Comparison between avidity test and real- time PCR in diagnosis of recent toxoplasmosis in pregnancy

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Abstract: Diagnosis of recently acquired toxoplasmosis must be improved to avoid its risks during pregnancy. The present study was done to evaluate the using of quantitative real-time PCR assay (qRT-PCR) in diagnosis of recent toxoplasmosis, and compare it with avidity IgG ELISA test. This study was carried out on 120 pregnant women taken from women attending Outpatient Clinics of the Obstetrics & Gynecology Department at Zagazig University Hospital, ranging in age from 20- 38 years. These women were divided into two groups, a case group of 100 complicated pregnancy and control group of 20 healthy pregnant women. IgM ELISA and *Toxoplasma* IgG avidity ELISA were performed and qRT-PCR technique was used to confirm the infection with *T.gondii* Fifty-one cases of the 100 complicated pregnancy women tested positive for IgM antibodies compared with 0% (none) in the normal control pregnancy group. While for avidity IgG antibodies, 29.1% of complicated pregnancy women tested positive compared with 1.6% positive IgG antibodies in the control group. Regarding qRT-PCR technique, (38cases) 31.6% of complicated pregnancy women and only one case from control group were positive for toxoplasmosis. The qRT-PCR -assay provides a rapid, sensitive, automated and quantitative way of detecting *Toxoplasma* DNA. It can detect the infection very early and can be used also in follow up of the treatment. But, it is very expensive and needs technical skills. So routine application of PCR– based methods, especially in developing countries, is difficult. Serological tests especially IgG avidity test are good screening test, cost effective and can be used to exclude recent infection in pregnancy.

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Key words: IgG avidity ELISA; Real-time PCR; *Toxoplasma gondii*.

1. Introduction

Toxoplasma gondii infection is widely spread in most countries either in human or animal (He *et al.,* 2016). It causes asymptomatic or milddisease. For many years, it was recognized as an infective agent that is responsible for congenital infection resulting in fetal abnormalities or even death (Soliman *et al.,* 2001), but also considered as a major pathogen of humans with HIV infection and organ transplant (Jones *et al.,* 2009). Misdiagnoses of infection in a new born baby lead to ocular, hearing impairment, neurological symptoms or mental retardation in early childhood (Karczewski and Golab, 2011). Ocular toxoplasmosis is the major cause of infective uveitis (Tong and Lu, 2015). Early diagnosis of *Toxoplasma gondii* in pregnant women is very important in order to initiate early therapy or other interventions to fetus (Iqbal and Khalid, 2007). Prevention and control of toxoplasmosis based on vaccination is one of the promising strategies (Xu *et al.,* 2015). Clinical features associated with toxoplasmosis are varied and nonspecific (Almushait *et al.,* 2014). Detection of *Toxoplasma* parasite in clinical specimens is very difficult. Moreover, the diagnosis of toxoplasmosis depends mostly on immunological tests, especially IgG, IgM and IgA antibodies (Cruceresc u and Lovin, 2002).

ELISA is the most widely used form for diagnosis of toxoplasmosis (Iddawela *et al.,*2015).In the past the diagnosis of recent infection depend on detection of specific IgM Antibodies but the detection of IgM cannot determine the actual time of toxoplasmosis as IgM antibodies may persist in the serum for long times after acute infection (Horvath *et al.,* 2005). Anti-*Toxoplasma* IgG avidity test is a good test for differentiation between recent and past infection (Remington *et al.,* 2004). The functional affinity of specific IgG antibodies can be measured by avidity test, which is low in early infection and increases during subsequent weeks and months by antigen-driven B cell selection. Dissociation of the antibody-antigen complex by Protein denaturing reagents, including urea, was performed. Determination of the avidity was done by comparison of curves of antibodies titre of treated sample and untreated one (Liesenfeld *et al.,* 2001). Inimmunocom promised patient, serological tests cannot be used in detection of specific anti-*Toxoplasma* immunoglobulin G (IgG) or IgM during the active phase of infection, due to a severe dysfunction of immune system (O'Driscoll and Holliman, 1991).

