A retrospective study using IgA in association with IgG and IgM for precise diagnosis of toxoplasmosis in suspected cases

khitam, Y.O. AL-dujaily[1];Athmar k.Alazawi[2] ; Haider, K .Shindakh[1] and Jaber, A.M.Taqi[1]

[1] Central Public Health Laboratories (CPHL) Ministry of Health. Baghdad [2] Department of parasitology, Faculty of Veterinary Medicine, University of Baghdad, Iraq

Accepted: 12/ 3 / 2012

Summary

In order to achieve precise diagnosis of toxoplasmosis in suspected cases of aborted women and infants with abnormal clinical manifestation, 1527 serum samples consisting of 1450aborted women and 77 infants were tested by enzyme linked immune sorbent assay (ELISA), using IgG, IgM and IgA platellat oxogondii kits, 865 (56.64%) were found positive, 848 (58.48%) were found in aborted women and 17 (22.07%) infants with a significant differences (P≤ 0.05).The immunoglobulin IgM , were detected in 72 (8.49%)cases of aborted women, IgM with IgG were found in 44 (61.11%) and with IgA in two (2.77%) cases ,whereas found in three(17.64%)cases of infants. While IgA were found in 77(9.08%) cases of aborted women, and 6 (35.22%) infants. IgG was detected in699 (82.42%)with a significant differences(p≤ 0.05),alone in602 (86.12%) ,and with IgA in 34(4.86%) case of aborted women and 8(47.05%)of infants. These results were support the importance of testing the suspected cases by all kinds of immunoglobulins (IgG, IgM and IgA) to achieve precise diagnosis for toxoplasmosis.

Keywords: Toxoplasmosis, IgG, IgM, IgA, Antibodies, Detection, Retrospective.

دراسة تراجعية باستعمال الضد I gA والاضدادIgM و IgG في التشخيص الدقيق لداء مقوسات كوندي (Toxoplasmosis) فى الحالات المشكوك بها

ختام يحيى الدجيلي[1] و اثمار خضير العزاوي[2] وحيدر شنداخ[1] وجابراحمد تقي[1]

[1]مختبرات الصحة المركزية - وزارة الصحة [2] فرع الطفيليات- كلية الطب البيطري - جامعة بغداد- العراق

الخلاصة

لاجل اجراء تشخيص دقيق للحالات المشكوك باصابتها بداء مقوسات كوندي فى النساء المجهضات والمرضعات التي اظهرت علامات سريرية . اجرى اختبارتقنية الامتزاز المناعي المرتبط بالانزيم (الاليزا) على 1527عينة مصل 1450 من النساء المجهضات و 77 عينة من المرضعات باستعمال العدة التشخيصية المعتمدة على الكشف عن الضدات نوع IgG و IgM وIgA وجد 865 (65.64%)حالة موجبة848 (58.48%) فى النساء المجهضات و17 (22.07%) فى الرضع وبفروقات معنوية (p0.05>) وجدالنوعIgM فى النساء المجهضات في 8.49%) (72حالة مع الضد IgGفى.61%44(11) حالة ومع الضد IgA في حالتين (2.77%) اما في الرضع فقد سجل في ثلاث حالات فقط (17.64%) وبفروقات معنوية (p0.05>) بينما تم الكشف عن الضد IgA فى 77 (9.08%) حالة للنساء المجهضات وفى 6 حالات للرضع (35.22%) .وسجل الضد IgG فى 699(82.42%) حالة وبفروقات معنوية (P<0.05) فقد سجل مفردا فى 602 (86.12%)ومع IgA في 34(4.86%) حالة لمصل النساء وبنسبة 8 (05. 47%) للرضع اثبتت النتائج اهمية الكشف عن انواع الضدات المختلفة IgGو IgMو IgAفي الحالات المشكوك بها لاجل الكشف المبكروالدقيق لحالات الاصابة بداء مقوسات كوندي.

الكلمات المفتاحية: الضد , الأضداد , داء المقوسات , النساء المجهضات والمرضعات .

