



Evaluation of B1 gene and G529 repeated element as targets for molecular diagnosis of toxoplasmosis in pregnant and aborted women in Erbil

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ABSTRACT: *Toxoplasma gondii* is a ubiquitous protozoan parasite that infect one-third of the world's human population, and diagnosed mainly by serological methods that are impeded by insufficient sensitivity. Therefore, it becomes necessary to rely on either direct discovery of the parasite or DNA detection by polymerase chain reaction (PCR). This study aimed to find out the prevalence of toxoplasmosis among women in Erbil and to organize molecular tools for toxoplasmosis using PCR targeting B1 gene and G529 comparing with Enzyme-linked immunosorbent assay (ELISA). The study also aimed to evaluate placenta versus blood specimens in molecular diagnosis.

A cross sectional study carried out in Erbil city, from November 2015 to September 2016, including convenience samples of 350 women who attended the Maternity Teaching Hospital, and some Primary Health Centers in Erbil city. The subjects involved pregnant women with and without history of abortion, and women at labor room. Peripheral blood samples were collected. DNA was extracted and the B1 gene and G529 of *T. gondii* were amplified by PCR.

Out of 350 samples tested, 38(10.9%) and 81(23.1%) were seropositive for anti-toxoplasma IgM and anti-toxoplasma IgG, respectively, and 7(2%) for both IgG and IgM. PCR, targeting B1 gene and G529, revealed positive reactions in 92 (26.3%) and 41 (11.7%), respectively. In 40 placenta specimens collected from abortive and non-abortive women, 1 (5.56 %) and 5 (27.77 %) of abortive women, versus 1 (4.55 %) and 5 (22.73 %) of non-abortive women revealed positive reactions for B1 and G529, respectively. Statistically, there was no significant ($P= 0.897$, $P= 0.533$) differences among the studied genes in respect to detect toxoplasmosis in the blood of whether abortive or non-abortive women. PCR along with serology as a confirmatory test is insisted demand for definitive diagnosis of toxoplasmosis, and PCR targeting G529 being more efficient than B1 gene in the molecular diagnosis in placenta specimens. Although not all pregnancies with toxoplasmosis terminated adversely, maternal infection during pregnancy is a serious condition and early diagnosis on time and proper treatment can lead to healthy offspring

Keywords: *Toxoplasma gondii*, Toxoplasmosis, PCR, Eliza, B1 gene, G529 repetitive element.



1. INTRODUCTION

Toxoplasma gondii (*T. gondii*) is an intracellular parasite, affecting both humans and a wide range of warm blood animals. Domestic cat is the only definitive host [1]. The infection has a universal distribution, approximately one third of all people have been exposed to this parasite. A wide range of incidence of toxoplasmosis has been reported variable factors such as socio-cultural and feeding habits, contact with domestic cats, as well as warm and moist climate, associated with wavy incidence of the disease worldwide [2]. This infection is responsible for significant morbidity and mortality in immunocompromised individuals [3]. The broad distribution of the parasite is due to its several mechanisms of transmission: ingestion of tissue cysts present in raw or poorly cooked meat; ingestion of oocysts in feces of Felidae that contaminate food as well as water and soil [4]. Accordingly, the transmission is either horizontal or vertical. The parasite is horizontally transmitted to humans by accidental intake of water, food, or soil contaminated with *T. gondii* oocysts or use of meat containing tissue cysts that is eaten raw or undercooked. Furthermore, can be vertically transmitted to the fetus during pregnancy and may cause a wide range of clinical manifestations in the offspring depending on the gestational age at which the primary maternal infection was acquired, the virulence of the parasite, and the immunologic development of the fetus [5].

Worldwide prevalence of toxoplasmosis is estimated to be between 15% to 85% of the humans that are asymptotically infected. Most cases of human infection are mild. However, destructive of disease can be occurred in immunocompromised individuals and in congenitally infected fetuses that develop serious neurological or ocular problems appear either early after labor or later on during life and may not become manifested until the second or third decade of life [6] [7]. The transmission through the mother to fetus may occur in women acquiring primary maternal infection during pregnancy. The rate of both transplacental transmission and severity of disease in the fetus are inversely correlated. Whereas early in pregnancy, the transmission rate is low, clinical manifestations are severe and may result in death of the fetus and spontaneous abortion. Late maternal infection is associated with a high rate of transmission, but clinical signs in the newborn are mild or absent upon first examination [8] [9].

