Original
ISSN 2521-8492

Antigenic Markers of *T. gondii* for Chronic Forms of Toxoplasmosis in Fertility Age Women

Suha A. AL-Fakhar[1*], Wifaq M. Ali[1], Saad Hasan Mohammed Ali[1], Khalil Ismail A Mohammed[1], Jinan M. Mousa[1], Nada Nuri Youns[2], Zahraa Mushtaq Jabbar[2]

[1]Clinical Communicable Diseases Research Unit, College of Medicine, University of Baghdad, Baghdad, Iraq.
[2]Central Public Health, Ministry of Health, Baghdad, Iraq.
*Correspondence to: Suha A. AL-Fakhar (E-mail: suhaabdullah2016@gmail.com)
(Submitted: 21 November 2023 – Revised version received: 13 December 2023 – Accepted: 19 January 2024 – Published Online: 26 March 2024)

Abstract
Objective: This study aimed to assess the avidity of IgG antibodies against *Toxoplasma gondii* in fertile-aged women with a history of abortion. Using the *recom*Line Toxoplasma IgG Avidity kit, the research investigated the relationship between antibody avidity, *T. gondii* antigens (GRA1 and SAG1), and the number of abortions to enhance understanding of infection dynamics in this population.
Methods: Forty (40) fertile aged women (18–37years) with history of abortion and had positive results of IgG Abs by minividus form October 2021 to April 2022, Kit of *recom*Line Toxoplasma IgG Avidity form Mikrogen / Germany, item No. 11010, was used, which is a qualitative *in vitro* test for the determination the avidity of IgG antibodies against *Toxoplasma gondii* in human serum or plasma.
Results: Phase II of toxoplasmosis was positive of antibodies against different antigens detected by *recom*Line assay, no other significant relation was observed. Also, that there was no significant relation between the period of infection and avidity of IgG–Abs against different antigens of *T.gondii* detected by *recom*Line test. In addition the results showed that there was a significant relation between of the number of abortions to GRA1-antigen ($P = 0.012$) and SAG1-antigen ($P = 0.003$).
Conclusion: There was a significant relation between of the number of abortions to GRA1 and SAG1-antigens detected by *recom*Line assay, where increase in the positive results with the increase in the number of abortions.
Keywords: *recom*Line toxoplasma IgG avidity, chronic toxoplasmosis and abortion, laboratory diagnosis of toxoplasmosis

Introduction

Toxoplasma gondii the organism protozoan which is the origin of toxoplasmosis. Although humanlike toxoplasmosis in thriving mature is normally symptomless, overserious sickness can happen in the cause of inborn transmission and disorder individualist.[1]

Currently, regular diagnosing of toxoplasmosis relies primarily along the use various serologic trial to detect specific antibodies in the serum samples of infected patients. The presence of a recent infection can be determined by detecting seroconversion of immunoglobulin M (IgM) or IgG antibodies, a substantial increase in IgG antibody titer, or a Toxoplasma serologic profile compatible with acute infection (using Toxoplasma serodiagnostic tests, including an IgG avidity test) in sequential serum samples of infected individuals.[2,3]

Recombinant antigens could therefore enhance the clinical usefulness of avidity assays in order to determine more accurately when *T. gondii* infection occurred. In addition, the standardization of an avidity test based on recombinant antigens, which have a more-constant quality than TLAs prepared from parasites grown either in a cell culture or in the peritoneal cavities of mice, should be easie.[4]

The *recom*Line *Toxoplasma* is worldwide the only test system which meets the requirements for the special diagnosis of toxoplasmosis especially for pregnancy. The optimized combination of using recombinant antigens for IgG and IgM and also 4 different phase specific avidity antigens is unique on the market for the determination of the status of a *Toxoplasma* infection.[5] The organism *Toxoplasma gondii* mightiness hurt the foetus if a female is infective during pregnancy. IgG seroconversion and fundamental gain in IgG antibody quantity in maternity tell parental transmission. Existence of toxoplasma gamma globulin M (IgM), gamma globulin G (IgG) and reduced IgG avidness in a individual blood serum sample inform achievable paternal contagion, merely positive toxoplasma Immunoglobulin M and low-level IgG avidness hawthorn continue for listing period of time and justified years.[6]

