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Comparative study for recurrent aborted women infected with *Toxoplasma gondii*

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Abstract

The study was conducted on 70 recurrent aborted women and twenty healthy women, whom have visited Al-Zahraa hospital Laboratory in Al-Najaf Governorate during the period from December 2017 till March 2018. The study was designed to comparative four diagnostic methods (LAT, ELISA IgG, lgM, nPCR from blood samples and nPCR from tissue samples by used *B1* primer) which determine the infection with *Toxoplasma gondii* in clinical suspected women in Al- Najaf governorate.

DNA of *T. gondii* parasite determined by nPCR, the results indicate that nPCR was the best method that can be used in diagnosis and to determine the prevalence of *T. gondii* and nPCR from tissue samples was the higher sensitivity and specificity (66,100) respectively in comparison with other methods nPCR from blood samples (83,100 no significant) LAT (37,100) respectively and ELISA IgG (53,100) respectively.

The present study conducted that the majority of age groups were at age category (21-27) by about (42.9%) for aborted women while for control was (28-35) by about (40%), most studied aborted women were don't read and write by about (61%) while control was middle school by about (45%), that aborted women most patients have (3-6) abortion (77.1 %) followed by double (18. 6%) aborted women ,the majority of (ABO) blood group system was A group for aborted women and the lowest was O group by about (41.4% and 4.3%) respectively ,the week of abortion was 9 up by about (78.6%) .

The current study concluded that nPCR technique from tissue samples was the best method that can be used in diagnosis and to determine the prevalence of *T. gondii* as well as the relationship between the A blood group, age, level of education, week of abortion and aborted women with toxoplasmosis disease.

INTRODUCTION

Toxoplasmosis is one of the most common parasitic diseases where approximately one-third of the world's population is affected [1] .Its capable of causing severe and life threatening conditions in pregnant women and immunocompromised individuals [2].

Human infections generally occur by the consumption of undercooked meat that contains tissue cysts or by water and food contaminated with Oocysts present in cat feces [3] .The infection is transmitted directly from the mother to the fetus during the transition of the active phase tachyzoite across the placenta, rarely through blood transfusions or organs transplantation [4] .

Congenital infection is one of the most important sequels of toxoplasmosis in pregnant women [5] .Congenital transmission of *Toxoplasma gondii* predominantly occurs at the first time during pregnancy [6] .The severity of congenital toxoplasmosis is highest in the first and second trimesters of pregnancy which usually results in abortion or stillbirth [7] .

Several serological diagnostic methods were used for *T. gondii* identification like Latex agglutination Test, enzyme linked immune sorbent assay [8].

Recently, the polymerase chain reaction (PCR) technique has been widely used due to its highly sensitivity and specificity of *T. gondii* detection [9]. Detection of *T. gondii* DNA by molecular methods, the polymerase chain reaction (PCR) is today frequently used to detect *T. gondii* DNA in clinical samples [10].

The most often used target for PCR detection is the 35 fold repetitive *B1* gene [11]. PCR is the only method that can detect *T. gondii* organisms in low numbers (10 organisms per ml) and can detect a partly destroyed parasite [12]

MATERIALS AND METHODS

The study was conducted on seventy aborted women who suffer from recurrent abortions .twenty healthy women as a control group, that pregnancy with normal delivery.

Samples were collected from suspected patients and a control group who attended AL-Zahraa Maternity and Child Teaching Hospital in Najaf Governorate from December-2017 to march 2018. They were 16-42years old age.

Blood samples:

Five ml of venous blood were drawn from vein of each suspected patient and control groups by using disposable syringes, three ml from this blood were collected in a sterile serum tube and left 30 minutes at room temperature to separate the serum which was collected into Eppendorf - tube by micropipette and stored at -20 °C until analysis (*Toxo* latex agglutination Test, Toxo IgG, IgM-ELISA Kit), the remaining 2 ml blood sample was used for DNA extraction**.**

Placental tissue sample:

Three to Five gram of placental tissue samples was collected from seventy recurrent aborted women and twenty healthy delivery women , kept with normal saline in sterile plastic containers and transferred to the research laboratory of Microbiology Department in College of science- Kufa University with cooling conditions (ice bags) for DNA extraction. The extracted DNA was kept in deep freeze at -20˚C, until use.