Detection of anti-toxoplasma antibodies in patients may assist diagnosis. There are abundant serological procedures available for the finding of humoral antibodies; these include the Sabin–Feldman dye test, indirect hemagglutination assay, indirect fluorescent antibody assay (IFA), direct agglutination test, latex agglutination test, enzyme-linked immunosorbent assay (ELISA), and immunosorbent agglutination assay test. The IgM antibodies appear sooner after infection than the IgG antibodies and disappear faster than IgG antibodies after recovery [10] [11]. Several molecular-based techniques have been developed for the diagnosis of toxoplasmosis using various clinical specimens, including amniotic fluid, blood, cerebrospinal fluid, and tissue biopsy [10]. Polymerase chain reaction (PCR) assay is an important technique to evaluate the prevalence of *T. gondii* reactivation when the detection of circulating DNA is the only clue to its reactivation [12].

Limited studies concerning molecular diagnosis of toxoplasmosis in pregnant women in Kurdistan region, have been conducted and little is known about the target genes that could be used for the molecular diagnosis of toxoplasmosis in local patients by polymerase chain reaction. The present study was aimed to investigate B1 gene and G529 repeated element as targets for molecular detection of toxoplasmosis in the whole blood and placenta using both already existing as well as new designed primers.

2. MATERIALS AND METHODS

2.1 STUDY DESIGN, SETTING AND DURATION

This cross-sectional study was carried out in Erbil city which is the capital city of Kurdistan region with population about 1,750 000 inhabitants [13]. In this study a convenience sample of 350 women who attended Maternity Teaching Hospital, Dr. Nazdar Bammarni, Dr. Najdi Haider and Mala Afendi Health Centers, within the period from November 2015 to September 2016, were invited to be part of the study. Inclusion criteria involved pregnant women with and without history of abortion, and women at delivery room. Ladies with autoimmune disease such as rheumatoid arthritis and systemic lupus erythematosus in addition to allergic conditions were excluded from the study. A close ended

questionnaire which included certain questions about sociodemographic characteristic of study samples, was used and filled through direct interview with the involved ladies.

2.2 COLLECTION OF CLINICAL SPECIMENS

A total of 350 blood samples were collected from the foregoing groups for detection of toxoplasmosis using Enzyme-linked immunosorbent assay (ELISA) and by polymerase chain reaction (PCR). A volume of 6 ml of blood samples were collected aseptically through vein puncture with a disposable plastic syringe and dispensed into two clean sterile screw plastic test tubes with and without anticoagulant. The anticoagulant free samples were centrifuged at 3000 rpm for 10 minutes. The harvested serum dispensed into two clean Eppendorf tubes (1.5 ml capacity) and stored at - 40 °C to be used for serological detection of toxoplasmosis by ELISA. Anticoagulant containing blood samples were stored at - 40 °C and used later for extraction of DNA and molecular detection of toxoplasmosis. Furthermore, biopsies of 40 placenta were collected in clean sterile screw cupped containers contained sterile normal saline (0.85%), stored at -40 °C and used later for DNA extraction and molecular detection of the parasite in the tissue.

2.3 DETECTION OF ANTI-TOXOPLASMA IgM AND IgG ANTIBODIES BY ELISA

Anti-toxoplasma IgG and IgM antibodies were detected in the sera of women using commercially available quantitative ELISA kits (Bioactiva diagnostic; Germany) in accordance of the manufacturer's instructions

2.4 MOLECULAR ASSAY

2.4.1 EXTRACTION OF DEOXYRIBONUCLEIC ACID (DNA) FROM BLOOD SAMPLES

Extraction of DNA was conducted using 3ml of blood, which was collected in tubes containing EDTA using DNA isolation kit from blood, according to the manufacturer's instructions. This was used to isolate highly purified DNA from the whole blood samples. The isolation procedure was based on magnetic bead technology. From the placenta samples, a tissue specific DNA extraction kit was used. Both kits were purchased from GeNet Bio- Global Gene; South Korea.

2.4.2 AMPLIFICATION OF B1 GENE

The amplification procedure that developed by Burg et al (1989) targeting toxoplasma B1 gene was followed. Nested PCR was carried out using the primer 5'- GGAAGTGCATCCGTTTCATGAG-3' (F) and 5'- TCTTTAAAGCGTTTCGTGGTC-3' (R); 5'-TGCATAGGTTGCAGTCACTG-3'(F) and 5'- GGCGACCAATCTGCGAATACACC-3' (R) for outer B1 gene with amplicon length of 200 base pair (bp) and inner B1 gene with amplicon length of 100 base pair (bp), respectively.