Polymerase chain reaction (PCR) is alternative method for diagnosis of toxoplasmosis as it was considered as rapid, qualitative, highly sensitive,

especially when nested PCR is used (Contini *et al.,* 1999). PCR based genotyping is also having a key role in epidemiological studies to identify of the source of *T. Gondii*in fection. QuantitativeRT-PCR has been developed to detect and quantitate *Toxoplasma gondii* (Jauregui *et al.,* 2001). The early diagnosis of toxoplasmosis can be depending on using qRT-PCR as it is a quantitative test and can be used for follow up of the treatment (Romand *et al.,* 2004; Martino *et al.,* 2005). The present study aimed to compare low avidity IgG and qRT-PCR for diagnosis of acute toxoplasmosis in pregnancy.

2. Material and methods

Study population

This study was carried out during the period from October 2014 to October 2015, the techniques of the study were carried out in Parasitology Department and Molecular biology unit, Faculty of Medicine, Zagazig University. This study was carried out on 120 pregnant women attending Outpatient Clinics of the Obstetrics & Gynecology Department at Zagazig University Hospital, ranging in age from 20- 38 years. These women were divided into 2 groups, case group of 100 complicated pregnancy states had one of the following criteria; women with repeated abortion (Congenial anomaly, Premature delivery, Still birth (S.B), Intrauterine fetal death (IUFD), some of them have previous history of *Toxoplasma* infection and control group of 20 healthy pregnant women. Other relevant and Demographic information were recorded for each woman. For PCR and ELISA techniques, 10 ml of venous blood were collected from each woman included in the study. Each blood sample was divided into two tubes, one heparinized tube was used for PCR and the second one serum was stored at -20°C until used for ELISA.

Type of study: case- control study.

Detection of *Toxoplasma* IgM was done by ELISA technique according to Turunen *et al*. (1983).

Principle of ELISA test

Anti-*Toxoplasma* IgM antibodies were tested by commercial available enzyme-linked immunosorbent assay (ELISA, Pishtazteb-Iran), according to the manufacturer's instructions, sera were diluted serially and added to the *T.gondii*antigen-coated microtiter plate and then anti-human IgM antibodies conjugated was added. After incubation and washing, the chromogenic substrate was added, and the optical densities were read by means of an automated ELISA-reader.

Detection of *Toxoplasma* by IgG Avidity ELISA technique was done according to Hedman *et al.* (1993).

A commercial ELISA kit for IgG avidity detection was used (Lab systems kit). For analyzing the serum sample, we made a duplicate row (row A and row B), 8 M urea was used to elute low-avidity antibodies from the *Toxoplasma* antigen. After 1 h incubation at 37°C, row A was washed three times, 5 minutes each wash, with 8 M urea in phosphate-buffered saline 0.05%. Row B was washed with the wash buffer provided by the kit manufacturer. The optical density of each well was read in a microtiter plate reader at 450nm against the differential wavelength of 600 nm. IgG avidity was calculated as the percentage of the row A titer and the row B titer and expressed as Avidity Index (%) = (OD of the sample treated with Urea / (OD of the sample treated without Urea) × 100. avidity was categorized into three groups: low avidity, lower than 40; high avidity, above 60; and border line, equal or lower than 60 and equal or higher than40.

Detection of *Toxoplasma* DNA was done by qRT-PCR technique according to (Delhaes *et al.,* 2013).

DNA was extracted from the whole blood samples of the study groups using a commercial purification system extraction kit (QIAamp DNA mini kit, QIAGEN Inc., Valencia, California, USA).