The Diagnostic Methods:

1- Serological tests

The two serological test were used in diagnosis of *Toxoplasma gondii* parasite (Latex Agglutination Test according Toxo latex kit cod 1201002 and ELISA IgM according kit with catalog NO. TOXG02 and IgG according kit with catalog NO.TOXG01)

2- Molecular detection test

Nested Polymerase chain reaction (nPCR)

Genomic DNA extraction of Blood Protocol was according DNA extraction kit with catalog NO.FABGK001-1) and Genomic DNA Kit of tissue Protocol was according DNA extraction kit with catalog NO.FATGK001-1), the procedure of nPCR and Gel electrophoresis were according [13]

Statistical Analysis

The data were analyzed by SPSS (version) 22. A chi-square test compared the sero-prevalence values with the genes *B1* of *T. gondii*. Confidential intervals at 95% and $P < 0.05$ were considered levels of significance [14].

RESULTS

The present study revealed that the highest aborted women was age category (21-27) by about30 (42.9%) respectively and the lowest was in category (36 Up) by about 12 (17.1%) respectively, as showed in table (1). The results of this study revealed that the highest aborted women were don't read and write by about 43 (61%) respectively and the lowest were in Preparatory School 9 (12.9%) respectively, as showed in table (2). The current study revealed that the highest aborted women were in (3-6) number of abortion by about 54 (77.1 %) respectively and the lowest were in 7 Up number of abortion by about 3 (4.3%) respectively, as

showed in table (3).The present study conducted that the highest aborted women according(ABO) blood group were A group by about 29 (41.4%) respectively and the lowest were in O blood group 3 (4.3%) respectively, as showed in table (4).The results of study revealed that the highest aborted women were in week of abortion 9 up by about 55 (78.6%) respectively and the lowest were in (4-8) week of abortion by about 15 (21.4%) respectively, as showed in table (5).The present study revealed that the highest specificity and Sensitivity the nPCR tissue was more sensitive and specific than other tests by about (66) and (100) respectively (*p* value 0.025) in comparison with other methods nPCR from blood samples (83,100 non sig.) respectively, LAT (37,100) respectively and ELISA IgG (53,100) respectively, as showed in figure (1) .also recorded that the detection of Toxoplasmosis by using specific primer *B1* for nPCR technique and also revealed highly specific in magnification of *Toxoplasma* DNA and fruitful in the detection of *Toxoplasma* DNA from tissue sample more than blood sample of aborted women infected with *T. gondii* , as showed in figure (2).

Table (1): showed the variable of age group of abortion women by *Toxoplasma gondii* comparison with healthy group.

Variables		Patient		Control		Chi-square df P-value (Sig.)
		No.	%	No.	%	
age group (Years)	<= 20	10	14.3%	1	5.0%	3.348 3 0.341 (NS)
	21 – 27	30	42.9%	6	30.0%	
	28 – 35	18	25.7%	8	40.0%	
	36 Up	12	17.1%	5	25.0%	
Total		70		20		

Table (2): Showed the variable of education of abortion women by *Toxoplasma gondii* comparison with healthy group.

Variables		Patient		Control		Chi-square df P-value (Sig.)
		No.	%	No.	%	
Education	Don't read and write	43	61.4%	1	5.0%	31.248 3 0.0001 (HS)
	Primary school	14	20.0%	3	15.0%	
	Middle school	4	5.7%	9	45.0%	
	Preparatory School	9	12.9%	7	35.0%	
Total		70		20		

Table (3): Showed the variable of(age)?? number of abortion in abortion women by *Toxoplasma gondii* comparison with healthy group.

Variables		Patient		Control		Chi-square df P-value (Sig.)
		No.	%	No.	%	
No. of abortion	<= 2	13	18.6%	20	100.0%	44.416 2 0.0001 (HS)
	3 – 6	54	77.1%	0	0.0%	
	7 Up	3	4.3%	0	0.0%	
Total		70		20		

Table (4): Showed the variable of blood group in abortion women by *Toxoplasma gondii* comparison with healthy group.

