- Petersen E, Borobio MV, Guy E, Liesenfeld O, Meroni V, Naessens A, et al. European multicenter study of the LIAISON automated diagnostics system for determination of *Toxoplasma gondii*-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. *J Clin Microbiol*. 2005; 43: 1570-1574.
- Ajayi GO. Chorionic Villus Sampling: analysis of the first 350 singleton pregnancies by a single operator.
- Clin Exp Obstet Gynecol*. 2009; 36: 251-253
- Ajayi G.O. Ultrasonography guided amniocentesis in singleton pregnancies: a review of the first 1000 cases by a single operator. *Clin Exp Obstet Gynecol*. 2011; 38: 405-408.
- Ajayi G.O. Ultrasonography guided Cordocentesis in the first 100 singleton pregnancy. 27 Deutscher Kongress Fur Perinatale Medizin 2015 PV046.
- Hohlfeld P, MacAleese J, Capella-Pavlousky M, Giovangrandi Y, Thulliez P, Forestier F, et al. Fetal toxoplasmosis: ultrasonographic signs. *Ultrasound obstet gynecol*. 1991; 1: 241-244.
- Hohlfeld P, Daffos F, Costa JM, Thulliez P, Forestier F, Vidaud M. Prenatal diagnosis of congenital toxoplasmosis with a polymerase-chain-reaction test on amniotic fluid. *N Engl J med*. 1994; 331: 695-699.
- Desmont G, Daffos F, Forestier F, Capella-Paviousky M, Thulliez P, Chartier M. Prenatal diagnosis of congenital toxoplasmosis. *Lancet*. 1985; 325: 500-504.
- Daffos F, Capella-Paviousky M, Forestier F. Fetal blood sampling during pregnancy with use of a needle guided by ultrasound: a study of 606 consecutive cases. *Am J Obstet Gynecol*. 1985; 153: 655-660.
- Ajayi GO, Omilabu SA, Alamu A, Agboola A. Fetal Diagnosis of infectious disease. A Lagos program Experience. In: The problem of the EPH- Gestosis. Eds. Rippmann ET Bagdanys, Budapest/ Hungary. 2003; 99 -103.
- Reis MM, Tessaro M, DAzevedo PA. *Toxoplasma* IgM and IgG avidity in single samples from areas with a high infection rate can determine the risk of mother - to- child transmission. *Rev Inst med trop Sao Paulo*. 2006; 48: 93 -98.
- Gras L, Gilbert R, E, Wallon M, Peyron, F Cortina - Borja M. Duration of the IgM response in women acquiring *Toxoplasma gondii* during pregnancy implications for clinical practices and cross sectional incidence studies. *Epidemiol infect*. 2004; 132: 541-548.
- Montoya JG, Liesenfeld O, Kinney S, Press C, Remington JS. Vidas test for avidity of *Toxoplasma* specific immunoglobulin G for confirmatory testing of pregnant women. *J Clin Microbiol*. 2002; 40: 2504-2508.
- Dupouy-Carnet J, Bougnoux ME, Lavaredade Souza S, Thulliez P, Dommergues M, Mandelbrot L, et al. Comparative value of polymerase chain reaction and conventional biological test for the prenatal diagnosis of congenital toxoplasmosis. *Ann BiolClin (Paris)*. 1992; 50: 315-319.
- Savva D, Morris JC, Johnson JD, Holliman RE. Polymerase chain reaction for diction of *Toxoplasma gondii* *J.Med Microbiol*. 1990; 32: 25-31.
- Grover CM, Thulliez P, Remington JS, Boothroyd JC. Rapid Prenatal diagnosis of congenital *Toxoplasma* infection by using polymerase chain reaction and amniotic fluid. *J Clin Microbiol*. 1990; 28: 2297-2301
- Van de Ven E, Melchers W, Galama J, Camps W, Meuwissen J. Identification of *Toxoplasma gondii* infections by B1 gene amplification. *J Clin Microbiol*. 1991; 29: 2120-2124.
- Cazenare J, Forestier F, Bessieres MH, Broussin B, Begueret J. Contribution of a new PCR assay to the prenatal diagnosis of congenital toxoplasmosis. *Prenat.Diagn*. 1992; 12: 119-127.
- Guay JM, Dubois D, Morency MJ, Gagnono S, Mercier J, Levesque RC. Detection of the pathogenic parasite *Toxoplasma gondii* by specific amplification of ribosomal sequences using co-multiplex polymerase chain reaction. *J Clin. Microbiol*. 1993; 31: 203-207.
- Couto JC F, Melo RN, Rodrigues MV, Leite JM. Diagnostico prenatal e tratamerito da toxoplasmosenagestacao Femina. 2003; 31: 85-90.
- Castro FC, Castro M.J.B.V, Cabral A.C.V, Geraldo BF, Ricardo WAV. Comparacao dos metodospara diagnostic da toxoplasmoscongenita. *Rev Bras Gynecol Obstet*. 2001; 23: 277 -282.
- Daffos F, Forestier F, Capella-Paviovsky M, Thulliez P, Aufrant C, Valenti D, et al. Prenatal management of 746 pregnancies at risk for congenital toxoplasmosis. *N Engl J Med*. 1988; 318: 271-275.
- Ruiz lopes, Daniela Dib Gonealves, Regina mitsuka-Bregano, Roberta Lemos, Freire, Itamar Teodorico Navarro. *Toxoplasma gondii* infection in pregnancy. 2007; 2: 1678-4391.
- Hill D, Dubey JP. *Toxoplasma gondii* transmission diagnosis and prevention. *Clin Microbial infect*. 2002; 6: 634-640.
- Logar J, Petrovec M, Novak-Antolic Z, Premru-Srsen T, Cizman M, Arnez M, et al. Prevention of congenital toxoplasmosis in Slovenia by serological screening of pregnant woman. *Scand J Infec Dis*. 2002; 34: 201-204.
- Spalding SM, Amendoeira MRR, Ribeiro LC Silveira, Cláudio, Garcia, et al. Estudo prospective gestantes e seusbebes com risco de transmissao de toxoplasmos congenital emmunicipio do rio Grande do sul. *rev soc*
- Bras med Trop*. 2003; 36: 483-491.

Cite this article

Ajayi GO (2017) Prenatal Diagnosis of *Toxoplasma Gondii* Infection During Pregnancy Using *Toxoplasma Gondii* Igg/Igm, Igg Avidity Index Serology and Polymerase-Chain-Reaction (PCR): A Lagos Prenatal Diagnosis and Therapy Centre Experience. *Ann Clin Pathol* 5(5): 1121.