Detection of *T.gondii* B1 gene by qRT-PCR

The forward primer (TOXO-F), reverse primer (TOXO-R), and Hybridization probe for real-time PCR amplification were designed with the Primer Express software (DT lit 4 Mx3005pReal Time PCR Systems) (Applied Biosystems, Foster City, California, USA) to specifically amplify the *T.gondii* B1 gene. The target DNA for real-time PCR amplification was the 35-fold repetitive B1 gene of the *T.gondii* RH strain **(Burg** *et al.,* **1989).** Template DNA was added to a reaction mixture containing 35 ml of PCR master mix, 5 ml of the forward primer primers B1F1 (5'-CCGTTGGTTCCGCCT CCTTC-3') 5ml of the reverse primer and B1R1 (5'-GCAAAACAGCGGCAG CGTCT-3') and 5ml of probe (2mM, 6FAM- CTGTGCAACTTTG GTGTATTCGCAG-TAMRA) in a final volume of 50 ml. The PCRs were performed with the Gen Amp ABI Prism®7500 Sequence Detection System (PE Applied Bio system). The cycle threshold value (CT), indicative of the quantity of target gene at which the fluorescence exceeds a present threshold. This threshold was defined as the standard deviation of the baseline fluorescent signal, where the negative samples not exceed a preset threshold.

The Light Cycler software performs all additional calculation steps necessary for generation of a standard curve. A threshold band is automatically set at a fluorescence level, at which the fluorescence signal development reflects that the PCR is in the log-linear phase. The software then calculates the logarithmic values by interpolating a straight line through 2 data

points above the threshold value, and the points of intersection.

Ethical consideration:

The Committee of Research, Publications and Ethics of the college of Medicine, Zagazig University, Egypt gave the ethical approval for this study. All procedures were explained, and patients gave written or thumb-printed informed consent.

Statistical analysis:

All data were subjected to statistical analysis using SPSS statistical package version 19 using Chi-square test to examine the relation between variables.

3. Result:

The results were tabulated and drawn as follow.

Table (1): The Results of ELISA anti-*Toxoplasma* (IgM) among tested groups.

Serum IgM ELISA	high risk cases (100)		Control (20)		Total (120)	
	No.	%	No.	%	No.	%
• *Positive*	51	(42.5)	0	(0)	51	(42.5)
• *Negative*	49	(40.8)	20	(16.6)	69	(57.5)
P	< 0.01					

Table (2): Comparison between results of ELISA (IgM) and IgG avidity test among tested groups.

IgM –ELISA / IgG-Avidity	(IgM) *Positive(51)*	(IgM) *Negative (69)*	Total (120)
Low	35 (29.1)	2(1.6)	37 (30.8)
borderline	10	8	18
High	6	59	65

There is positive correlation between low IgG-Avidity and positive IgM and vice versa.

Table (3): Results of qRT-PCRtechnique among tested groups.

RT- PCR	High risk cases(100)		Control(20)		Total(120)	
	No.	%	No.	%	No.	%
Positive	38	(31.6)	1	(0.83)	39	(32.5)
Negative	62	(51.6)	19	(15.8)	81	(67.5)
P	< 0.01					

Table (4): Comparison between (ELISA) IgM and IgG-avidity results in the detection of recent toxoplasmosis according to RT- PCR *results* in Studied groups (*n* = 120 samples).

(ELISA)				RT- PCR	
IgG-avidity		IgM		PCR	
		+ve(51)	-ve(69)	+ve(39)	-ve(81)
Low	37	35	2	37	0
borderline	18	10	8	1	17
High	65	6	59	1	64

Table (5) Validity of ELISA IgG Avidity andRT- PCRin diagnosis of recent toxoplasmosis in comparison to IgM.