Variables		Patient		Control		Chi-square df P-value (Sig.)
		No.	%	No.	%	
Blood group	A	29	41.4%	5	25.0%	10.683 3 0.014 (S)
	B	28	40.0%	4	20.0%	
	AB	10	14.3%	8	40.0%	
	O	3	4.3%	3	15.0%	
Total		70		20		

Table (5): Showed the variable of week of abortion in abortion women by *Toxoplasma gondii* comparison with healthy group.

Variables		Patient		Control		Chi-square df P-value (Sig.)
		No.	%	No.	%	
Week of abortion	<= 3	0	0.0%	20	100.0%	90.000 2 0.0001 (HS)
	4 – 8	15	21.4%	0	0.0%	
	9 Up	55	78.6%	0	0.0%	
Total		70		20		

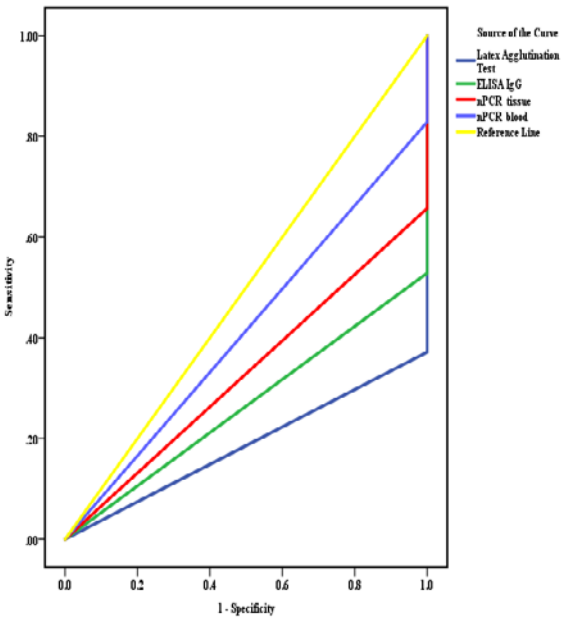
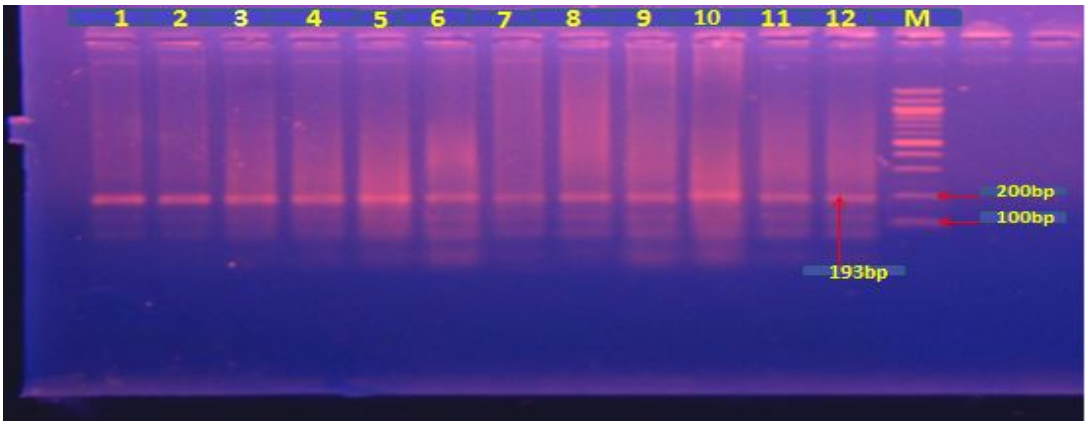


Figure (1): showed the receiver operating characteristic curve of Latex agglutination, ELISA IgG, nPCR tissue and nPCR blood in abortion women by *Toxoplasma gondii* comparison with healthy group.



The Figure (2): Amplification of toxoB1 *gene* of *Toxoplasma gondii* DNA from the blood of recurrent abortive women. Lane-M, molecular weight marker (100 bp Marker, 100 to 1200bp), Lanes 1-12 positive samples at 193bp. Running conditions: Agarose gel (2%), 80 volt for 90 Minutes, stained with ethidium bromide.

