Detection of Toxoplasma Gondii IgG Antibody in Patients Infected with Toxoplasma by ELISA

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ABSTRACT
Aim the current study to diagnosis of the IgG antibody to Toxolasma gondii, serum samples isolated from patients humans and healthy reviewers to AL-Diwaniyah hospital and different laboratories.
90 samples were collected from human infected with Toxoplasmosis including 60 samples of patients and 30 samples of healthy (control).
The results of this study showed that concentration of the IgG antibody in patients infected with T.gondii were 47.43 while the concentration in control were 8.14 and the percentage of infection in patients (24/60) (40%) and in control (4/30) (13.33%).
The results showed that IgG antibody found in both patients and control but its concentration in patients was higher this indicates a high concentration of IgG antibody with toxoplasmosis.

1. Introduction

Toxoplasma gondii is one of the parasitic protozoa which results from a zoonotic disease known as toxoplasmosis [1]. Toxoplasmosis is usually a symptomatic and spread worldwide [2].

This disease is transmissible by eating infected animals that are not cooked well, food or water contaminated with cat stool also through transmission from mother to fetus [3,4]. Contamination of water, soil, fruits and vegetables with oocysts play an important role in infecting humans and animals with toxoplasmosis [5,6]. In immunocompetent individuals, the infection is an self-limiting illness and asymptomatic [7]. The infection can be severe in two status, pregnancy where cause congenital toxoplasmosis and cell- mediated immunodeficiency like transplantation, infection with AIDS and malignant diseases those receiving immunosuppressive therapy [4] . In both definitive and intermediate hosts, the infection can be occur through the consumption of uncooked meat of intermediate hosts contain tissue cysts and through the congenital transmission of tachyzoites (8,9). The Congenital toxoplasmosis can cause several disturbances in the fetus, like abortion, chorioretintis, cerebral calcifications, hydrocephalus, microcephaly and lymphadenopathy and cause mortality in immune compromised individuals [5,10,11].

It is difficult to isolate this protozoan, since the diagnosis is generally based on serological tests [7,12,13]. The serological detection of parasite specific antibodies is used as common method to identify this disease in animals and humans [14,15]. IgG antibodies to T.gondii continue for several years and can used to the diagnose of previous exposure to this parasite as reliable serological biomarker [16,17,18].

ELISA (Enzyme Linked Immunosorbent Assay) is one of serological methods that used to detecting T.gondii specific antibodies in animals and humans [14,19].

This method is most used for diagnose exposure of this parasite, and more reliable, practical and economical of all the techniques used [20,21].

2. Materials and Methods

Samples collections

90 serum samples were collection from patients with toxoplasma gondii and healthy control individual from Al-Diwanyiah Hospital, and done according to company instructions (Toxoplasma IgG EIA Test Kit, foresight, ACON Laboratories, Inc. USA) as following steps:

1. Add Sample: 100µL of calibrator standards sets were added to standards wells, 100µL specimen dilute were added per samples wells and blank well. Then 5µL serum sample were added to each sample wells. After that, the solutions are mixed gently, the plate is covered with stopper and then incubated at 37°C for 30 minutes.

2. Wash: all wells of the plates were aspirated, washed, and this process was repeated three times. The process of washing was done by filling each well with wash buffer (almost 350µL) with a spray bottle.

4. HRP Conjugate: added 100µL of HRP Conjugate working solution to each well. Then, covered with the plate stopper and incubated at 37°C for 30 minutes.

5. Wash: repeated the washing process 5 times as in step three.

6. Substrate: added 50µL of substrate A Solution to each well. Then, covered with a new Plate stopper, and incubated at 37°C for 15 minutes.

7. Stop: added 50µLof Stop Solution to each well. the color turned immediately yellow.

8. OD Measurement: to detect (OD value) the optical density of each well immediately used a micro-plate reader set at 450 nm.

3. Results

Among 60 patients with toxoplasmosis had high concentration of IgG antibody to T. gondii were (47.43) and in control concentration of IgG antibody in 30 samples were less (8.14). the statically analysis showed that there were a significant differences in both control and patients human infected with toxoplasmosis as a table (1):

Table (1). concentration of IgG antibody to T.gndii in both control and patients human

Groups	IgG concentration
Control	8.14 ± 11.28^{A}
patients	47.43 ± 8.71^{B}
T test	2.675
P value	0.009

Also the results of present study showed the infection percentage in patients infected with toxoplasmosis were 40% while in control were 13.33% as table (2):

Table (2). the percentage of IgG antibody to T.gondii in both control and patients human

Groups	Total number	Positive N.	%
Control	30	4	13.33
patients	60	24	40
X^2	9.905		
P value	0.002		

The ELISA qualification results were calculated according to (Index value: specimen /cut off value) and quantification results depend on a standard curve plot (OD values as X axis) and (calibrator standards concentrations sets as Y value) . as in the curve:

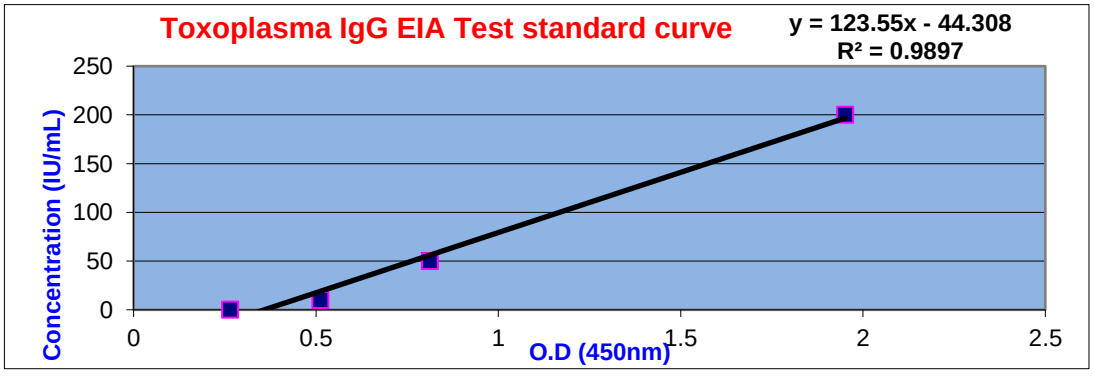

Figure (1). Toxoplasma IgG ELISA Test standard curve

4. Discussion

toxoplasmosis infection are spread around the world at different level 15-77% depending on the transmission resources and expose to risk factor [22-23]. The immune assay used for detecting antibodies to pathogenic organisms have been shown to be particularly sensitive in identifying toxoplasmosis infection [24]. (IgG) Immunoglobulin G is used to identify Toxoplasmosis infection, though can persist IgG antibodies for lifetime in immune competent toxoplasma people [25] .

This study showed the concentration of IgG antibody to T.gondii in patients were 47.43 while the concentration in control were 8.14 and the percentage of infection in patients (24/60) (40%) and in control (4/30) (13.33%).

[26] refer to IgG avidity ELISA and IgG ELISA tests can confirm the stages of toxoplasmosis and refer to that toxoplasma specific avidity test performed on specific IgM positive samples can diagnose both chronic and acute stages. (4) suggested measurement of IgG avidity can distinguish between chronic and acute stages of toxoplasmosis.

[27] in a study of seroprevalence in Jahrom, Iran proved a higher prevalence of toxoplasma IgG antibody was demonstrated in women with frequent miscarriage compared to control group. [28] Toxoplasma IgG and IgM antibodies have been demonstrated in women with still birth or history miscarriage in Sari.

[29] in Bandar Abbas, South Iran in women with a history of miscarriage in patients with proven presence of Toxoplasma IgG and IgM antibodies, the prevalence of IgG antibody may increase within 2-4 weeks after infection and rise gradually for several weeks and then remain at a low concentration for a short time, while repeatedly exposed to infection. Infection may cause high levels of antibody for a long time.

[30] demonstrated the frequency of Toxoplasma antibodies among women with miscarriage and normal pregnancy was 44.1%, 45.5% respectively for IgG and 21%, 46.5% for IgG. [31] demonstrated the prevalence of IgM and IgG antibodies in women with uncomplicated miscarriage is 33.3%, 44.9% respectively.

[32] in Pakistan found in 100 serum samples from pregnant women tested through latex agglutination test and ELISA that prevalence rate were 12% , 23% respectively and are refer to seroprevalence detection of toxoplasmosis in humans varies greatly between geographical areas, different countries and different group living in the same region of the world.

5. Conclusion

We conclude from this study IgG antibody found in both patients and control but its concentration in patients was higher this indicates a high concentration of IgG antibody with toxoplasmosis .

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Reference

[1] Slana I, Bier N, Bartosova B, Marucci G, Possenti A, Mayer-Scholl A, Jokelainen P, Lalle M (2021) Molecular Methods for the Detection of Toxoplasma gondii Oocysts in Fresh Produce: An Extensive Review. Microorganisms 9(1):167. https:// doi. org/ 10. 3390/ micro organ isms9 010167

[2] Leite M, Siciliano S, Rocha LS, Justa MT, César KR, Granato CF. Correlation between specific IgM levels and percentage IgG-class antibody avidity to Toxoplasma gondii. Rev Inst Med Trop Sao Paulo 2008; 50: 237-242.

[3] Iqbal J, Khalid N. Detection of acute Toxoplasma gondii infection in early pregnancy by IgG avidity and PCR analysis. J Med Mi¬crobiol 2007; 56(Pt 11): 1495-1499.