IgM	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)	Accuracy(%)
IgG Avidity	68.6	97.1	94.5	80.7	85
RT-PCR	76	100	100	85.1	90

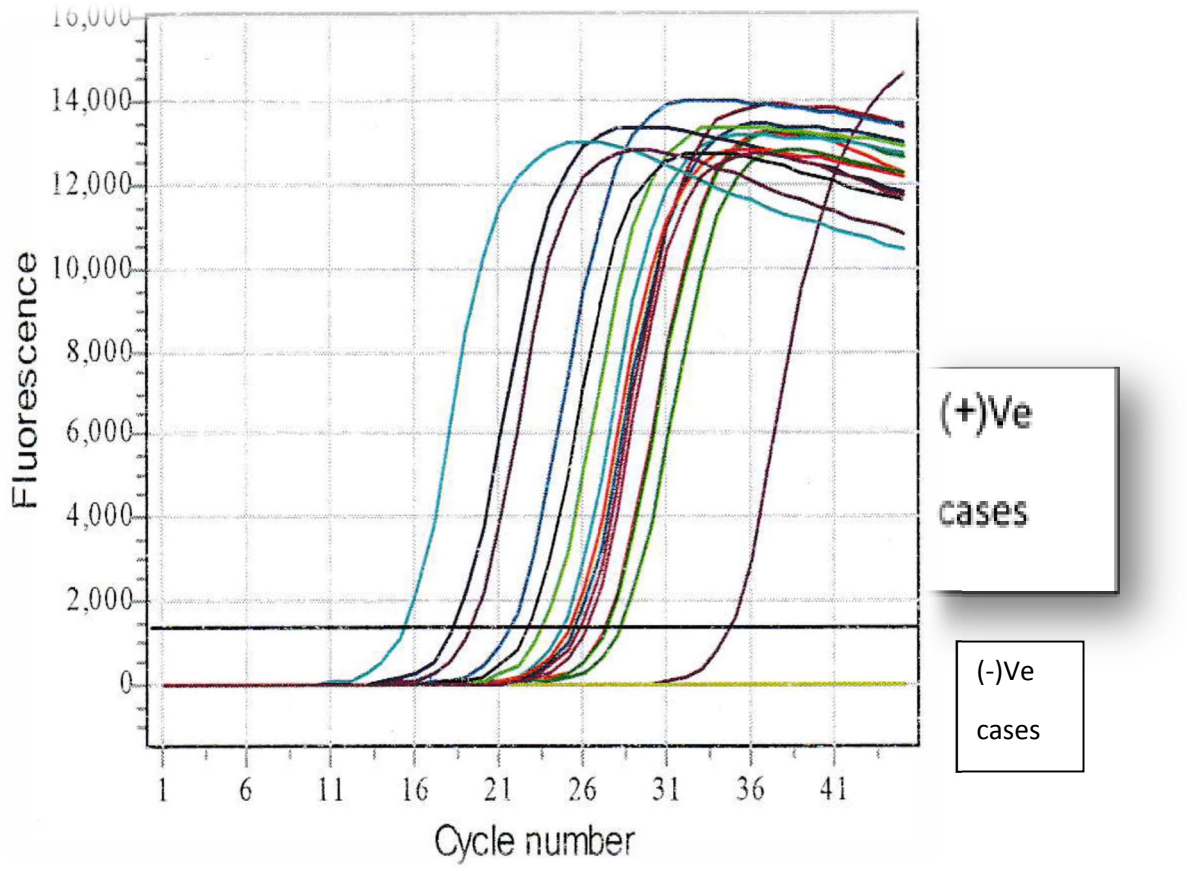

Fig.(1): Amplification plots Real-Time PCR show the positive and negative *cases.*

Table (6) Ranking of the diagnostic procedures for diagnosis of recent toxoplasmosis.

Techniques	Ranking for the attributes				
	Sensitivity& Specifiity	Time taken	Cost	Interpretation and use	Batch testing
(ELISA) IgM	2	2	3	4	5
IgG-avidity	3	2	3	4	5
qRT-PCR	5	4	1	3	5