Introduction

Toxoplasmosis is a zoonotic parasitic disease found frequently in human, the prevalence of infection in population might differ depending upon the country and age. The estimated worldwide infection is about half a billion individuals carrying antibodies against *Toxoplasma gondii*(1).The disease is usually a symptomatic and congenital presentation may

lead to abortion or fatal malformation or significant morbidity and mortality in the developing fetus , if the mother acquires acute infection during pregnancy (2,3 and 4). Serologic confirmations of *T. gondii* an antibody are indicative of exposure to the parasite and have widely become accepted as a mean to determine immune status and susceptibility to infection. Children's toxoplasma immunoglobulin (IgG) cannot distinguished from maternal IgG acquired across placenta during intrauterine life, and transmitted IgG persisted for several months in the child, while a specific immune response of the infant indicates that the infection is markedly active by the presence of antibodies, which don't cross placenta such as IgM or IgA antibodies(5 ,6 ,7,8 and 9). This study was done to confirm the importance role ofIgM,IgG and IgA in diagnosis of toxoplasmosis in acquired and congenital cases .

Materials and Methods

Total number of 1527 blood samples were collected from suspected cases comprising 1450 aborted women and 77 infants with congenital features of abnormal clinical manifestation jaundice and pyrexia of unknown origin (PUO) were admitted to the serology unit of Central Public Health laboratories (CBHL) from official and private clinics of Baghdad from April to July 2009.Serum samples were separated and frozen at -20 C untill used. The platella ELISA diagnostic kits for detection IgM,* IgG** and IgA*** against Toxoplasma. gondii were performed to all collected sera according to the procedures mentioned in the kits. Results of IgM and IgG antibodies was considered positive when ratio of absorbance were above or equivocal to cutoff value of 9 IU/ ml and negative when less of that .While IgA results considered positive when ratio equal or above 1 IU/ml .Comparison of results was made according to the platella data base.

Student (t) test and the chi-square test (x^2) were used for analytic assessment between ratios. The differences were regarded statistically significant when the P value less than 0.05 (10).

IgM* kit Platella company, France, Cat NO C 2033
IgG ** kit Platella company, France, Cat NO C 2033
IgA*** kit Platella company, France, Cat NO D 2034

Results and Discussion

In this study ELISA test was standardized for detection IgM, IgA and IgG. Results revealed that out of 1527 serum samples, 865(56.64%)were positive which include 848(58.48%) cases of aborted women and 17 cases(22 .07%) of infants which represented a significant differences (P< 0.05),(Table,1).

Table (1). Number of seropositivecases of Toxoplasma gondii in aborted women and infants.

Total serum samples	No of positive cases	%	Aborted women	No. of positive cases	%	Infants	No. of positive case	%
1527	865	56.64	1450	*848	58.48	77	*17	22.07

* p< 0.05

The finding of antibodies to T.gondii in one serum sample only established that the host had been infected at some time in the past(11).Serological diagnosis of congenital toxoplasmosis is most commonly done by demonstration of serum IgM or IgA antibodies in the new born(12).In aborted women IgM was detected in72(8.49%) cases, alone in 7(9.72%) cases, with IgA only in two cases (2.77%) , and with IgG and IgA were detected in 19 (

26.38%) cases .In the sera of the infants, IgM was detected only in 3 cases (17.64%),one case(33.33%) alone, also with IgG and with IgG and IgA (33.33 %) (Table, 2).

Table (2). Detection ofIgM anti Toxoplasma. gondii in association with IgG and IgA in aborted women and infants sera.

NO of cases	Total IgM	%	Alone	%	IgM +IgG	%	IgM + IgA	%	IgM+IgG +IgA	%
Aborted women	72	*8.49	7	* 9.72	44	* 61.11	2	*2.77	19	* 26.38
Infants	3	* 17.64	1	33.33	1	33.33	-	-	1	33.33

* **p< 0.05between aborted women and infants .Similar effect was observed within IgM ratio in aborted women. Differences within percentages of infection in infants were not significant (p> 0.05).**

The found of IgM in the sera was indicated an acute acquired or congenital infection. Remington *et al.*, (13) mentioned that IgM positive sera were either, recently acquired or infection acquired in the distal past or false positive results. While IgM appeared sooner after infection than the IgG and disappeared faster than IgG after recover (12), T. gondii IgM are detected first in recently acquired infection then titers become negative within few months. That some newly born with congenital toxoplasmosis may be negative for IgM or IgA antibodies or both during the neonates period (9). The kit for measuring IgM often has low specificity (8).Remington *et al.*, (9) demonstrated that the usefulness of anti T gondii IgM testing to determine the acuity of the maternal infection is limited because these antibodies may persist for more than one year. It has been reported to persist as long as 12 years, that they may reflect an infection acquired many months before pregnancy (8).Persistence of these antibodies does not appear to have any clinical relevance and those patients should be considered chronically infected. Women who contracts toxoplasmosis during pregnancy has about 50% chance of passing the infection to her fetus, however, the risk and severity of the baby's infection depend upon when the infection occurs in the pregnancy (13).Studies suggested that, when mothers are infected in the first trimester about 10% of fetuses become infected as compared to about 30% and 60% in the second and third trimesters (12).

