Combination of ELISA and RT-PCR tests in the diagnosis of toxoplasmic infection in aborted women and congenitally infected infants.

دمج تفاعلي الامدصاص المناعي الانزيمي والبوليمري المتسلسل في تشخيص الاصابة بداء المقوسات لدى النساء المجهضات والمواليد المشوهين خلقيا

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Abstract

The diagnosis of toxoplasmic infection in aborted women and congenitally infected infants suspected to have toxoplasmosis infection can be difficult due to similarity symptoms with other diseases. A combination of symptoms, serology and polymerase chain reaction (PCR)may facilitate diagnosis of toxoplasmosis in some patients. The present study compare the detection of toxoplasmosis infection by ELISA IgA and IgG antibodies with Real Time polymerase chain reaction (RT-PCR)in the study subjects. A total of 81 sera samples, 57(70.3%) samples from aborted women and 24(29.7%)samples from congenitally infants have been studied. 49(86%) samples from the aborted women were positive and 8(14%) samples were negative as diagnosed by one or two of ELISA markers (IgAand IgG).The ELISA results indicated that 15(62.5%) samples from infants were positive and 9(37.5%) samples of them were negative. RT-PCR tests indicated that 33(67.3%) from the mothers and 6(40%) from the infants were agreed with ELISA positive samples. For ELISA negative samples, RT-PCR detected toxoplasmosis DNA in 4 (50%) and 2 (22.2%) for the mothers and infants respectively. Therefore, ELISA and RT-PCR can make a good combination tests in detection toxoplasmopsis infection.

Key words: ELISA, toxoplasmic infection

المستخلص

ان تشخيص الاصابة في النساء المجهضات والاطفال المشوهين خلقيا بداء المقوسات قد يكون ذا صعوبة بسبب تشابه الاعراض مع بقية الامراض. لذا فان دمج الاعراض والتشخيص المصلي والتفاعل البوليمري المتسلسل قد يسهل تشخيص الاصابة بداء المقوسات في بعض المرضى. في الدراسة الحالية، تم مقارنة تشخيص الاصابة بداء المقوسات بوساطة تفاعلي الامدصاص المناعي الانزيمي (الضد IgA وIgG) والبوليمري المتسلسل على العينات. تم دراسة 81 نموذج مصلي منها 57 (70,3%) نموذج من النساء المجهضات و 24 (29,7%) من المواليد المشوهين خلقيا شخصت اصابة 49(86%) نموذج من النساء المجهضات باحد او كلا الاضداد في فحص الامدصاص المناعي الانزيمي و8(14%) كانت سالبة بنفس الفحص، كما اظهر الفحص 1(62,5%) نموذجا موجبا و9(37,5%) سالبا في المواليد. اظهر التفاعل البوليمري المتسلسل ان 33(67,3%) نموذجا من الامهات و 6(40%) نماذج من المواليد اتفاقا مع فحص الامدصاص المناعي الانزيمي بينما تم تشخيص الحامض النووي الداوكسيرايبوز في النماذج السالبة لفحص الامدصاص المناعي الانزيمي في 4(50%) و2(22,2%) للامهات والمواليد على التوالي. لذا فان كلا الفحصين يمكن ان يكونا مزيجا جيدا لتشخيص الاصابة بداء المقوسات.

الكلمات المفتاحية: الامدصاص المناعي الانزيمي، داء المقوسات

Introduction

Congenital toxoplasmpsis manifesting when pregnant women contract the primary infection or experience reactivation of the disease during pregnancy [1]. Dsease in fetus can be sever, culminating in aborting, stillbirth , sever neonatal disease or prematurity [2] or the symptoms may be present at birth or develop later in life leading to blindness. Psychomotor retardation and hearing difficulties [3,4,5]. Currentdiagnosis of toxoplasmosis relies either on serological detection of specific anti –*Toxoplasma* immunoglobulin, on culture of amniotic fluid or blood, mouse inoculation or tissue culture of the clinical specimen may confirm the infection but it is require several days to obtain results and labor intensive [6,7,8]. Serological diagnosis of congenital toxoplasmosis in newborns made by demonstration of IgM or IgA antibodies [9], but in some cases IgM persist after birth for up 24 months while IgA detection may be useful for the diagnosis of some recently acquired infection and for diagnosis and follow up of fetus and neonates infection [10]. PCR have been successfully used to diagnosis congenital and ocular toxoplasmosis .PCR of amniotic fluid or urine

sample was useful to prove or disprove fetal toxoplasmosis and to detect infection in lymphadenopathy reactivation of the infection after allogenic stem cell transplantation [11,12,13,14].