2.4.3 AMPLIFICATION OF G529 REPEATITIVE ELEMENT

G529 repeat element (NCBI gene bank accession number: AF146527.1) was used as second target for molecular detection of toxoplasmosis using the primers 5'-GCTTTTCCTGGAGGGTGGAA-3' (F) and 5'-ATTCTCTCCGCCATCACCAC-3' (R) with amplicon length of 84 bp. The amplification protocol for toxoplasma G529 was as follows. The PCR mixture involved 2.5µl of each primer, 5µl of DNA sample and 12.5 µl green master mix which in turn contained Taq DNA polymerase, reaction buffer (pH=8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM MgCl₂. Green master mix reaction buffer is a proprietary buffer containing a compound that increase sample density, and yellow and blue dyes, which function as loading dyes when reaction products are analyzed by agarose gel electrophoresis. The amplification protocol composed of denaturation 95C° for 5 minutes followed by 35 cycle's 95C° for 30 seconds, Annealing 56 C° for 30 seconds, extension 72 C° for 30 seconds. The PCR products were interpreted on 2% (2 gm agarose in 100 ml of TBE buffer) agarose gel electrophoresis.

2.5 ETHICAL CONSIDERATION

The project was approved by the research ethics committee in the College of Medicine, Hawler Medical University and verbal consent was obtained from each participant with assuring for those who agreed to participate in the study, the obtained information will be kept confidential and not be used for any other purpose apart from the study.

2.6 STATISTICAL ANALYSIS

The collected data were entered into the Microsoft excel sheets, translated into codes using a specially designed coding sheet, and converted into SPSS database. An expert statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 19.0. Descriptive statistics approach used to find out frequencies and percentage while in analytic approach, X^2 test and fisher exact test used to find out association between categorical variables. P value ≤ 0.05 was considered statistically significant.

3. RESULTS

Out of 350 samples that were assessed by ELISA, 38(10.9%) of the samples were positive for IgM, 81(23.1%) for IgG and 7(2%) for both IgG and IgM, among the studied women (Fig.1).

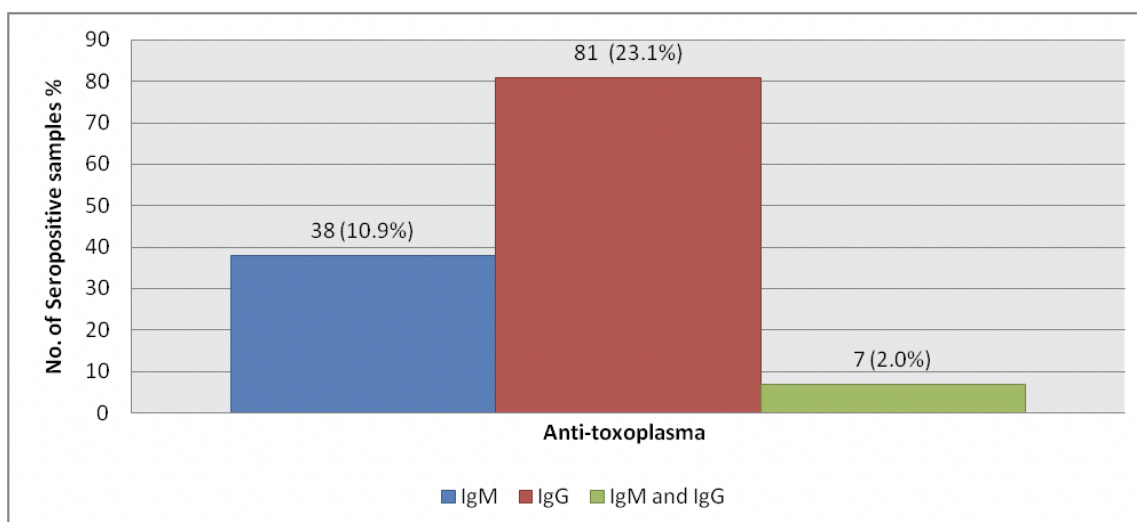


FIGURE. 1- Prevalence of anti-toxoplasma IgG and IgM antibodies among 350 women by ELISA