4. Discussiondd

The parasite, *Toxoplasma gondii,* affects about 30% of the global human population (Hide 2016). Currently, the diagnosis of toxoplasmosis in Egypt depends mainly on detection of IgM, IgG by ELISA technique (Alsammani 2014).Diagnosis of recent toxoplasmosis may be difficult although the presence of many serologic techniques (Pour Abolghasem *et al.,* 2011). In the current study, selection of 100 cases of complicated pregnancy and 20 of normal pregnant females as a control group were done. IgM was positive in 42.5 % in a complicated pregnancy group. Maternal toxoplasma infection is usually asymptomatic; and the presence of (IgM) antibodies suspects recent infection. But the presence of toxoplasma immunoglobulin (IgG) antibodies confirms past infection, IgM antibodies remain positive for long time after acute infection (Montoya and Liesenfeld 2004). Depending on IgM assay leads to false positive results which lead to improper diagnosis and treatment and causing many complications during pregnancy (Julliac *et al.,* 2010).

Estimation of the time of infection of toxoplasmosis is very difficult, so a number of different methods have been used to determine toxoplasma IgG avidity to improve the diagnosis (Jenun *et al.,* 1997). In the current study, avidity test is better than detection of IgM in diagnosing of recent infection. It showed 2 cases of low avidity IgG in IgM negative group. These finding were supported by other studies which stated that IgG avidity technique is a good test in excluding a recently toxoplasmosis in the-positive sample of IgM (Emelia *et al.,* 2014). Also Hashooshand Majeed (2014*)* stated that combination of IgG-avidity and ELISA IgG/IgM help in diagnosis of recent infection in pregnant women. The first method to differentiate between acute and chronic toxoplasmosisin the first trimester of pregnancy is IgG avidity test (Jenum *et al.,* 1997; Pelloux *et al.,* 1998). Diagnosis of affection of the mother by IgG avidity test and the foetus by multiplex nested PCR in the amniotic fluid was useful for diagnosis of a high risk pregnancy and subsequent avoiding congenital anomalies Hideto Yamada *et al.* (2011). The low IgG avidity may persist in the blood of infected females for about 5-14 months Petersen *et al.* (2005). On the other hand Gry Findal *et al.* (2015) reported that the IgG avidity method has a limited ability to diagnose the onset of acute infection, but the presence of high IgG avidity can exclude recent infection moreover, If no change in IgG avidity in early pregnancy and remains low and stable, there is low risk of affection of the mother and the foetus so the need for another test is needed.

During recent years, accurate diagnosis of toxoplasmosis has been developed by detection of of *Toxoplasma*DNA via different PCR techniques (Pujol-Rique *et al.,* 1999; Lee *et al.,* 1999; Jones *et al.,* 2000). PCR technique is a good technique but conventional PCR showed many disadvantages. It is time-consuming and not provide quantitative data, nevertheless, conventional primers have shown lack of specificity (Kompalic-Cristo *et al.,* 2004).

In this study, qRT-PCR assaywas applied to detect *Toxoplasma* and, as a result, *Toxoplasma* DNA was detected in 39% of suspected blood samples. Real time - PCR can detect 37 cases out of 51 (+veIgM) and one case out of (-veIgM). This is in agreement with Costa *et al.,* 2000 who reported that qRT- PCR can be used to follow up of the treatment and monitor its efficacy and can correlate the quantity of toxoplasma DNA with clinical symptoms. Also Edvinsson *et al.* (2005) demonstrated that qRT- PCR using the 529-bp repeat element of *T. gondii* can be used to detect low concentrations *of T.gondii* DNA. The low positivity of RT- PCR (32.5%)in comparison to the IgM positive samples (42.5%) with low sensitivity(76%) could be due to IgM can persist in the blood for a long time after acute infection in some patient Ho-Yen *et al.* (1992).

In the current study, the multi-attribute evaluation method proved that in relation to time, qRT- PCR was the best test and this result is in accordance with Buchbinder *et al.,* 2003; Simon *et al.,* 2004; Cassaing *et al.,* 2006 who reported that qRT-PCR take short time to obtain result.