DISCUSSION

Several previous studies were recorded that abortion by toxoplasmosis women and the disease is more prevalent among women with [15, 16,17,] Most of aborted women were in the age groups (21-27) years old, this finding may be due to that this age group represents an optimum period of fertility, thus, this critical period of women's life has higher chances for activation of latent infection of *T.gondii* that can be transmitted vertically to the fetus, which was considered as one cause of abortion as mentioned by [27]. This age (21-27) represents a mother's stage and the prenatal detection of antibodies against *T. gondii* in pregnant women. It was critical with regard to the management of serious congenital complication including abortion, [28].

Education level it might be the abortion associated with education level as in previous studies were conducted consist with [29] in Diyala, [30] and [31] in Turkey, [32]]found the occurrence of the disease was higher among uneducated people than educated ones.

In United States it was found that the seroprevalence was significantly higher among those with education below college level in United States [33].One cause of high incidence of toxoplasmosis among low level education may be due to that the legal obligation of education was not found in my country and another important causes Statistical association was not found between *T. gondii* seroprevalence and the education level of the women and the disease is more prevalent among women with school education and illiterate patients almost in all the tests and lower seroprevalence was present between women had college education because increased knowledge results in awareness, which consequently results in changes in risky behavior and decline in infection rates [30] .

This finding was agreement with a most recent study conducted in Diyala [34]. These results were statistically significant difference (*P* value= 0.014). Certainly, the molecules that define ABO blood group phenotypes consist of carbohydrate that are present in the glycoproteins structures and expressed in red blood cells and other tissues [35]. The adherence mechanism of micro-organisms to mucous membranes of hosts is not totally clear, but it is likely that glyco-conjugates of the ABO group system are involved in this process [36].

It is of interest that the present study revealed an association between blood group system and Toxoplasma infection with highest prevalence among blood donors. This study was agreement with modern study conducted in Baghdad [37]. Alternatively, four studies reported an association between infection by this parasite and B blood groups, these studies

proposed that the B antigen could act as potential receptor for *T. gondii*. However, two other similar investigations did not find any evidence of this association [38].

This may be due to the fact that first trimester of the pregnancy is considered as a critical period in which the fetus is not well established in the uterus and it is threatened for abortion whenever the mother is expose to any risky factor such as reactivation of latent infection as *T. gondii* that result from immunosuppressant concomitant with pregnancy which can lead to placental infection and next placental insufficiency, with subsequent embryonic death [39].This study constant with [40] , but not constant with [20].

The sensitivity and specificity of the PCR depend on multiple factors, such as the characteristics of the DNA sequence that is amplified, The DNA extraction protocol and the optimization of the reaction conditions [41] .The immunodiagnostic method of toxoplasmosis disease is widely used for screening pregnant women in order to prevent its congenital spread. Screening tests are ubiquitous in contemporary practice, yet the principles of screening are widely misinterpreted [42]. Screening is the testing of apparently well people to find those at increased risk of having a disease, some studies revealed high sensitivity was found by ELISA IgG and this method used in 40% of laboratories diagnosing toxoplasmosis were using this kit [43] .

The helpfulness of diagnostic tests, that is their capability to differentiation a person with disease without disease, is usually described by terms such as sensitivity, specificity, positive predictive value, and negative predictive value [44].

Sensitivity and specificity are important measures of the diagnostic accuracy of a test but cannot be used to estimate the probability of disease in an individual patient. The sensitivity of a test only tell us how good the test is for identifying people with disease when only looking at those with disease. Sensitivity tells us nothing about whether or not some people without the disease would also test positive and if so, in what proportion [45].

The sensitivity and specificity of a test cannot be used to estimate the probability of disease in a patient, but the parameters could be combined into one measure called the probability ratio which may be used in conjunction with disease prevalence to estimate an individual patient's probability of having disease [46].

Results conducted that nPCR assay from tissue and blood samples more sensitivity and specificity than ELISA IgG assay and LAT this may be due to delay or failure the body to produce antibodies or for presence some inhibitory substances such as Calmodulin, myosin, actin and tubulin intra cytoplasmic of *T. gondii* may be elucidate false positive results through serological diagnosis of parasite infections [47].