First contagion with *Toxoplasma gondii* during gestation whitethorn effect in intense alteration to the foetus if the parasites are transmissible through with the reproductive structure.[7] The hazard of infection and the intensity of craniate illness rely on gestational years at the instance of motherlike incident.[8] The craniate get on infective during the period of time of motherly parasitaemia, earlier the evolution of toxoplasma-specific antibodies.[7] Parental antibodies defend the fetus, and incident preceding to gestation does not impact the vertebrate.[9] Motherlike toxoplasma pathologic process is commonly symptomless; hence, the identification relies principally on serological tests assembled through with covering arrangement or unselected investigation. The existence of toxoplasma immune serum globulin G (IgG) antibodies steady current or past transmission, and the existence of toxoplasma human gamma globulin M (IgM) antibodies inform a realizable current transmission. Even so, differentiation betwixt ancient and recent incident is ambitious, as an single can be positive for toxoplasma IgM antibodies for various period of time or time of life later essential incident.[10-12]

In recent decades, *Toxo* IgG avidness assay has been utilized as a standardized designation technique for a better estimation of the transmission acquiring period of time and recognition of the essential *T. gondii* contagion during maternity.[13] Also, finding of toxoplasma IgG avidness has been enclosed as a common characteristic instrument to alter the estimate of the period of time of incident getting. The IgG avidness trial measures antibody attraction power, which is

reduced in the primal phase later original transmission but mostly increases with time. High toxoplasma IgG avidity indicates that an infection likely occurred at least four months earlier. Nevertheless, different written document have shown that IgG avidness canful persist low-level for a yearner period of time pursuing infection. This may be a natural response later transmission in few individualist, owing to immunologic modification during gestation or a reaction to antibacterial management. Hence, toxoplasma contagion is unachievable to support during maternity supported exclusively along low-level toxoplasma IgG avidness.[14-16]

Laboratory tests that help classify recent primary toxoplasmosis are important tools for the management of pregnant women suspected to have *T. gondii* exposure. Detection of *Toxoplasma* IgM (*Toxo* IgM) is a sensitive indicator of primary toxoplasmosis, but the indicator specificity is low because sometimes natural IgM antibodies react with Toxoplasma antigens in the absence of the infection. In recent decades, *Toxo* Immunoglobulin G avidness test was used as a standardized diagnostic method for a better estimation of the transmission getting time and determination of the essential *T. gondii* incident during pregnancy. Avidity is described as the aggregate strength; by which, a miscellany of polyclonal IgG particles reacts with doubled epitopes of the proteins. This parameter matures gradually within 6 months of the primary infection. A high Toxo IgG avidity index allows a new transmission (fewer than four months) to be excluded, whereas a low *Toxo* IgG avidity index indicates a probable recent infection with no exclusions of the older infections. This minireview is based on various aspects of *T. gondii* IgG avidity testing, including (i) description of avidity and basic methods used in primary studies on *T. gondii* IgG avidity and primary infections; (ii) importance of IgG avidity testing in pregnancy.[17]

Materials and Methods

Forty (40) fertile aged women (18–37 years) with history of abortion and had positive results of IgG Abs by minividus form October 2021 to April 2022, Kit of *recom*Line *Toxoplasma* IgG Avidity form Mikrogen / Germany, item No. 11010, was used, which is a qualitative *in vitro* test for the determination the avidity of IgG antibodies against *Toxoplasma gondii* in human serum or plasma.

Preparation of Solutions

Preparation of ready-to-use wash buffer A

This buffer was required for serum and conjugate dilution as well as washing stages. Prior to dilution, the volume of wash buffer A was determined for the corresponding number of tests. First, the skimmed milk powder was dissolved in wash buffer A concentrate, and then deionised water was added to bring the solution up to the final volume (dilution: 1 + 9).