[4] Candolfi E, Pastor R, Huber R, Filisetti D, Villard O. IgG avidity assay firms up the diagnosis of acute toxoplasmosis on the first serum sample in immunocompetent pregnant women. Diagn Microbiol Infect Dis 2007; 58: 83-88.

[5] Matsuo J, Kimura D, Rai SK, Uga S (2004) Detection of Toxoplasma oocysts from Soil by Modified Sucrose Flotation and PCR Methods. Southeast Asian J Trop Med Public Health 35:270–274 (PMID: 15691123).

[6] Waap H, Cardoso R, Leitao A, Nunes T, Vilares A, Gargate MJ, Meireles J, Cortes H, Angelo H (2012) In Vitro Isolation and Seroprevalence of Toxoplasma gondii in Stray Cats and Pigeons.

[7] Lisbon. Portugal Vet Parasitol 187(3–4):542–547. https:// doi. org/10. 1016/j. vetpar. 2012. 01. 022

[8] Crucerescu E, Lovin DR. Study on specific IgG avidity as a tool for recent primary Toxoplasma gondii infection diagnosis. J PrevMed 2002; 10: 56-62.

[9] Amany MA, Merwad AM (2012) Epidemiology and Molecular Detection of Zoonotic Toxoplasma gondii in Cat Feces an Seroprevalence of Anti-Toxoplasma gondii antibodies in pregnant women and sheep. Life Sci J 9:133–146

[10] Rohmah, M. K., Salahdin, O. D., Gupta, R., Muzammil, K., Qasim, M. T., Al-Qaim, Z. H., ... & Abarghouei, S. (2022). Modulatory role of dietary curcumin and resveratrol on growth performance, serum immunity responses, mucus enzymes activity, antioxidant capacity and serum and mucus biochemicals in the common carp, Cyprinus carpio exposed to abamectin. Fish & Shellfish Immunology, 129, 221-230.

[11] Margiana, R., Alsaikhan, F., Al-Awsi, G. R. L., Patra, I., Sivaraman, R., Fadhil, A. A., ... & Hosseini-Fard, S. (2022). Functions and therapeutic interventions of non-coding RNAs associated with TLR signaling pathway in atherosclerosis. Cellular Signalling, 100, 110471.

[12] Arif, A., Alameri, A. A., Tariq, U. B., Ansari, S. A., Sakr, H. I., Qasim, M. T., ... & Karampoor, S. (2023). The functions and molecular mechanisms of Tribbles homolog 3 (TRIB3) implicated in the pathophysiology of cancer. International Immunopharmacology, 114, 109581.

[13] Lei, Z., Alwan, M., Alamir, H. T. A., Alkaaby, H. H. C., Farhan, S. S., Awadh, S. A., ... & Nekuei, A. (2022). Detection of abemaciclib, an anti-breast cancer agent, using a new electrochemical DNA biosensor. Frontiers in Chemistry, 10, 980162.

[14] Bashar, B. S., Kareem, H. A., Hasan, Y. M., Ahmad, N., Alshehri, A. M., Al-Majdi, K., ... & Qasim, M. T. (2022). Application of novel Fe3O4/Zn-metal organic framework magnetic nanostructures as an antimicrobial agent and magnetic nanocatalyst in the synthesis of heterocyclic compounds. Frontiers in Chemistry, 10, 1014731.

[15] M Abbas, M., W Abooud, K., Qasim Mohammed, A., Hasan Al-Zubaidi, S., Hussain, A., M Hameed, N., ... & Ahmad Batayneh, K. (2022). Effects of various irrigation levels and biochar-based fertilizers on peanut production. Journal of Nuts, 13(4), 289-300.

[16] Hussein, H. A., Khudair, S. A., Alwan, M., Aljawahiry, T., T Qasim, M., & V Pavlova, I. (2022). Impact of pollution caused by salmon breeding centers on river water quality. Caspian Journal of Environmental Sciences, 20(5), 1039-1045.

[17] Lafta, H. A., AbdulHussein, A. H., Al-Shalah, S. A., Alnassar, Y. S., Mohammed, N. M., Akram, S. M., ... & Najafi, M. (2023). Tumor-Associated Macrophages (TAMs) in Cancer Resistance; Modula-tion by Natural Products. Current topics in medicinal chemistry.

[18] Al-Jassani, M. J., Sayah, M. A., Qasim, M. T., Kadhim, A. J., & Muhammad, E. H. (2022). Isolation and Evaluation of Antibacterial Agents Produced by Soil Bacillus SP. and Study Some of their Immunological Parameters. Revista Electronica de Veterinaria, 23(4), 105-111.