In the present study qRT-PCR was the only method able to detect one case from the control group, this may be due to the presence of low immunoglobulin at the time of sample examination due to the *Toxoplasma* infection was very early before the appearance of immunoglobulin or another explanation that those patients are immunocom promised, this result was in agreement with Hawraa *et al.,* 2014.The specificity of qRT-PCR is 100% in our study so it considered a good negative test.

In conclusion

The qRT-PCR based method described in this study is a rapid, specific, sensitive, automated and quantitative way of detecting *Toxoplasma* especially very early infection and can be used in follow up of the treatment. However, it is costly and needs good trained personnel. So, routine application of PCR–based methods is very difficult, Serological test especially avidity test is cost effective and can be used as screening test especially in developing country.

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References

1. Almushait M A, Bin Dajem SM, Elsherbiny NM, Eskandar MA, Al Azraqi TA•. Makhlouf L M (2014) Seroprevalence and risk factors of *Toxoplasma gondii* infection among pregnant women in south western, Saudi Arabia. J Parasit Dis 38(1):4–10.
2. Alsammani M A(2014) Sero-epidemiology and risk factors for *Toxoplasma gondii* among pregnant women in Arab and African countries. J Parasit Dis 2639-014-0558-8.
3. Buchbinder S, Blatz R, Rodloff AC (2003) Comparison of real-time PCR detection methods for B1 and P30 genes of *Toxoplasmagondii*. Diagn Microbiol Infect Dis 45:269–271.
4. Burg J L, Grover CM, Pouletty P, Boothroyd J C (1989) Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J Clin Microbiol 27:1787–1792.