The highest rates, 44 (12.6 %) and 21 (6 %) were observed among women who were urban inhabitants by both ELISA-IgG, and ELISA-IgM, respectively. However, statistical analysis revealed no significant differences among urban and rural inhabitants for both anti-toxoplasma IgG and IgM ($P= 0.169$, $P= 0.454$) antibodies, respectively (Table 1). Comparably, the prevalence of anti-toxoplasma IgM was the highest (9.5 %) being at more risk to get toxoplasmosis among illiterate than educated level (Table 2), however, statistically, there was no significant differences among illiterate and educated women ($P=0.687$, $P=0.697$) in respect to having anti-toxoplasma IgG and IgM antibodies, respectively.

Table 1.- Seroprevalence of toxoplasmosis among women according to residency by ELISA

Residency	No. of examined samples	IgM		IgG	
		Seropositive samples No. (%)	Seronegative samples No. (%)	Seropositive samples No. (%)	Seronegative samples No. (%)
Urban	213	21 (6.0)	192 (54.9)	44 (12.6)	169 (48.2)
Rural	137	17 (4.9)	120 (34.2)	37 (10.57)	100 (28.6)
Total	350	38 (10.9)	312 (89.1)	81 (23.1)	269 (76.9)
P. value		0.454		0.169	

As it can be seen in Table 3, no significant ($P= 0.548$, $P= 0.311$) association of toxoplasmosis and history of abortion was observed on screening for both anti-toxoplasma IgG (26.2 % vs 21.42 %) and IgM (9.52 % vs 11.6 %) antibodies by ELISA in women with and without history of abortion. Regarding the socioeconomic level of the patients, the results revealed highly significant ($P= 0.005$) association of anti-toxoplasma IgM antibodies and low socioeconomic level (9.2 %) comparing with medium status (1.7 %) and welfare (0.0 %) women. However, no significant association of anti-toxoplasma IgG and socioeconomic level was observed (Table 4).

Table 2. - Seroprevalence of toxoplasmosis among women according to educational level by ELISA

Education	No. of examined samples	IgM		IgG	
		Seropositive samples No. (%)	Seronegative samples No. (%)	Seropositive samples No. (%)	Seronegative samples No. (%)
Illiterate	287	33 (9.42)	254 (72.6)	68 (19.4)	219 (62.6)
Primary	5	0 (0)	5 (1.4)	2 (0.6)	3 (0.9)
Secondary	6	1 (0.3)	5 (1.4)	1 (0.3)	5 (1.4)
University	52	4 (1.1)	48 (13.7)	10 (2.9)	42 (12.0)
Total	350	38 (10.9)	312 (89.1)	81 (23.1)	269 (76.9)
P. value		0.687		0.697	

Table 3. - Seroprevalence of toxoplasmosis among women according to history of abortion by ELISA

History of Abortion	No. of examined samples	IgM		IgG	
		Seropositive samples No. (%)	Seronegative samples No. (%)	Seropositive samples No. (%)	Seronegative samples No. (%)
Yes	126	12 (9.52)	114 (90.48)	33 (26.2)	93 (73.8)
No	224	26 (11.6)	198 (88.4)	48 (21.42)	176 (78.58)
Total	350	38 (10.9)	312 (89.1)	81 (23.1)	269 (76.9)
P. value		0.548		0.311	

Table 4. - Seroprevalence of toxoplasmosis among women according to socioeconomic level by ELISA

Socioeconomic Level	No. of examined samples	IgM		IgG	
		Seropositive samples No. (%)	Seronegative samples No. (%)	Seropositive samples No. (%)	Seronegative samples No. (%)
Low	210	32 (9.14)	178 (50.85)	49 (14.0)	161 (46.0)
Medium	139	6 (1.7)	133 (38.0)	32 (9.1)	107 (30.6)
High	1	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.3)
Total	350	38 (10.9)	312(89.1)	81 (23.1)	269 (76.9)
P. value		0.005		0.858	

Comparably, the results revealed that, the highest rate of anti-toxoplasma (IgG, 12.6 %; IgM, 6.9 %) antibodies were among women with ages ranged between 21-30 years old. However, statistical analysis revealed no significant, differences in the levels of both IgG (P= 0.274) and IgM (P= 0.191) antibodies among the studied age groups (Table 5).