[19] Sane, S., Mahoori, A., Abdulabbas, H. S., Alshahrani, S. H., Qasim, M. T., Abosaooda, M., ... & Darvishzadehdaledari, S. (2023). Investigating the effect of pregabalin on postoperative pain in non-emergency craniotomy. Clinical Neurology and Neurosurgery, 226, 107599.

[20] Al Anazi, A. A., Barboza-Arenas, L. A., Romero-Parra, R. M., Sivaraman, R., Qasim, M. T., Al-Khafaji, S. H., ... & Gono, R. (2023). Investigation and Evaluation of the Hybrid System of Energy Storage for Renewable Energies. Energies, 16(5), NA-NA.

[21] Hjazi, A., Nasir, F., Noor, R., Alsalamy, A., Zabibah, R. S., Romero-Parra, R. M., ... & Akram, S. V. (2023). The pathological role of CXC chemokine receptor type 4 (CXCR4) in colorectal cancer (CRC) progression; special focus on molecular mechanisms and possible therapeutics. Pathology-Research and Practice, 154616.

[22] Althomali, R. H., Al-Hawary, S. I. S., Gehlot, A., Qasim, M. T., Abdullaeva, B., Sapaev, I. B., ... & Alsalamy, A. (2023). A novel Pt-free counter electrode based on MoSe2 for cost effective dye-sensitized solar cells (DSSCs): Effect of Ni doping. Journal of Physics and Chemistry of Solids, 182, 111597.

[23] Hjazi, A., Ahsan, M., Alghamdi, M. I., Kareem, A. K., Al-Saidi, D. N., Qasim, M. T., ... & Mirzaei, R. (2023). Unraveling the Impact of 27-Hydroxycholesterol in Autoimmune Diseases: Exploring Promising Therapeutic Approaches. Pathology-Research and Practice, 154737.

[24] Gupta, J., Suliman, M., Ali, R., Margiana, R., Hjazi, A., Alsaab, H. O., ... & Ahmed, M. (2023). Double-edged sword role of miRNA-633 and miRNA-181 in human cancers. Pathology-Research and Practice, 154701.

[25] Al-dolaimy, F., Kzar, M.H., Hussein, S.A. et al. (2023). Incorporating of Cobalt into UiO-67 Metal–Organic Framework for Catalysis CO2 Transformations: An Efficient Bi-functional Approach for CO2 Insertion and Photocatalytic Reduction. J Inorg Organomet Polym. https://doi.org/10.1007/s10904-023-02860-0

[26] Al-Hawary, S. I. S., Kadhum, W. R., Saleh, E. A. M., Yacin, Y., Abdullah, E. A., Qasim, M. T., ... & Alsalamy, A. (2023). Tunneling induced swapping of orbital angular momentum in a quantum dot molecule. Laser Physics, 33(9), 096001.

[27] Gaffar Sarwar Zaman, Ibrahem Waleed, Ruaa Ali Obeid, Shaymaa Abdulhameed Khudair, Saafa Abaas Abd Al-Kahdum, Kadhum Al-Majdi, Ahmed S. Abed, Ali Alsalamy, Maytham T. Qasim, Ahmed Hussien Radie Alawadi. (2023). Electrochemical determination of zearalenone in agricultural food samples using a flower like nanocomposite-modified electrode, Materials Chemistry and Physics,Volume 305,127986. ISSN 0254-0584, https://doi.org/10.1016/j.matchemphys.2023.127986.

[28] M. Sharif and A. Ajami, “Serological survey of toxoplasmosis in women with abortion or still birth referring to women clinic in Sari, 1997-1998,” Journal of MazandaranUniversity of Medical Science, vol. 26, pp. 13–18, 1999.

[29] A. S. Jahromi, “Anti-toxoplasma antibodies in women with abortion or still birth,” Journal of Jahrom University of Medical Science, vol. 4, pp. 47–52, 2007.

[30] M. Saeedi, S. B. Nosrat, A. Moradi, S. M. H. Mofidi, and N. Behnampoor, “Comparative study of Cytomegalovirus, Listeria monocytogen and Toxoplasma gondii infections in successful and non-successful pregnancy in Gorgan,” Medical Laboratory Journal, vol. 3, no. 1, pp. 25–30, 2009 (Persian).

[31] B. Bobic, D. Sibalic, and O. Djurkovic-Djakovic, “High levels of IgM antibodies specific for Toxoplasma gondii in pregnancy 12 years after primary toxoplasma infection. Case report,” Gynecologic and Obstetric Investigation, vol. 31, no. 3, pp. 182–184, 1991.

[32] Mabood, H., Shams, S., Ayaz, S., Rasool, I., Ali, S., & Hamayun, M. (2022). Microscopic and ELISA Based Detection of Toxoplasmosis and its Associated Risk Factors in Pregnant Women of District Mardan. Pakistan. Biosciences Biotechnology Research Asia, 15(3), 635-643.