Preparation of conjugate solutions

The conjugate solution was prepared just before use. It is not possible to store the ready-to-use conjugate solution. One part of the conjugate concentrate was diluted with 100 parts of the ready to use wash buffer A (1 + 100).

Test Procedure

1. All reagents were tempered at room temperature for about 30 minutes (18–25°C).
2. 2 ml of the ready to use wash and dilution buffer were pipetted into each incubation well. A test strip was then carefully placed in each of the wells filled with wash buffer using a forceps. The strip number was faced upwards.
3. 20 µl of undiluted sample (human/plasma) were pipetted into each of two incubation wells for each incubation charge.
4. The incubation tray was covered with plastic lid and was incubated at room temperature for 1 hour while shaking gently. The incubation temperature was between (18–25°C).
5. The plastic lids were carefully removed from the incubation trays.

Washing procedure

1. Following incubation the plastic lids were carefully removed.
2. The reaction solution was carefully aspirated from individual wells.
3. Then 2 ml of the ready to use wash buffer was placed into each well and was incubated for 5 minutes while the plate was shaking gently. The wash buffer was aspirated after the washing procedure.

Second incubation

2 ml of the ready to use avidity solution were added to the first charge and wash buffer were added to the second charge, followed by an incubation for 3 minute (the low avidity antibodies were washed off). The solutions were aspirated from the incubation wells and the strips were washed again, whereby step 3 in washing procedure was carried out of a total three times.

Third incubation

After the strips were washed, 2 ml of the appropriately prepared conjugate solution was added to each incubation well and was incubated while shaking gently for 45 minutes, whereby the incubation tray was covered by the plastic lid.

Washing

The solutions were aspirated from the incubation wells and the strips were washed again three times.

Fourth incubation

1. 1.5 ml of substrate solution were placed in each well, then were incubated for 5–15 minutes at room temperature while was shaked gently.
2. After the substrate solution had been aspirated, the strips were rinsed three times with deionized water.
3. The strips were carefully removed from the water using a foreceps and were placed on absorbent paper to dry for 2 hours. Then the strips were adhesively attached to enclosed evaluation sheet.

Results

Statistical Analysis

Statistical analyses were performed using SPSS statistical package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA). Mean and standard deviation were used to describe the age. Count and percentage were used to study the

different marker results. Chi-square test was used to study the relation of pregnancy and abortions to the studied markers. *P* value <0.05 was considered statistically significant.

The results of the present study showed in Table 1 the distribution of IgG Abs avidity for different antigens *recom*Line assay within phase I and II.

All phase II were +ve of antibodies against different antigens of *recom*Line assay, no other significant relation was observed. Since the results showed in Table 1 that GRA-1 had a significant relation with phase ll with GRA-1 ($P < 0.05$), since there were 4(100%) had GRA-1 & had phase II of *T.gondii* ($P = 0.028$) using Chi-square, while there was no significant relation between other antigens and phase II of toxoplasmosis.

The results showed in Table 2 that there were no significant relation between the period of infection and avidity of IgG–Abs against different antigens of *T.gondii* detected by *recom*Line test.

Table 3 showed the number of abortion with different antigens of toxoplasmosis, and the results showed that there was a significant relation of the number of abortions to GRA1-antigen ($P = 0.012$) and SAG1-antigen ($P = 0.003$) where increase in the positive results with the increase in the number of abortions. No other significant relation was observed with the other markers ($P > 0.05$), Chi-Square test was used.