Materials and methods

Total of 81 selected sera samples 57(70.3%)samples from aborted women and 24(29.7%)samples from congenitally infants and children attended to the central public health laboratories (Baghdad/ Iraq), were tested for specific anti *Toxoplasma* IgA and IgG antibodies by (IgA and IgG ELISA Platelia ,UK.) also tested by Toxoplasma gondii (T. gondii) RT-PCR kit(Sacace , Biotechnologies ,Italy).Thermal cycles system using Applied Biosystem 7300 RT-PCR APPLERIA).

The principle of anti *Toxoplasma* IgA is a solid – phase immunoenzymatic double sandwich method, with capture of the IgA on the solid phase (micro plate wells coated with anti human alpha chain antibodies).The peroxidase labeled monoclonal antibodies specific to *Toxoplasma gondii* ia directed against a surface antigen pritein. The sample consider to be negative if the ratio (by dividing optical density of the sample on cut-off) is below 0.8 and positive if it is equal or above 1,while the principle of anti *Toxoplasma* IgG is designed to detect IgG antibodies to *Toxoplasma* in human serum using indirect ELISA immune -enzymatic method. *T.gondii antigen* is used for coating the micro plate. A monoclonal antibody labeled with peroxides which is specific for human gamma chain (anti- IgG) is used as a conjugate ,if the titer of IgG antibody less than 6 IU/ml, the sample is negative and it will be positive if it is equal or higher than 9 IU/ml. For RT-PCR reaction mixture consist of PCR –mix-1Toxoplasma gondii/Glob, PCR-buffer-FRT and Taq F polymerase. The target region 529bptandem repeat. For reading the results, internal control (IC)is detected on (6-carboxyflourescene(FAM, Green channel) and *Toxoplasma gondii* on the 4-5-dichloro-6-carboxyflourescene (JOE ,Yellow channel).The results are interoperated through the presence of crossing of florescence curve with the threshold line, the sample is considered to be positive for *Toxoplasma gondii* if in the channel JOE (Yellow) the value of Ct is different from zero(Ct<40)and it consider to be negative if in the channel JOE (Yellow) the value of Ct is not determined (florescence curve does not cross the threshold line)and in the result table on the channel FAM(Green) the Ct value is lower than 30. Thermal cycle used as manufactures instructions (as shown below):

stage	Profile	cycles
1	95 C -15min.	1
2	95 C - 5 sec.	5
	60 C - 20 sec.	
	72 C - 15 sec.	
3	95 C - 5 sec.	40
	*60 C - 30 sec.	
	72 C - 15 sec.	

***equation(fluorescent signal detection)**

Statistical Analysis:

Chi-square test (x^2) were used for analytic assessment between ratio of ELISA antibodies and RT-PCR results. The differences were regarded statistically significant when the P value less than 0.05.

Results and discussion

In the present study,81 selected samples of sera distributed as 57(70.3%) samples from aborted women and 24 (29.3%) samples from congenitally infants and children, were tested by ELISA IgA and ELISA IgG then by RT-PCR. As shown in Table(1), positive ELISA IgA or IgG or both were 49/57(86%) for aborted women and 15/24 (62.5%) for congenital cases. Negative ELISA IgA and IgG, distributed as 8/57(14%) and 9/24 (37.5%) for mothers and congenital cases respectively. All subjects were tested by RT-PCR to find the correlation with ELISA. RT-PCR matched with positive ELISA results in 33/49(67.3%),6/15(40%)for aborted women and congenital cases respectively and for negative ELISA results, PCR detect DNA in 4/8(50%) for aborted women and 2/9(22.2%)for congenital cases.

Table (1): The correlation between ELISA and RT-PCR for the total toxoplasmosis cases.

Source of samples	ELISA IgA(-)IgG(+) or IgA(+)IgG(-) or IgA(+)IgG(+)	RT-PCR		ELISA IgA(-) IgG(-)	RT-PCR	
		+ve	ve-		ve+	-ve
Aborted women	49 86%	33 67.3%	16 22.7%	8 14%	4 50%	4 50%
Congenitally infants and children	15 62.5%	6 40%	9 60%	9 37.5%	2 22.2%	7 77.8%

Table (2): The correlation between ELISA and RT-PCR for the positive toxoplasmosis cases.