This result corresponded with the study by [48, 49]. The two researchers diagnosed *T. gondii* by serological and molecular test and they found PCR method was more sensitive and specific than IgG and IgM specific ELISA test.

A successful PCR technique in detection of parasite DNA in acute infection may belong to PCR assay not dependent on the viability of parasite which detected all the *T. gondii* dead and viable. In peripheral blood of human *T. gondii* rapidly killed by the immune system but the DNA remains for some time in peripheral blood of human [50].

Negative PCR of blood samples may be due to few number of *T. gondii* In the peripheral blood short remain time of parasitaemia or small size of blood sample which used to DNA is extracted compared to the total volume of blood in the human body and presence some inhibitory substance in human blood that may impede the reaction of PCR assay such as hemoglobin, haem, immunoglobulin G and Lactferrin [48].

The results of the present study corresponded with the study of [48], which that reported that molecular diagnosis from blood specimens giving from women suspected of acute infection with *T. gondii* more specific and sensitive the serological assay (*T. gondii* specific IgM and IgG ELISA test), found about (29%) of the suspected specimens with DNA of *Toxoplasma* was identified in compared to (20%) positive bioassay. Similar results have been conducted by [51] that reported the negative serological test for women with low-avidity to Abs and negative IgM were sure negative for *T. gondii* DNA by PCR technique.

This results may be due to small volume of blood specimens or DNA molecules of *T. gondii* which used as source of *T. gondii* DNA in compared to whole blood in the body of human and small number of parasite in peripheral blood as well as due to many inhibiter materials in the blood lead to inhibit the PCR reaction, such as hemoglobin, Lactoferrin, immunoglobulin G and haeme[52] . Also may be due to few quantity of *Toxoplasma* DNA may be extracted from clinical samples [35].

The highest sensitivity of (B1) primers maybe because that *B1* primer was specific to strain of *T. gondii* found in Iraq [54]. Results by [55, 56] agreed with results of the current study which found *B1* gene and 18S have high specificity and sensitivity, therefore, the have been used in diagnosis of *T. gondii* parasites by PCR technique.

REFERENCES

1- Robert-Gangneux, F., Brenier-Pinchart, M. P., Yera, H., Belaz, S., Varlet-Marie, E., and Bastien, P. (2017). Evaluation of Toxoplasma ELITe MGB Real-Time PCR Assay for Diagnosis of Toxoplasmosis. Journal of clinical microbiology, 55(5), 1369-1376.

2-Opsteegh, M., Maas, M., Schares, G., and Giessen, J. (2016). Relationship between seroprevalence in the main livestock species and presence of Toxoplasma gondii in meat (GP/EFSA/BIOHAZ/2013/01) An extensive literature review. Final report. EFSA Supporting Publications, 13(2).

3-Jafari, R., Sadaghian, M., d an Safari, M. (2012). Seroprevalence of Toxoplasma gondii infection and related risk factors in Tabriz city, Iran, 2008. Journal of research in health sciences, 12(2), 119-121.

4-Vimercati, A., Chincoli, A., De Gennaro, A., Carbonara, S., Scarasciulli, M., and Cicinelli, E. (2017). Clinical Management of Infections in Pregnancy: Update in Congenital Cytomegalovirus and Toxoplasmosis. In Management and Therapy of Late Pregnancy Complications (pp. 339-358). Springer, Cham.

5-Mahmood, O. I. (2016). Effect of Toxoplasmosis on hematological, biochemical and immunological parameters in pregnant women in Tikrit city, Iraq. Tikrit Journal of Pure Science, 21(3).

6-Paquet and Yudin, M. H. (2013). Toxoplasmosis in pregnancy: prevention, screening, and treatment. J. Obstet. Gynaecol Can.;35(1):78-79.

7-Ghasemi, F. S., Rasti, S., Piroozmand, A., Bandehpour, M., Kazemi, B., Mousavi, S. G. A., and Abdoli, A. (2016). Toxoplasmosis-associated abortion and stillbirth in Tehran, Iran. The Journal of Maternal-Fetal & Neonatal Medicine, 29(2), 248-251.