Table 1. **Distribution of the avidity of the IgG Abs for antigens: P30, MAG-1, GRA-1 & r SAG-1 with phase I and phase II of toxoplasmosis**

		Phase				*P*-value
		I		II		
		Count	%	Count	%	
P30	+ve	28	100.0%	4	100.0%	–
	–ve	0	0.0%	0	0.0%	
MAG-1	+ve	14	50.0%	4	100.0%	0.113
	–ve	14	50.0%	0	0.0%	
GRA1	+ve	10	35.7%	4	100.0%	0.028*
	–ve	18	64.3%	0	0.0%	
r SAG1	+ve	16	57.1%	2	50.0%	0.788
	–ve	12	42.9%	2	50.0%	

Table 2. **The distribution of the avidity of IgG Abs of *T.gondi* against: P30, MAG-1, GRA-1, r SAG with the period of infection of *T.gondii***

		Period of infection				*P*-value
		>2 months		<3 months		
		Count	%	Count	%	
P30	+ve	14	100.0%	18	100.0%	–
	–ve	0	0.0%	0	0.0%	
MAG-1	+ve	6	42.9%	12	66.7%	0.283
	–ve	8	57.1%	6	33.3%	
GRA1	+ve	6	42.9%	8	44.4%	0.928
	–ve	8	57.1%	10	55.6%	
rSAG1	+ve	6	42.9%	12	66.7%	0.283
	–ve	8	57.1%	6	33.3%	

Discussion

The parasite *Toxoplasma gondii*, is an obligate intracellular organism, can infect nearly entirely warm-blooded animals, including human beings. Man and animals become infective by ingestion undercooked or unprocessed meat include cysts, or by ingestion food polluted with sporulated oocysts. Conclusive identification of *T. gondii* transmission by mouse immunization, or immunohistochemical analyses is optimal. However, these tests are time-consuming, involved in experimental animals, and may have a low sensitivity. Multiple tests, such as indirect haemagglutination (IHA), modified agglutination test (MAT), latex agglutination test (LAT), indirect fluorescent antibody test (IFAT), and enzyme-linked immunosorbent assay (ELISA), are useful to demonstrate *T. gondii* infection in humans and animals. Despite the satisfactory results of serological tests, development of reliable and standard reagents remains a major constraint in serodiagnosis of *T. gondii* infection. Most conventional tests using tachyzoites grown in mice or in tissue culture are usually difficult to standardize, making the test results difficult to evaluate.[18]

The *recom*Line Toxoplasma IgG is only used to determine the a vidity of the IgG Abs for the following antigens: P30, MAG-1, GRA-1, and r SAG-1 (the time of infection can only be delimited by determining the avidity of the IgG antibodies for these antigens. Avidity cannot be used to determine the time of infection for the antigens ROP-1c, MIC3, GRA7, and GRA8.[19]

The parasite *Toxoplasma gondii* might harm the fetus if a woman is infected during pregnancy. IgG seroconversion and significant increase in IgG antibody amount in pregnancy indicates maternal infection. Presence of toxoplasma immunoglobulin M (IgM), immunoglobulin G (IgG) and low IgG avidity in a single serum sample indicates possible maternal infection, but positive toxoplasma IgM and low IgG avidity may persist for months and even years.[6] The results showed in Table 1 that GRA-1 antigen had significant relation with phase II of toxoplasmosis $P = 0.028$, and these results were in agreement with that reported by (Fatemeh Rezaei et al., 2019) that many studies are focused on using of various excretory secretory antigens (ESA); and among them dense granule antigens (GRAs) being involved in parasite survival, virulence and replication processes.[20]

Also, *T. gondii* dense granule antigen proteins (GRAs) are secretory proteins expressed by both tachyzoite and bradyzoite. GRA1 is secreted into the parasitophorous vacuole (PV), which has Ca^{2+} binding domain, becoming a physiological important factor to invade in the host cells, and GR7 is secreted into the cytoplasm of bradyzoite-infected cells and within the PV and the PV membrane in tachyzoite-infected cells. GRA1-based ELISA shows a sensitivity of about 60%, but the specificity can reach 98% in humans.[21] Table 2 showed that there was no significant relation between the period of infection of toxoplasmosis with different antigens detected in *recom*Line assay and most of antigens were positive in all period of infection of toxoplasmosis, these results in compatible with that reported by (Groβ U et al) that in most cases determining the avidity for individual antigens makes it possible to determine