Source of samples	ELISA IgA(-) IgG(+)	RT-PCR +VE	RT-PCR -VE	ELISA IgA(+) IgG(-)	RT-PCR +VE	RT-PCR -VE	ELISA IgA(+) IgG(+)	RT-PCR +VE	RT-PCR -VE
Aborted women	39	27	12	6	3	3	4	4	/
Congenitally infants and children	4	/	4	5	2	3	6	4	2

Toxoplasmosis infection could be fatal to the fetus or it may causes medical problems to pregnant women such as abortion or congenitally newborns depending on the strain, dose of the pathogen and stage of pregnancy. Congenital infection resulting in both economic and social concerns [15]. Serological testing has been one of the major diagnostic technique for toxoplasmosis but it has many limitation. PCR has been successfully used to diagnosis congenital and ocular toxoplasmosis in immunocompromised patients [9] by using specimen such as amniotic vitreous, bronchaleveolar lavage, pleural, peritoneal and Cs fluids, placental and brain tissue, whole blood , urine. In the present study, RT-PCR correlate with negative ELISA IgA and IgG in 4/8(50%)2/9(22.2%) for mothers and congenital cases respectively Table (1), Chi- square analysis revealed high significant difference ($P \leq 0.05$,df 3). Failing in detection Toxoplasmosis antibodies by ELISA, could be explained due to the late production in these antibodies until after several weeks of parasitemia or the test may fail to detect *T.gondii* infection in certain immunocompromised patients due to the fact that the titers of specific anti *Toxoplasma* IgG or IgM may fail to rise in this type of patients [16,17],RT-PCR detect parasitemia earlier than ELISA in these samples while RT-PCR matched with ELISA for negative samples is that may be due other pathogens that causes abortion and congenital problems other than toxoplasmosis, so negative results obtained by ELISA and RT-PCR rule out the infection with toxoplasmosis [16].

In Table (2), the correlation between the two tests was 27/39(69.2%) for ELISA (IgA-ve,IgG +ve) , 3/6(50%) ELISA (IgA+ve,IgG-ve) and 4/4(100%) ELISA (IgA+ve,IgG+ve) for abortive women and ELISA (IgA+ve,IgG+ve) 4/6(%) and ELISA (IgA+ve,IgG-ve) 2/5(%) for congenital cases, Chi- square analysis revealed high significant difference ($P \leq 0.05$,df 3). IgG is an indicator that *Toxoplasma* cyst already present in body tissue [18] and some tachyzoites release from cyst and be the source of parasitemia [16], in the meanwhile IgA detection may be useful for the diagnosis of some recently acquired infection or diagnosis and follow up of fetus and neonates infection [10], this may be the reason for this correlation between the two tests [19] explained the limitation value of PCR detection *T.gondii* DNA after anti parasitic therapy and this could be the reason of absence the correlation for the other subjects in the same group Table (2). Another reason for RT-PCR negative results is that may be due to untreated serum samples by microwave heat to increase sensitivity of RT-PCR as done by [20] when he exposed sera to 800 watt microwave oven for 2-3

minutes or until when sera desiccated. For negative RT-PCR results and ELISA(IgA-ve,IgG+ve)0/4 for congenital cases, this may be due to the same explanation by [19] or clearance of parasitemia by immune system since [13] found the clearance time for *Toxoplasma* DNA from blood with acute lymphoadenopathy estimated to be 5.5-13 weeks, and this may be the same for congenital samples from such subjects.

The conclusion come out for this study, that the combination of serological test and RT-PCR make diagnosis of toxoplasmosis be more precisely.