8-Pittman, K. J., and Knoll, L. J. (2015). Long-term relationships: the complicated interplay between the host and the developmental stages of *Toxoplasma gondii* during acute and chronic infections. Microbiology and molecular biology reviews, 79(4), 387-401.

9-Sultan, B. A., and AL-Fatlawi, S. N. (2016). Relationship between *Toxoplasma gondii* and abortion in aborted women in Najaf province. journal of Karbala university, 14(1), 177-185.

10-Yera, H., Filisetti, D., Bastien, P., Ancelle, T., Thulliez, P., and Delhaes, L. (2009). Multicenter comparative evaluation of five commercial methods for toxoplasma DNA extraction from amniotic fluid. Journal of clinical microbiology, 47(12), 3881-3886.

11-Burg, J. L.; Grover, C. M.; Pouletty, P. and Boothroyd, J. C. (1989). Direct & sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J. Clin. Microbiol., 27(8): 1787-1792.

12-Turkey, S. A., Al–Ani, S. F., and Al-hadithi, R. J. (2014). Comparison Between the Efficiency of nested PCR Analysis and IgG Avidity Test in Detection Of Acute Toxoplasma gondii Infection in Early Pregnancy in Ramadi city. Journal of university of Anbar for Pure science, 8(3):1-6.

13-Mousavi M.; Saravani R.; Modrek M. J.; Shahrakipour M. and Sekandarpour S.(2016). Detection of *Toxoplasma gondii* in Diabetic Patients Using the Nested PCR Assay via RE and B1 Genes. Jundishapur J. Microbiol. ; 9(2): 29493.1-6.

14-Aljanaby AAJ and Alhasnawi HMRJ.(2017).phenotypic and molecular characterization of multidrug resistant *Klebsiella Pneumonia* isolated from different clinical sources in Al-najaf province-Iraq.Pak J Biol Sci;20(5):217-32.

15-AL-Doski, B. D. A. (2000). Seroepidemiological study of toxoplasmosis among different groups of population in the Duhok city by using Latex