This study was showed that the important role of IgA as a confirmatory indicator for acute cases beside the IgM, which indicate an acute infection of toxoplasmosis during pregnancy can lead to severe sequlae in fetus (11).In the sera of aborted women ,IgA were detected in 77(9.08%) cases , 22(28.57%) cases were alone , two(2.59%) with IgM , 34(44.15%) cases with IgG and 19(24.67%) with IgG and IgM. In case of infants IgA were found in 6(35.22%) cases ,4(66.66%) alone and in one case(16.66%) with IgG also with IgG and IgM.(Table,4). Detection of IgA in infants may be due to infection during trimester of gestation, and either at birth or in the following weeks together with IgM. in contrast, when the infection occurred in the first term of gestation.The infant at birth will beat the end of the acute phase of fetal infection which characterized by absence of IgM (probably appeared during fetal life and disappeared before birth) ,meanwhile the appearance of IgA (14). In women, the infection can be recorded as chronic cases, because IgA can persist for many months to more than one year, for this reason it is of a little additional assistance for diagnosis of the acute infection in adult (15). Diagnosis of IgA as well as IgM for congenital toxoplasmosis represented an advanced infection of infants (Table, 3).

Table (3).Detection of IgA anti Toxoplasma gondii in association with IgM and IgG in aborted women and infants sera .

NO of CASES	Total IgA CASES	%	IgA Alone	%	IgA +IgG	%	IgA+ IgG	%	IgA+IgG+ IgM	%
Aborted women	77	*9.08	22	*28.57	2	*2.59	34	*44.15	19	* 24.67
Infants	6	*35.22	4	66.66	-	-	1	16.66	1	16.66

*** p< 0.05 between aborted women and infants .Similar effect was observed within IgA ratio in aborted women . Differences within percentages of infection in infants were not significant (p> 0.05).**

Diagnosis can be made by demonstration of IgG in the new born, since maternal antibodies are passively transferred to fetus in utero (9).From all positive cases IgG was revealed in highest rate 699(82.42%) in sera of aborted women,602 (86.12%) cases alone, with IgM in44(4.29%)cases, with IgA in 34(4 .86%)cases and with IgG and IgA in19(2.71%) cases. While in the sera of infants, IgG were detected in 8(47.05%) cases, alone in 5 (62.50%) , one case(12.50%)with IgM, and with IgM and IgA. Significant differences ($p<0.05$) were found between the types of antibodies (Table, 3). This may be an indicative of chronic infection in women and infants. The titer of IgG may be detectable till the end of life and may indicate the chronic stage (16).Determined of Toxoplasma specific IgG in the new bone is not helpful because of passively transmitted antibodies from mother. Most passively transmitted maternal antibodies in the infant will have disappeared by 8-10 month of age as they decrease by approximately to the half each month of life (17).Positive titer after that time suggests that congenital infection is possible with negative maternal antibodies suggesting acquired infection.

Table (4).Detection of IgG anti Toxoplasma gondii in association with IgM and IgA in aborted women and infants sera.

No of cases	Total IgG cases	%	IgG Alone	%	IgG +IgM	%	IgG + IgA	%	IgG+IgM +IgA	%
Aborted women	699	*82.42	602	*86.12	44	*4.29	34	*4.86	19	*2.71
Infants	8	*47.05	5	62.50	1	12.50	1	12.50	1	12.50

*** p< 0.05 between aborted women and infants .Similar effect was observed within IgG ratio in aborted women . Differences within percentages of infection in infants were not significant (p> 0.05).**

The data obtained above established the importance of using IgA accompanied with IgM and IgG as confirmatory tests for more efficient diagnosis of toxoplasmosis in aborted women and infants.