References

1. Gaimaraes, A.C.S, M., Borges, M.M, Tolegano, J.E. and Andrade, Jr. (1993). Regional variation in toxoplasmosis sero - negativity in the Sao Paulo metropolitan region. Rev. Inst. Med Trop.S. Paulo. 35: 479 – 483.
2. Leitem, M., Siciliano, S., Rochal, S.A, justam, T.R, Cesar, K.R. and Granato, C.F.H. (2008). Correlation between specific IgM and percentage IgG Class antibody avidity to *Toxoplasma gondii*. Rev. Inst. Med. Trop. S.Paulo. 50:231-242.
3. Remington, J. S. Mcleod, R., Thullie, P., Desmonts, G. (2005). Toxoplasmosis in: Remington J.S., Baker, C, Wilson, E., Klein, J .O ..eds. infectious diseases of the fetus and newborn infant.6th ed. philadelphia: WB SAUNERS: 947-1091.
4. Montoya, J.G. and Liesenfeld,O. (2004).Toxoplasmosis. Lancet. 363:1965-1978.
5. Petersen, E. Toxoplasmosis. (2007). Semin Fetal Neonatal Med. 12:214-2123.
6. Luft, B.J. and Remington, J.S. (1992).Toxoplasmosis encephalitis in AIDS. Clin. Infect. Dis. 15:211-222.
7. James, G.S., Sintchenko, V. G, Dickeson, D.J. and Gilbert, G.L. (1996). Comparison of cell culture mouse inoculation and PCR for detection for *Toxoplasma gondi* : effect for storage condition on sensitivity. J. Clin. Microbial. 34:1572-1.
8. Remington, J.S., McLeod, R. and Desmontes, C. (1995). Toxoplasmosis in Remington JS, Klein J.O, eds. infectous disease of the fetus and new born infant 4th ed. Philadelphia, pa:the W. B. Saunders Co. 140-267.
9. Remingtion, J.S. Thulliez , Ph. and Montoya, J.G. (2004). Recent developments for diagnosis of toxoplasmosis. J. Clin. Microbiol. 47:941.94.
10. Bessieres, M.H., Roques, C., Berrebi, A., Barre, V. and Cazaux, M. (1992). IgA antibodies response during acquired and congenital toxoplasmosis. J. Clin. Pathol. 45.605-60.
11. Romands, S.,Wallon, M., Franch, J. and Thullies, P. (2001). Prenatal diagnosis using polymerase chain reaction on amniotic fluid for congenital toxoplasmosis.obstet. gynecol. 97. 296-300.
12. Fuentes, I., Rodrigues, M., Domingo, C.J., Cas tillo F., Del Juncosa, T. and Alvar, J. (1996). Urine sample used for congenital toxoplasmosis diagnosis by PCR. J. Clin. Microbiol. (34):2368-2371.
13. Guy, E.C. and Joynson, D.H. (1995). Potential of the polymerase chain reaction in the diagnosis of active *toxoplasma* infection by detection of parasite in blood. J. Infect. Dis. 172.319-322.
14. Costa, J.M., Pautes, C., Ernault, P and Foult, F. (2000). Real Time PCR for diagnosis and follou-up of toxoplasma reactivation after allogenic stemcell transplantation using fluorescence resonance energy transfero hybridization probs.J. Clin. Microbiol. 38.2929-2932.
15. Khurana Bagga, R., Aggarwal, A., Lyngdoh, V., Diddi, K. A. and Malla, N. (2010). Serological screening for neonatal Toxoplasma infection in India. Indian j. Med. Microbial. 28.143-146.
16. Al-Rawi, Kh.H.Z. (2009). Detection for B1 antigen from blood of pregnant and abortive women infected with *Toxoplasma gondii*. Athesis submitted to the college of Science, Baghdad university.
17. Lin, M.H., Chen,T.Ch., Kuo, T.T,Tseng, Ch.Ch. and Tseng, Ch.P. (2000). Real Time PCR for quantitave detection of *Toxoplasma gondii*. J. Clin. Microbiol.38.4121-4125.
18. Dupon, M.,Casenave, J., Regnaud, J., Cheyrou, A., Fischer, I., LENG, b., Lacut, J. (1995). Detection for *Toxoplasma gondii* by PCR and tissue culture in cerebrospinal fluid and blood of human immunodeficiency virus. Sero positive patients.J.Clin. Microbial. 33:2421-2426.
19. Contini,C., Culterara, R.,Seraceni,S., Segala, D., Romani, R., Fainardi, E., Cinque, P., Lazzaarin, A and Delia, S. (2002).The role for stage-specific oligonudeotide primers in providing effective laboratory support for the molecular diagnosis of reactivated *Toxoplasma gondii* encephalitis in patients with AIDS. j. Med microbial. 51:879-890.
20. Meganthan, P., Singh,S.,Ling, L.Y., Singh, J., Subrayan,Vand Nissapatron,V. (2010). Detection of *Toxoplasma gondii* DNA by PCR following Microwave treatment of serum and whole bloo. Southeast Asian J.Trop. Med. Publichealth.91:265-27.