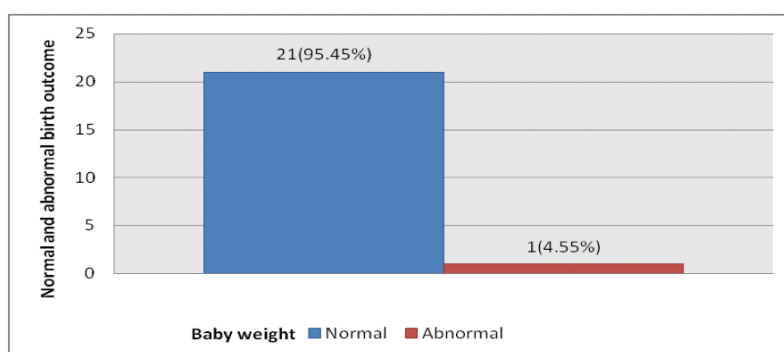
Table 5. - Age distribution of anti-toxoplasma antibodies among women by ELISA

Age (year)	No. of examined samples	IgM		IgG	
		Seropositive samples No. (%)	Seronegative Samples No. (%)	Seropositive samples No. (%)	Seronegative samples No. (%)
≤20	48	5 (1.4)	43 (12.3)	12 (3.4)	36 (10.3)
21-30	204	24 (6.9)	180 (51.4)	44 (12.6)	160 (45.71)
31-40	89	7 (2.0)	82 (23.4)	23 (6.57)	66 (18.85)
≥41	9	2 (0.6)	7 (2.0)	2 (0.6)	7 (2.0)
Total	350	38 (10.9)	312 (89.1)	81 (23.1)	269 (76.9)
P. value		0.191		0.274	

Table 6 shows, the relationship between toxoplasmosis and the birth weight, out of 100 women at delivery theatre and who tested for toxoplasmosis by PCR targeting B1 gene, 22(22%) revealed positive reactions for toxoplasmosis. The birth weight ranged between 1 to 4.5 kg. Of those 22 women who revealed positive test for toxoplasmosis, 21 (95.45%) and 1 (4.55%), were delivered normal and abnormal pregnancy outcome, respectively (Fig. 2). Statistically, no significant association (P= 0.635) of toxoplasmosis and birth weight outcome was detected by PCR targeting B1 gene.

Table 6. - Association of toxoplasmosis and birth weight in women at delivery theatre by PCR targeting B1 gene.

Birth weight (Kg)	No. of baby	Toxoplasmosis	
		No. PCR positive samples (%)	No. PCR negative samples (%)
1 – 2.4	2	0 (0)	2 (2)
2.5 - 4	93	21 (21)	72 (72)
≥ 4.1	5	1 (1)	4 (4)
Total	100	22 (22)	78 (78)
P. value		0.635	

**FIGUR 2. - Birth weight outcome in 22 women with toxoplasmosis by PCR targeting B1 gene.**

As it can be seen in Table 7 that, comparably, the highest rate of anti-toxoplasma (IgG, 12.9%; IgM, 6.0%) antibodies were among women with gestational ages ranged between 31- 41weeks. However, statistical analysis revealed no significant, differences in the levels of both IgG (P= 0.131) and IgM (P= 0.422) antibodies among the studied women at different gestational stages.

Table 7. - Gestational age distribution of anti-toxoplasma antibodies among women by ELISA

Gestational age/weeks	Number of examined sample	IgM		IgG	
		Seropositive samples No. (%)	Seronegative samples No. (%)	Seropositive samples No. (%)	Seronegative samples No. (%)
1-10	95	14 (4.0)	81 (23.1)	21 (6.0)	74 (21.14)
11-20	39	1 (0.3)	38 (10.85)	5 (1.4)	34 (9.7)
21-30	32	2 (0.6)	30 (8.6)	10 (2.9)	22 (6.3)
31-41	184	21 (6.0)	163 (46.6)	45 (12.85)	139 (39.7)
Total	350	38 (10.9)	312 (89.1)	81 (23.1)	269 (76.9)
P. value		0.422		0.131	

Table 8 shows the prevalence of toxoplasmosis among the studied women by ELISA- IgM, and ELISA- IgG and by PCR targeting Toxoplasma B1 gene and G529 repeat element. The highest rate of infection was detected by nested PCR targeting B1 gene that detected 26.3 % of the cases comparing with 10.9 %, 23.1 % and 11.7 % by IgM- ELISA, IgG- ELISA and PCR targeting G529 repeat element, respectively.