5. He JJ, Ma J, Song HQ, Zhou DH, Wang JL, Huang SY, Zhu XQ(2016) Transcriptomic analysis of global changes in cytokine expression in mouse spleens following acute *Toxoplasma gondii* infection. Parasitol Res 115(2):703-12.
6. Cassaing S, Bessières M H, Berry A, Berrebi A, Fabre R, Magnaval JF (2006) Comparison between two amplification sets for molecular diagnosis of toxoplasmosis by real-time PCR. J Clin Microbiol 44:720–724.
7. Contini C, Seraceni S, Cultrera R (1999) Different PCR systems to detect *Toxoplasma gondii*tachyzoites or bradyzoites in clinical specimens from patients with and without overt disease. J Eukaryot Microbiol 46:S77–S78.
8. Costa JM, Pautas C, Ernault P, Foulet F, Cordonnier C, Bretagne S (2000) Real-time PCR for diagnosis and follow-up of Toxoplasma reactivation after allogeneic stem cell transplantation using fluorescence resonance energy transfer hybridization probes. J Clin Microbiol 38: 2929–2932.
9. Crucerescu E, Lovin DR (2002) Study on specific IgG as a tool for recent primary Toxoplasma infection diagnosis. J Prev Med 10: 56-62.
10. Delhaes L, Year H, Ache S, Tsatsaris, Houfflin-Debarge V (2013) Contribution of molecular diagnosis to congenital toxoplasmosis. DiagnMicrobiolInfect Dis 76: 244–247.
11. Gry Findal1 Babill Stray-Pedersen1, Ellen K. Holter, Tone Berge, Pål A, Jenum L (2015) Persistent Low *Toxoplasma* IgG Avidity Is Common in Pregnancy: Experience from Antenatal Testing in Norway. PLOS ONE 10(12): e0145519.
12. Edvinsson B, Lappalainen M, Evenga˚ B (2005) Real-time PCR targeting a 529-bp repeat element for diagnosis of toxoplasmosis. Clin Microbiol Infect 12: 131–136.
13. Emelia O, Rahana AR, Mohamad Firdaus A, Cheng HS, Nursyairah MS, Fatinah AS, Azmawati MN, Siti NAM, Aisah MY(2014) IgG avidity assay: A tool for excluding acute toxoplasmosis in prolonged IgM titer sera from pregnant women. Tropical Biomedicine 31(4): 633–640.
14. Hashoosh DA and Majeed IA (2014) Comparison of two assays in the diagnosis of toxoplasmosis: immunological and molecular. Eastern Mediterranean Health Journal 20: 1.
15. Hedman K, Lappalainen M, Sönderlund M, Hedman L (1993). Avidity of IgG in serodiagnosis of infectious diseases. Rev Med Microbiol 4: 123-129.
16. Hide G (2016). Role of vertical transmission of *Toxoplasma gondii* in prevalence of infection. Expert Rev Anti Infect Ther 14(3):335-44.
17. Hideto Y, Akira N, Tomohiro Y, Yuka M, Takashi Y(2011) Prospective Study of Congenital Toxoplasmosis Screening with Use of IgG Avidity and Multiplex Nested PCR Method. J Clin Micr 49 (7):2552–2556
18. Ho-Yen DO, Joss AW, Balfour AH, Smyth ETM, Baird D, Chatterton JMW (1992) Use of the polymerase chain reaction to detect *Toxoplasma gondii* in human blood samples. J Clin Pathol 45:910–913.
19. Horvath KN, Szénási Z, Danka J, Kuscera I (2005) Value of the IgG avidity in the diagnosis of recent toxoplasmosis :a comparative study of four commercially available anti- *Toxoplasma gondii* IgG avidity assay. Acta Parasitologica 50(3):255-260.
20. Liesenfeld O, Montoya JG, Kinney S, Press C Remington JS (2001) Effect of testing for IgG avidity in the diagnosis of *Toxoplasma gondii* infection in pregnant women: experience in a US reference laboratory. The Journal of Infectious Diseases 183(8): 1248-1253.
21. Iddawela D, Ehambran K, Kumarasiri PV, Wijesundera S (2015). Development and validation of an Enzyme Linked Immunosorbent Assay (ELISA) test for the diagnosis of toxoplasmosis in Sri Lanka. Ceylon Medical Journal 60(3):82–86.
22. IqbalJ, Khalid N (2007) Detection of acute *Toxoplasma gondii* infection in early pregnancy by IgG avidity and PCR analysis. Journal of Medical Microbiology 56(11): 1495-1499.
23. Jauregui LH, Higgins J, Zarlenga D, Dubey JP, Lunney JK (2001) Development of a real-time PCR assay for detection of *Toxoplasma gondii* in pig and mouse tissues.J ClinMicrobiol 39(6):2065-71.
24. Jauregui LH, Higgins J, Zarlenga D, Dubey JP, Lunney JK, O'Driscoll JC, Holliman RE (1991) Toxoplasmosis and bone marrowtransplantation. Rev Med Microbiol 2:215–222.
25. Jenum PA, Stray-Pedersen B, Gundersen AG (1997) Improved diagnosis of primary *Toxoplasma gondii* infection in early pregnancy by determination of anti-*Toxoplasma* immunoglobulin G avidity. J Clin Microbiol 35(8):1972–7.
26. Jones CD, Okhravi N, Adamson P, Tasker S, Lightman S (2000) Comparison of PCR detection methods for B1, P30, and 18S rDNA genes of *T. gondii*in aqueous humor. Investig Ophthalmol Vis Sci41:634–644.