agglutination test and indirect hemagglutination test. M.Sc. Thesis, college of Medicine. Duhok University.
16-Hasson, K.F. (2004). Sero- epidemiological study of toxoplasmosis among pregnant women with gynecological and obstetrical problems in Najaf city. M.Sc. Thesis , college of medicine , Kufa university.
17-Al-Ani, S. K. (2004). Epidemiological and immunological study of toxoplasmosis among aborted women in Ramadi City. M.Sc. Thesis, college of Medicine. Al-Anbar Univeristy.
18-Al- Addlan, A. A. J. (2007). Diagnostic and serological study on Toxoplasma gondii for women whom had abortion by using PCR technique in Thi-Qar governorate. M. Sc. Thesis, college of Education.
19-Al-Rubaia, Z.A.A. (2008). Comparative between enzyme linked immunosorbent assay and enzyme linked fluorescent assay diagnosis of *T.gondii* in pregnant women and its relationship with abortion cases and abnormalities in Diwanyah Province M.Sc.Thesis, college of Science. Al-Qadisiya University.Thi-Qar university.
20-Mohammed, G. J. (2008). JA study the role Toxoplasmosis, cytomegalovirus and anti-phospholipids antibodies in cases abortion among women in Hilla city (Doctoral dissertation, M. Sc. thesis. College of Medicine. Babylion Univeristy.
21-Mohammed Kareem Ghali (2011).Some Serological and Molecular tests used to identify Toxoplasmosis among Women with Abortion. Ph.D.Thesis, college of medicine. Kufa university.
22-Aaiz, N.N. (2010). Genotyping Analysis to determine the main lineages types of *T.gondii* with the study of autoantibodies production by toxoplasmosis, PhD thesis. College of Medicine. Kufa University.
23-Alghamdi , J., Elamin, M. H., and Alhabib, S. (2016). Prevalence and genotyping of *Toxoplasma gondii* among Saudi pregnant women in Saudi Arabia. Saudi Pharmaceutical Journal, 24(6), 645-651.
24-Coelho, R. A. ;Kobayashi, M. and Carvalho, L. B.(2003). Prevalence of IgG antibodies specific to Toxoplasma gondii among blood donors in Recife. Northeast Brazil. Rev. Inst. Med. trop. S. Paulo., 45(4):229-231.
25-Al-Hindi, A.I. and Lubbad, A.H. (2009). Seroprevalence of toxoplasmosis among Palestinian aborted women in Gaza. Ann Alquds Med .5: 39-47.
26-Shin, D.; Cha, D.; Hua, Q.J.; Cha, G. and Lee, Y. (2009). Seroprevalence of *Toxoplasma gondii* infection and characteristic of seropositive patients in general hospitals in Daejeon, Korea. Korean J. Parasitol., 47(2):125-130.
27-Remington, J.S.; McLeod, R.; Thulliez, R. and Desmonts, G. (2001). Toxoplasmosis In: Remington, J.S. and Klein, J. O. (eds) Infectious diseases of the fetus and newborn infants. 5th Ed. Philadelphia: WB Saunders, pp: 205–346.
28-Han, K., Shin, D., Lee, T., and Lee, Y. (2008).Seroprevalence of *Toxoplasma gondii* infection and risk factors associated with seropositivity of pregnant women in Korea. J.Parasitol.,94(4):963-9650.
29-Al-Griari, A. J. A. (2007). A seroepidemiological study of toxoplasmosis in Diyala province/Iraq (Doctoral dissertation, M. Sc. Thesis, College of Education, Diyala University)
30-Ertug, S., Okyay, P., Turkmen, M., and Yuksel, H. (2005). Seroprevalence and risk factors for Toxoplasma infection among pregnant women in Aydin province, Turkey. BMC public health, 5(1), 66.
31-Sert, M., Ozbek, S., Paydas, S., and Yaman, A. (2007). Is there any relationship between toxoplasma infection and reactive arthritis. Journal of postgraduate medicine, 53(1), 14 .
32-Mohammed, H.H. (2012).Serological detection of antitoxoplasma antibodies in apparently healthy blood donors in Nineveh province. MSc Thesis, College of Medicine, Mosul Univ.
33-Jones, J. L., Kruszon-Moran, D., Wilson, M., McQuillan, G., Navin, T., and McAuley, J. B. (2001). *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. American journal of epidemiology, 154(4), 357-365.
34-Darweesh N.H., Hussein R.A., Salman S.T. and Shaker M.J. (2018).“Immunological and Molecular study of *Toxoplasma gondii* from aborted women in Diyala / Iraq”. Sci. J. Med. Res. 2 (6): 75-82.
35-Schenkel-Brunner, H.(2000).Human blood group chemical and biochemical basis of antigen specificity. 2PndP. Springer.,New York pp 637.
36-Henry,S.M.(2001).Molecular diversity in the biosynthesis of GI tract. Glycoconjugates. receptors.Transt. Clin. Biol. 8(1):226-30.