Table 3. **Correlation between abortion and antigens markers of toxoplasmosis detected by *recom*Line assay**

		Abortions								*P*-value
		–ve		1		2		3		
		N	%	*N*	%	*N*	%	*N*	%	
IgG +ve	–ve	4	25.0%	4	22.2%	0	0.0%	0	0.0%	0.614
	+ve	12	75.0%	14	77.8%	2	100.0%	4	100.0%	
ROP	–ve	12	75.0%	16	88.9%	2	100.0%	4	100.0%	
	weak +ve	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0.470
	+ve	4	25.0%	2	11.1%	0	0.0%	0	0.0%	
MIC	–ve	12	75.0%	14	77.8%	2	100.0%	4	100.0%	
	weak +ve	4	25.0%	2	11.1%	0	0.0%	0	0.0%	0.550
	+ve	0	0.0%	2	11.1%	0	0.0%	0	0.0%	
GRA7	–ve	6	37.5%	10	55.6%	2	100.0%	2	50.0%	
	weak +ve	4	25.0%	2	11.1%	0	0.0%	0	0.0%	0.575
	+ve	6	37.5%	6	33.3%	0	0.0%	2	50.0%	
GRA8	–ve	8	50.0%	14	77.8%	2	100.0%	2	50.0%	
	weak +ve	2	12.5%	4	22.2%	0	0.0%	0	0.0%	0.078
	+ve	6	37.5%	0	0.0%	0	0.0%	2	50.0%	
P30	–ve	2	12.5%	6	33.3%	0	0.0%	0	0.0%	
	weak +ve	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0.255
	+ve	14	87.5%	12	66.7%	2	100.0%	4	100.0%	
MAG1	–ve	6	37.5%	6	33.3%	2	100.0%	0	0.0%	
	weak +ve	4	25.0%	2	11.1%	0	0.0%	0	0.0%	0.142
	+ve	6	37.5%	10	55.6%	0	0.0%	4	100.0%	
GRA1	–ve	8	50.0%	8	44.4%	0	0.0%	0	0.0%	
	weak +ve	4	25.0%	2	11.1%	2	100.0%	0	0.0%	0.012*
	+ve	4	25.0%	8	44.4%	0	0.0%	4	100.0%	
SAG1	–ve	8	50.0%	6	33.3%	0	0.0%	0	0.0%	
	weak +ve	0	0.0%	4	22.2%	2	100.0%	0	0.0%	0.003*
	+ve	8	50.0%	8	44.4%	0	0.0%	4	100.0%	

the infection status more accurately. This is particularly important for distinguishing between an acute and subsiding *Toxoplasma* infection with persistent IgM antibodies. The fact that the IgG antibody reactions against specific antigens typically start in various phases of infection and that these IgG antibodies successively mature from low avidity to high avidity usually makes a differentiated view of the infection status possible by analysing the IgG bands and avidity patterns.[22]

Also the results in Table 3 showed that there was a significant relation of the no. of abortion to GRA-1, $P = 0.012$ and rSAG-1 ($P = 0.003$) where increase in the positive results with the increase in the no. of abortion no other significant relation was observed with other markers $P > 0.05$, these results in agreement that reported by (Nam HW, 2009) that *T. gondii* dense granule antigen proteins (GRAs) are secretory proteins expressed by both tachyzoite and bradyzoite. GRA1 is secreted into the parasitophorous vacuole (PV), which has Ca^{2+} binding domain, becoming a physiological important factor to invade in the host cells. GRA1-based ELISA shows a sensitivity of about 60%, but the specificity can reach 98% in humans.[23]