Table 8. - Overall rate of toxoplasmosis among women by ELISA and PCR in blood sample

Diagnostic method	No. of examined samples	Seropositive samples No. (%)	Seronegative samples No. (%)
ELISA- IgM	350	38 (10.9)	312 (89.1)
ELISA- IgG	350	81 (23.1)	269 (76.9)
B1	350	92 (26.3)	258 (73.7)
G529	350	41 (11.7)	309 (88.3)

Table 9 shows the results of molecular diagnosis of toxoplasmosis by PCR using blood and tissue (placenta) as sources of DNA. Out of 40 blood and placenta samples that collected from women at delivery, 40 % and 15 % versus 5 % and 25 %, revealed positive reactions for B1 and G529, when blood and placenta used as source of parasite genome, respectively. Statistically, PCR targeting B1 gene was more efficient ($P=0.013$) than G529 in the detection of the infection when blood was used as source of DNA, however, the rate of the positive reactions was dramatically diminished by B1 gene in favor of G529 ($P=0.005$) in the tissue. Accordingly, PCR targeting G529 repeat element in Placenta sample is more efficient ($P=0.001$) for diagnosis of toxoplasmosis in women.

Table 9. - Molecular detection of toxoplasmosis in the blood and placenta specimens by PCR targeting B1 and G529 genes.

		Blood			Tissue			
		No. of examined samples	Seropositive samples No. (%)	Seronegative samples No. (%)	No. of examined samples	Seropositive samples No. (%)	Seronegative samples No. (%)	
B1	40	16 (40.0)	24 (60.0)	40	2 (5.0)	38 (95.0)		*
G529	40	6 (15.0)	34 (85.0)	40	10 (25.0)	30 (75.0)		**
P. value			0.013			0.005		

* Comparison B1 positivity in blood and tissue.

** Comparison G529 in blood and tissue.

Table 10 shows molecular diagnosis of toxoplasmosis in blood samples of abortive and non-abortive women. Out of 40 samples that were tested by PCR, 7(38.88%) and 2(11.12%) abortive women revealed positive reactions by B1 and G529, respectively, versus 9 (40.90 %) and 4 (18.18%) of non-abortive women. Statistically, there was no significant ($P= 0.897$, $P= 0.533$) differences among the studied genes in respect to detect toxoplasmosis in the blood of whether abortive or non-abortive women.

Table 10. - Molecular detection of toxoplasmosis in 40 blood specimens in term of history of abortion

History of abortion	No. of examined sample	B1		G529	
		Seropositive samples	Seronegative samples	Seropositive	Seronegative samples
		No. (%)	No. (%)	Samples No. (%)	No. (%)
Yes	18	7 (38.88)	11 (61.12)	2 (11.11)	16 (88.88)
No	22	9 (40.90)	13 (59.10)	4 (18.18)	18 (81.82)
Total	40	16 (40.0)	24 (60.0)	6 (15.0)	34 (85.0)
P. value		0.897		0.533	

Table 11 shows molecular diagnosis of toxoplasmosis in 40 placenta specimens collected from abortive and non-abortive women at labor. Of those 40 samples, 1 (5.56 %) and 5 (27.77 %) of abortive women, versus 1 (4.55 %) and 5 (22.73 %) of non-abortive women revealed positive tests for B1 and G529, respectively. Statistically, no significant association ($P=0.884$, $P=0.714$) between toxoplasmosis and history of abortion was detected by PCR targeting both B1 gene and G529 repeat element when placenta sample used as source of DNA among the studied genes in respect to detect toxoplasmosis in the placenta of whether abortive or non- abortive women.

Table 11. - Molecular detection of toxoplasmosis in 40 placenta specimens in term of history of abortion

History of abortion	No. of examined samples	B1		G529	
		Seropositive samples	Seronegative samples	Seropositive samples	Seronegative samples
		No. (%)	No. (%)	No. (%)	No. (%)
Yes	18	1 (5.56)	17 (94.44)	5 (27.78)	13 (72.22)
No	22	1 (4.55)	21 (95.45)	5 (22.73)	17 (77.27)
Total	40	2 (5.0)	38 (95.0)	10 (25.0)	30 (75.0)
P. value		0.884		0.714	

4. DISCUSSION

Toxoplasmosis is a zoonotic disease with potential transmission to people from livestock and vice-versa [2]. Approximately one-third of the world's population is infected with the protozoan parasite *T. gondii* [8] [15], infection may lead to serious illness when the organism is contracted while pregnancy or when it is reactivated in immune-suppressed persons [10].