37-Suhad H. M. Al-Saadii.(2013). The Effect of Toxoplasmosis on The Level of Some Male Sex Hormones In Samples from National Blood Transfusion Center/Baghdad. M. Sc. Thesis, College of Science, University of Baghdad.
38-Rodrigues, A. C. F., Uezato, S., Vono, M. B., Pandossio, T., Spegiorin, L. C. J. F., Oliani, A. H., ... and de Mattos, L. C. (2011). Non-association between anti-*Toxoplasma gondii* antibodies and ABO blood group system. Journal of Venomous Animals and Toxins including Tropical Diseases, 17(2), 184-189.
39-Nawrass J. AL-Salihi ;Saad M. AL-Aaraji (2011). A Study of the Some Causes Associated with Single and Recurrent Spontaneous Abortion in Al-Hindia City. Medical Journal of Babylon,8(4),497-506.
40-Hacker, N. F., Gambone, J. C., and Hobel, C. J. (2010). Hacker and Moore's Essentials of Obstetrics and Gynecology (Essentials of Obstetrics and Gynecology). Elsevier Health Sciences.
41-Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., and Vandesompele, J. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinical chemistry, 55(4), 611-622.
42-Grimes DA. and Schulz KF (2002) Uses and abuses of screening tests. Lancet 359: 881-884.
43-Kodym P (2005) Se´rologie toxoplasmo´zy Zpra´vy Centra Epidemiology Mikrobiology. SZU´ Praha 14: 39-41.
44-Steurer J, Fischer, JE, Bachmann LM, Koller M . and ter Riet G (2002).Communicating accuracy of tests to general practitioners: a controlled study.BMJ 324: 824-826.
45-Mayer D (2010) Essential evidence based medicine. 2ndedn Cambridge: Cambridge University Press.
46-Akobeng AK (2007) Understanding diagnostic tests 1: sensitivity, specificity, and predictive values. Acta Paediatr 96: 338-341.
47-Hadi F. A. and Al-Hadrawy S. K.(2015).Comparative study of Immunological and Molecular for *Toxoplasma gondii* by using (*B1, 18s and P30*) in Al –Najaf Al- Ashraf Governorate. M.Sc. A thesis. college of science. Kufa university.
48-Vujanić M (2012). Molecular detection and genotyping of Toxoplasma gondii strains isolated in Serbia. PhD thesis. University of Belgrade, Serbia,158pp.
49-Hashoosh A. and I.A. Majeed. (2014).Comparison of two assays in the diagnosis of toxoplasmosis: immunological and molecular.Eastern Mediterranean Health Journal,Vol. 20 No. (1).
50-Guy E. and Joyson D (1995). Potential of the Polymerase Chain Reaction in the Diagnosis of Active Toxoplasma Infection by Detection of Parasite in Blood. J. Infect. Dis. 172, 1: 319- 322.
51-Montoya J. G., Liesenfeld O., Kinney S., Press C. and Remington J. S. (2002). VIDAS test for avidity of Toxoplasma-specific immunoglobulin G for confirmatory testing of pregnant women. J .Clin .Microbiol. 40, 2504–2508.
52-Ajzenberg D, Yera H, Marty P, Paris L, Dalle F, Menotti J, Aubert D, Franck J, Bessieres MH,Quinio D, Pelloux H, Delhaes L, Desbois N, Thulliez P, Robert-Gangneux F,Kauffmann Lacroix C, Pujol S, Rabodonirina M, Bougnoux ME, Cuisenier B, Duhamel C, Duong TH, Filisetti D, Flori P, Gay-Andrieu F, Pratlong F, Nevez G, Totet A, Carme B, Bonnabau H, Darde ML and Villena I (2009): Genotype of 88 Toxoplasma gondii isolates associated with toxoplasmosis in immunocompromised patients and correlation with clinical findings. J. Infect .Dis .199, 8: 1155-1167.
53-Nowakowska D, Colon I, Remington JS, Grigg M., Golab E, Wilczynski J .and Sibley LD (2006). Genotyping of Toxoplasma gondii by multiplex PCR and peptide-based serological testing of samples from infants in Poland diagnosed with congenital toxoplasmosis. J. Clin .Microbiol .44, 4: 1382-1389.
54-Colin D. Jones, Narciss Okhravi, Peter Adamson, Sharron Tasker, and Susan Lightman.(2000).Comparison of PCR Detection Methods for B1, P30, and 18S rDNA Genes of *T. Gondii* in Aqueous Humor.Investigative Ophthalmology & Visual Science, , Vol. 41, No. 3.
55-Adriana Calderaro, Giovanna Piccolo, Chiara Gorrini, Simona Peruzzi, Laura Zerbini, Simona Bommezzadri and Giuseppe Dettori, Carla Chezzi.(2006).Comparison between two Real-time PCR assays and a nested-PCR for the detection of *Toxoplasma gondii*.ACTA BIOMED; 77; 75-80.
56-Elisabeth Chabbert, Laurence Lachaud, Lucien Crobu and Patrick Bastien.(2004).Comparison of Two Widely Used PCR Primer Systems for Detection of Toxoplasma in Amniotic Fluid, Blood, and Tissues.Journal of Clinical microbiology, Vol. 42, No. 4.