The results of the present study showed that there were 16 patients had high avidity to P30 Abs and 16 patients had high avidity to MAG-1 Abs and 16 patients had high avidity to GRA-1 Abs and 16 had high avidity to r SAG-1 Abs, these results were in agreement with that reported by Harning D. et al., 1996 that the fact that the IgG antibody reactions against specific antigens typically start in various phases of the infection and that these IgG Abs successively mature from low avidity to high avidity usually makes a differentiated view of the infection status possible by analysing the IgG bands and avidity patterns. Also, the negative results dose not exclude the possibility of a toxoplasma infection. False negative results can occur if the sampling is made before the initial reaction of the immune system.[24]

In addition, where the serum test results are negative for pregnant women a follow up samples should be taken and tested after 8–12 weeks, and it must be into account that treatment may result in delayed IgG and / or IgM antibody formation, thus also influencing the IgG avidity.[25]

There were 10 patients had low avidity to MAG-1 and 12 patients had low avidity to (GRA-1) and 10 patients had low

avidity to r SAG-1. These results in compatible with that reported by Holec–Gasior, 2013 that avidity describes the binding strength of specific antibody to Ag, since it was found to be low in the first phase after primary infection.[26]

Finally, the major advantages of recombinant antigens for the diagnosis of *T.gondii* infections are follows: (i): the precise antigen composition of the test is known, (ii): more than one defined antigen can be used, and (iii) the method can easily be standardized. In addition, selected antigens that are characteristic for acute and chronic stages of the infection could serve as tool to discriminate between the two stages.[27]

Conflict of Interest

None.