In the present study screening of toxoplasmosis among 350 pregnant and abortive women with and without history of abortion, the serological test by ELISA showed 38(10.9%) of the samples were positive for IgM, 81(23.1%) for IgG and 7 cases (2%) were positive for both IgG and IgM (Fig.1).

However previous studies which carried out in Erbil city, recorded different results. [16] reported 8 (5.3%), 23 (15.3%) for IgM and IgG respectively. In Baghdad the infection rate was found to be 4.16% for IgM, 25.83% for IgG and 13.33% for both IgM and IgG as reported by Mahmood et al. (2010). In Saudi Arabia, the sero-prevalence of toxoplasmosis was found to be 32.5% and 6.4% for IgG and IgM, respectively [17]. In Iran, 5(0.63%) and 341 (42.7%) of women were revealed positive sero-reactivity for acute and chronic infections, respectively [18].

In the current study, the highest rates of toxoplasmosis, 44 (12.6 %) and 21 (6 %) of toxoplasmosis were observed among women who were urban inhabitants by both ELISA-IgG, and ELISA-IgM, respectively. The frequency of toxoplasmosis in the human population is associated directly or indirectly with numerous risk factors. An increased prevalence of toxoplasmosis in the urban area could be attributed to the consumption of poorly washed vegetables or treated with unsafe water supply that was also identified among other risk factors or at least to expose to the parasite infective stages, in addition to some other risk factors, such as personal hygienic practices, feeding habits and socio-economic status [19]. Association of *Toxoplasma gondii* sero-reactivity with lower educational levels might be attributed to low level of sanitation, health awareness and due to direct or indirect handling potentially contaminated, outdoor and even indoor cats and cat's feces which is only known definitive host for this parasite [4].

In the current study unexpectedly, no significant association between anti-toxoplasma seropositivity and history of abortion was noticed, which was inconsistent with the findings that reported by [20] in Northeast India. It is suggested that encysted forms of *T. gondii* in long-term infected uteri and their rupture in subsequent placentation may lead to infection of the fetus in the first trimester and subsequent abortions. However, there is insufficient data to prove the link between *toxoplasmosis* and recurrent or habitual abortions [21] [20] .

In the present study, significant association observed between anti- toxoplasma IgM antibodies and low socioeconomic level (9.2 %) comparing with medium status (1.7 %) and welfare (0.0 %) women (Table 4). However, no significant association of anti-toxoplasma IgG antibodies and socioeconomic level was observed. Women of low-income group could be at risk of repeated infections attributable to the unhygienic environment in which they reside. Furthermore, a low socioeconomic level may be linked to malnutrition, and this factor might impair the host defense against *T. gondii* infection [19].

Regarding the age, our study observed highest rate of anti-toxoplasma (IgG, 12.6 %; IgM, 6.9 %) antibodies were among women with ages ranged between 21-30 years old. However, statistical analysis revealed no significant association (Table 5). [22] in Brazil reported that women at childbearing age are more susceptible to get *T. gondii* infection, and demonstrated that women presenting before pregnancy have a 1.74 times higher risk of toxoplasmosis than non-previously pregnant women. This greater vulnerability of pregnant women to the parasite is probably due to alterations in the immune mechanisms inherent to gestation, resulting from suppression of immune response because of the necessity of tolerance to the graft (fetus) and/or as a result of hormone imbalances characteristic of the gestational condition [23].