27. Jones JL, Dargelas V, Roberts J, (2009)Risk factors for *Toxoplasma gondii* infection in the United States. Clin Infect Dis 49: 878-84.
28. Julliac B, Théophile H, Begorre M, RichezB Haramburu F (2010) Side effects of spiramycin masquerading as local anesthetic toxicity during labor epidural analgesia. International Journal of Obstetric Anesthesia 19(3): 331-332.
29. Karczewski G, Golab E (2011) Diagnostic problems with congenital toxoplasmosis. Przeglad Epidemiology 65(3): 451-454.
30. Kompalic-Cristo A, Nogueira SA, Guedes AL, Frota C, González LF, Brandão A, Amendoeira MR, Britto C, Fernandes O (2004) Lack of technical specificity in the molecular diagnosis of toxoplasmosis. Trans R Soc Trop Med Hyg 98:92–95.
31. Lee PYC, Mangan, Holliman R E Butcher PD (1999) Quantitation of *Toxoplasma gondii* DNA in a competitive nested polymerase chain reaction. J Clin Pathol 52:61–64.
32. Liesenfeld, O, Montoya, JG, Kinney S, Press C, Remington JS (2001) Effect of testing for IgG avidity in the diagnosis of *Toxoplasma gondii* infection in pregnant women: experience in a US reference laboratory. The Journal of Infectious Diseases 183(8): 1248-1253.
33. Nassef NE, Abd El-Ghaffar M M, El-Nahas NS, Hassanain M A, Shams El-DinSA, AmmarAI (2016). Seroprevalence and genotyping of *Toxoplasma gondii*in Menoufia governorate. Menoufia Medical Journal 28:617–626.
34. Martino R, Bretagne S, Einsele H, MaertensJ, Parody R, Schumacher U, Pautas C, Theunissen K, Schindel C, Munoz C, Margali N, Cordonnier C (2005) Early detection of *Toxoplasma* infection by molecular monitoring of *Toxoplasma gondii* in peripheral blood samples after allogeneic stem cell transplantation. Clin Infect Dis 40:67–78.
35. Montoya J G, Liesenfeld O (2004) Toxoplasmosis. Lancet 363(9425):1965–76.
36. Pelloux H, Brun E, Vernet G (1998) Determination of anti–*Toxoplasma gondii* immunoglobulin G avidity: adaptation to the Vidas system (BioMe´rieux) Diagn Microbiol Infect Dis 32:69–73.
37. Pujol-Rique M F, Derouin A, Garcia-Quintanilla M E, Valls ME, Miro JM, Jimenez de Anta M T (1999) Design of a one-tube hemi-nested PCR for detection of *Toxoplasma gondii*and comparison of three DNA purification methods. J Med Microbiol 48:857–862.
38. Petersen E, Borobio MV, Guy E, Liesenfeld O, Meroni V, Naessens A (2005) European multicentre study of the LIAISON automated diagnostic system for determination of *Toxoplasma gondii*-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. J Clin Microbiol 43(4):1570–4.
39. Remington, JS, Thulliez, P& Montoya, JG)2004)Recent development of diagnosis of toxoplasmosis. J Clin Microbiol 42(3): 941-945
40. Romand S, Chosson M, Franck J, Wallon M, Kieffer F, Kaiser K, et al. (2004) Usefulness of quantitative polymerase chain reaction in amniotic fluid as early prognostic marker of foetal infection with *Toxoplasma gondii*. Am J Obstet Gynecol 190:797–802.
41. Simon A, Labalette P, Ordinaire I, Fréalle E, Dei-Cas E, Camus D, Delhaes L (2004) Use of fluorescence resonance energy transfer hybridization probes to evaluate quantitative real-time PCR for diagnosis of ocular toxoplasmosis. J Clin Microbiol 42:3681–3685.
42. Soliman M, Nour-Eldin MS, Elnaggar HM, El-Ghareb ME, Ramadan NI (2001) *Toxoplasma* antibodies in normal and complicated pregnancy. J Egypt Soc Parasitol. Aug; 31(2):637-46.
43. Tong X, Lu F(2015) IL-33/ST2 involves the immunopathology of ocular toxoplasmosis in murine model. Parasitol Res 114(5):1897-905.
44. Turunen H, Vuorio K, Leinikki P (1983) Determination of IgG, IgM and IgA antibody responses in human toxoplasmosis by enzyme-linked immunosorbent assay (ELISA). Scandinavian Journal of Infectious Diseases 15(3): 307- 311.
45. Xu Y, Zhang NZ, Wang M, Dong H, Feng SY, Guo HC, Zhu XQ (2015) A long-lasting protective immunity against chronic toxoplasmosis in mice induced by recombinant rhoptry proteins encapsulated in poly (lactide-co-glycolide) microparticles. Parasitol Res 114(11):4195-203.

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