References

1. Lefecre, M.; Lecam, S.; Wallen, M. and Peyron, F.(2006). Delayed maturation of immunoglobulin G avidity: implication for the diagnosis of toxoplasmosis in pregnant women. Europ. J. Clin. Microbiol. Infect. Dis., 25:687-693.
2. Luft, BJ. and Remington, JS.(1992).Toxoplasmic encephalitis in AIDS. J. Clin. Inf. Dis., 15 (2): 211-222.
3. Leite, M.; Siciliano, S.; RochaL, S.A.; Justa, MT.; Cesar, KR.; and Granato, F.H. (2008). Correlation between specific IgM levels and percentage IgG-class antibody avidity to Toxoplasma gondii.Rev.Inst.Med.Trop.SaoPaulo.,50(40):237-242.

4.Khurana, S.;Baggu, R.;Aggarwal, A.;Lyngdoh, V.;Shivapriga, S.;Diddi, K. and Malla, N. (2010). Serological screening for antenatal Toxoplasma infection in Indian. J. Med. Parasitol., 28 (2):143-146.

5.Desmonts, J.; Naot,Y. and Remington, J. S.(1980). Immunoglobulin M – immunosorbent agglutination assay for diagnosis of infectious diseases: diagnosis of acute congenital and acquired Toxoplasma infection .J. Clin. Microbiol., 13:859-864.

6.Pinon, JM.; Thoannes, H.; Poulettyp, H.; Poirriez, J.; Damiens, J. and Pelletier, P. (1986). Detection of IgA specific for toxoplasmosis in serum and cerebrospinal fluid using anon enzymatic IgA. capture assay. Diagn. Immunol., 4:223-227.

7.Stepick-Biek, P.; Thulliez, P.; Araujo, GA.; and Remington, JS. (1990). IgA antibodies for diagnosis of acute and acquired congenital toxoplasmosis.J.Infec. Dis.,162:270-273.

8-Turunen, H.; VuorioK, A. and Leinikki, PO.(1983). Determination of IgG,IgM and IgA antibody response in human toxoplasmosis by enzyme–linked immune sorbent assay (ELISA). Scand. J. Infec. Dis., 15:307-311.

9.Remington, J.S.; McLeod, R.M.;Thulliez, P. and Desmonts,G.(2001).Toxoplasmosis. In: Infectious diseases of the fetus and newborn. Remington, J. S. and Klein, J. 5th ed. W.B. Saunders Philadelphia, Pp: 205- 346.

10 .Johnson, R. and Bhattacharyya, B.(1985). Statistical principles and methods. John Wiley and Sons. New York.

11.Remington, JS.; Thulliez, P. and Montoya, JG.(2004). Recent development for diagnosis of toxoplasmosis. J. Clin. Mcrobio., 42(3):941-945.

12.Singh ,S.(2003).Mother-to-chilled transmission and diagnoses of Toxoplasma gondii .infection during pregnancy. Indian .J.Med.Microbiol.,21.(2):69-76.

13. Remington, JS.; and Montoya, JG.(2009).laboratory test for the diagnosis of toxoplasmosis , Aguid for clinicians. Palo. Alto. Med. Found. U.S.A

14-Bessieres, MH.; Roques, C.; Berrebi, A.; Barre, V.; Cazaux, M . and Pseguela, J. (1992) . IgA response during acquired and congenital toxoplasmosis .J.Clin.Pathol.,45:605 -608.

15.Decccoster, A.; Slizewicz, B.; Simon, J.; Bazin, C.; Darcy, FV.; Tttu ,G.; Boulanger, C.; Champeau, Y.; Demory, J.; Duhamel, M. and Carbon, A. (1991). Platella-toxo IgA, A new kit for early diagnosis of congenital toxoplasmosis by detection of anti-P30 immunoglobulin A antibodies.J.Clin .Microbiol., 29 (10):2291-2295

16.Horvath, KN.; Zenasi, SZ.; Danka, J.; and Kucsera, I. (2005). Value of IgG avidity in the diagnosis of recent toxoplasmosis: A comparative study of four commercially available ant-Toxoplasma gondii IgG avidity assays. Acta. Parasitol., 50:255-260.

17.Liesenfeld, O.; Montoya, JG.; Kinney, S.; Press, C.; and Remington, JS. (2001). Effect for testing IgG avidity in the diagnosis of Toxoplasma gondii infection in pregnant women experience in a US reference laboratory. J. Infec. Dis., 183:1248-1253.