References

1. Holec-Gasior L. 2013. *Toxoplasma gondii* Recombinant Antigens as Tools for Serodiagnosis of Human Toxoplasmosis: Current Status of Studies. Clin Vaccine Immunol. 20(9): 1343–1351.
2. Montoya JG. 2002. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. J. Infect. Dis. 185:S73–S82.
3. Suzuki LA, Rocha RJ, Rossi CL. 2001. Evaluation of serological markers for the immunodiagnosis of acute acquired toxoplasmosis. J. Med. Microbiol. 50:62–70.
4. Dunn D, Wallon M, Peyron F, Petersen E, Peckham C, Gilbert R. 1999. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. Lancet 353:1829–1833.
5. Burg JL et al. 1988. Molecular analysis of the gene encoding the major surface antigen of Toxoplasma gondii. J Immunol. 141p. 3584–3581.
6. Findal G, Stray-Pedersen B, Holter EK, Berge T, Jenum PA. 2015. Persistent Low Toxoplasma IgG Avidity Is Common in Pregnancy: Experience from Antenatal Testing in Norway, PLoS One. 10(12): e0145519.
7. Remington JS MR, Wilson CB, Desmonts G. 2011. Toxoplasmosis. In: Remington JS KJ, editor. Infectious diseases of the fetus and newborn infant. 7th ed Philadelphia, PA: Saunders/Elsevier. p. 918–1041.
8. Jenum PA, Stray-Pedersen B, Melby KK, Kapperud G, Whitelaw A, Eskild A, et al. 1998. Incidence of *Toxoplasma gondii* infection in 35,940 pregnant women in Norway and pregnancy outcome for infected women. J Clin Microbiol. 36(10):2900–6.
9. Dunn D, Wallon M, Peyron F, Petersen E, Peckham C, Gilbert R. 1999. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. Lancet. 353(9167):1829–33.
10. Montoya JG, Liesenfeld O. 2004. Toxoplasmosis. Lancet. 363(9425):1965–76.
11. Sensini A, Pascoli S, Marchetti D, Castronari R, Marangi M, Sbaraglia G, et al. 1996. IgG avidity in the serodiagnosis of acute *Toxoplasma gondi*i infection: a multicenter study. Clin Microbiol Infect. 2(1):25–9.
12. Wong SY, Remington JS. 1994. Toxoplasmosis in pregnancy. Clin Infect Dis. 18(6):853–61; quiz 62.
13. Aref Teimouri, Sina Mohtasebi, Elham Kazemirad, Hossein Keshavarz. 2020. Role of *Toxoplasma gondii* IgG Avidity Testing in Discriminating between Acute and Chronic Toxoplasmosis in Pregnancy, J Clin Microbiol. 24;58(9):e00505–20.
14. Hedman K, Lappalainen M, Seppaia I, Makela O. 1989. Recent primary toxoplasma infection indicated by a low avidity of specific IgG. J Infect Dis. 159(4):736–40.
15. Jenum PA, Stray-Pedersen B, Gundersen AG. 1997. Improved diagnosis of primary *Toxoplasma gondii* infection in early pregnancy by determination of antitoxoplasma immunoglobulin G avidity. J Clin Microbiol. 35(8):1972–7.
16. Murat JB, L'Ollivier C, Fricker Hidalgo H, Franck J, Pelloux H, Piarroux R. 2012. Evaluation of the new Elecsys Toxo IgG avidity assay for toxoplasmosis and new insights into the interpretation of avidity results. ClinVaccine Immunol. 19(11):1838–43.
17. Lindsay DS, Dubey JP. 2011. *Toxoplasma gondii*: the changing paradigm of congenital toxoplasmosis. Parasitology 138:1829–1831.
18. Zedong Wang, Wei Ge, Si-Yang Huang, Jiping Li, Xing-Quan Zhu & Quan Liu. 2014. Evaluation of recombinant granule antigens GRA1 and GRA7 for serodiagnosis of *Toxoplasma gondii* infection in dogs, BMC Veterinary Research volume 10, Article number: 158.
19. Beghetto E.et al. 2003. Use of an immunoglobulin G avidity assay based on recombinant antigens for diagnosis of primary Toxoplasma gondii infection during pregnancy. J. Clin. Microbiol. 41 p.5414–5418.
20. Fatemeh Rezaei, Mahdi Sharif, Shahabeddin Sarvi, Seyed Hossein Hejazi, Sargis Aghayan, Abdol Sattar Pagheh, Samira Dodangeh, Ahmad Daryani. 2019. A systematic review on the role of GRA proteins of *Toxoplasma gondii* in host immunization, Journal of Microbiological Methods, Volume 165, 105696.
21. Zhou P, Chen Z, Li HL, Zheng H, He S, Lin RQ, Zhu XQ. 2011: *Toxoplasma gondii* infection in humans in China. Parasit Vectors. 4: 165.
22. Groβ U et al. 1992. Improved Serological Diagnosis of *Toxoplasma gondii* Infection by Detection of Immunoglobulin A(IgA) and IgM Antibodies against P30 Using the Immunoblot Technique. J. Clin. Microbiol. p.1436–1441.
23. Ho-Woo Nam: GRA Proteins of *Toxoplasma gondii*. 2009. Maintenance of Host-Parasite Interactions across the Parasitophorous Vacuolar Membran; Korean J Parasitol. 47(Suppl): S29–S37.
24. Harning D. et al. 1996. Recombinant *Toxoplasma gondii* surface antigen 1(P30) expressed in Escherichia coli is recognized by human Toxoplasma specific immunoglobulin M (IgM) and IgG antibodies. Clin. Diagn. Lab. Immunol., 3: p.355–357).
25. Carvalho FR.et al. 2008. Reverse Enzyme–Linked Immunosorbent Assay Using Monoclonal Antibodies against SAG1-Related Sequence, SAG2 and P97 Antigens from *Toxoplasma Gondii* To Detect Specific Imuunoglobulin G(IgG), IgM and IgA Antibodies in Human Sera, Clin. Vac. Immunol. p.1265–1271).
26. Holec–Gasior L. 2013. *Toxoplasma gondii* Recombinant Antigens as Tool for Serodiagnosis of Human Toxoplasmosis: Current Status of Stadies, Clin. And Vac. Immunol., 20p.1343–135.
27. Holec–Gasior L, Ferra B, Drapala D. 2012. MIC1-MAG1-SAG1 chimeric protein. A most effective antigen for detection of human toxoplasmosis. Clin. Vaccine Immunol. 19:1977–1979.