The relationship between toxoplasmosis and the birth weight shows in (Table 6), out of 100 ladies at delivery theatre and who tested for toxoplasmosis by PCR targeting B1 gene, 22(22%) revealed positive reactions for toxoplasmosis. The birth weight ranged between 1 to 4.5 kg. Out of those 22 women who revealed positive test for toxoplasmosis, 21 (95.45%) and 1 (4.55%), were out normal and abnormal pregnancy outcome, respectively. The results of PCR, using *T. gondii* B1 gene as a target, revealed non-significant association of toxoplasmosis and baby birth weight. Birth weight is the single most predictive factor of mortality in the first few months of life, and a baby's birth weight is an important indicator of his/ her health [24]. The World Health Organization (WHO) defined low birth weight as that below 2,5kg [25]. The standard body weight from intrauterine life until adulthood, is reflected by individual's genetic structure. Of a total of at least 24,000 genes that constitute the human blueprint, over 250 of those genes may help determine the body weight [26]. Population genetic examination based on restriction fragment length polymorphisms (RFLPs) indicated that most strains of *T. gondii* in North America and Europe belong into one of three clonal lineages, designated types I, II, and III, which happen in both animals and humans with type II associated with more than 70% of human disease cases of toxoplasmosis [27]. So strain variation is a crucial factor for determine normal and abnormal pregnancy outcome in infected women. This feature could justify the normal delivery and normal birth outcome in women

already revealed positive tests for toxoplasmosis. Accordingly, molecular characterization and studying molecular diversity of toxoplasma in local population is a crucial clinical and epidemiological demand.

Although statistical analysis revealed no significant differences among women at different gestational stages in respect to have anti-toxoplasma seropositivity, the highest rate of seropositivity was detected among women at gestational ages ranged between 31-41 weeks (Table 7). Acquiring toxoplasmosis is more frequent during third trimester; however, it is very rare for congenital toxoplasmosis to be developed in offspring, if the immunocompetent mother is infected at least three months prior to conception. The rate of transmission during pregnancy that leads to congenital toxoplasmosis depends upon the gestation period of primary infection and transmission rate of over 60% may be observed, if primary *T. gondii* infection occurs in the last trimester of pregnancy [28] [20].

In regards to the rate of toxoplasmosis among women by ELISA and PCR, and compared PCR results with serological results by detection of IgM and IgG to Toxoplasma by ELISA. Out of 350 examined samples, 38(10.9%) were positive for IgM and 81(23.1%) were positive for IgG.

Routine serologic diagnosis of toxoplasmosis provides high sensitivity, but specificity difference depending on the test used. Anti-Toxoplasma IgM antibodies may persist for up to one year post infection. Diagnosis of primary infection with *T. gondii* in early pregnancy can be more reliable if detected by anti-toxoplasma IgG avidity test, which has the ability to distinguish between recent and prior infections [29]. Therefore, when recent toxoplasmosis is assumed, the serum should be examined for IgG and IgM antibodies that are sometimes unreliable. While severe immune system dysfunction results in a lack of production of antibodies, therefore, advanced molecular methods are strongly recommended tests to be done for precis diagnosis [30]. Rasti et al. (2015) observed that pathogenesis, number of transferred organisms, infant's age, gestational age at which infection occurred and maturity of the infant's immune system plays an important role in their positive serology.

In the current study, out of 40 samples that were tested by PCR, 7(38.88%) and 2(11.12%) abortive women revealed positive reactions by B1 and G529, respectively, versus 9 (40.90 %) and 4 (18.18%) of non-abortive women. Statistically, no significant differences observed among the studied genes in respect to detect toxoplasmosis in the blood of whether abortive or non-abortive women. The PCR set up and primer type might affect the results of PCR [31].

Molecular diagnosis of toxoplasmosis in 40 placenta specimens revealed positive reactions in 1 (5.56 %) and 5 (27.77 %) of abortive women, versus 1 (4.55 %) and 5 (22.73 %) of non-abortive for B1 gene and G529 repeat element, respectively. Higher rate (27.77%) of positive reactions were detected when G529 repeat element was used as target in placenta samples among women who had history of abortion. Statistically, no significant association of toxoplasmosis and history of abortion were detected by PCR targeting both B1 gene and G529 repeat element. PCR using the placental tissue is useful for the rapid and accurate diagnosis of congenital toxoplasmosis [31] [31]. Since induction of abortion is multifactorial, so it is not a condition that the cause of abortion is toxoplasmosis but many etiological factors have been attributed as a cause of abortion such as chromosomal abnormalities, uterine factors, immunological problems, hormonal disturbances, infection and chronic systemic disease [32].

5. CONCLUSION

Not all pregnancy necessarily leads to adverse termination, and Polymerase chain reaction targeting G529 being more efficient than B1 gene in the molecular diagnosis in placenta specimen. Studying the strain variation and molecular characterization of local strain of *T. gondii* is insisted demand in order to understand the virulency and association of toxoplasmosis and abnormal pregnancy outcome.

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