



The Role of GRA7 Protein as a Diagnostic
Indicator for Toxoplasmosis in Breast Cancer
Women and Thalassemia Patients

Haider Rehman and Zainab Sulaiman

EasyChair preprints are intended for rapid
dissemination of research results and are
integrated with the rest of EasyChair.

March 31, 2025

The Role of GRA7 Protein as a Diagnostic Indicator for Toxoplasmosis in breast cancer women and thalassemia patients

Abstract

Background: Toxoplasmosis is a significant global illness that represents a life-threatening risk to immunocompromised individuals.

Objectives: Estimation of Recombinant dense granular protein(GRA7) in serum of breast cancer and thalassemia patients.

Materials and methods: This study was conducted on 50 women with breast cancer and 50 patients with thalassemia who attended the Oncology and Haematology center, and 50 sample collected from healthy people as a control group . Informed consent was obtained from the University of Tikrit/College of medicine and from the Ministry of Health (approval number 22187 on 19/5/2024).

Results

Fifty blood samples were obtained from women diagnosed with breast cancer, and an additional fifty samples were gathered from thalassemia patients admitted to the Oncology and Hematology center, and fifty blood samples were obtained from healthy persons to serve as a control group. The blood samples were properly handled and analyzed for *Toxoplasma gondii* GRA7, IgM, and IgG using the enzyme-linked immunosorbent assay (ELISA) technique.

Conclusion: toxoplasmosis is a disease commonly associated with cases of immune suppression, such as in individuals with cancer or thalassemia. Additionally, the GR7 protein has been identified as a biomarker useful for detecting the acute stage of toxoplasmosis.

Keywords: GRA7, *T.gondii*, ELISA, breast cancer, toxoplasmosis

Introduction

Toxoplasma gondii is among the most prevalent parasites in people globally, with serological investigations revealing that over one-third of the world's population is infected⁽¹⁾. Humans can acquire *T. gondii* infection by ingesting undercooked meat from intermediate hosts, such as pork and beef, which contains tissue cysts, or by consuming food or water contaminated with oocysts from definitive hosts, as well as through blood transfusion⁽²⁾. Infection in immunocompromised individuals is often asymptomatic or presents with mild, self-limiting symptoms; nevertheless, the

parasite may remain dormant for years and reactivate when the immune system is impaired⁽³⁾. Resulting additionally in an active disease but also in the potential for hematogenous transmission⁽⁴⁾. *Toxoplasma gondii* has a complex life cycle, including three main different stages, namely tachyzoites (trophozoite), tissue cyst (bradyzoites) and oocysts⁽⁵⁾. Domestic and wild cats are essential in the transmission of toxoplasmosis, as they are the unique animals capable of excreting millions of environmentally resilient oocysts in their feces⁽⁶⁾. Consequently, it is logical to believe that cat owners possess a greater concentration of anti-toxoplasma antibodies compared to generally healthy individuals of the same age. Nevertheless, research assessments suggest that cat ownership is not consistently a significant risk factor for acquiring this parasite illness⁽⁷⁾. Immunocompromised persons, particularly those with cellular immunity deficiencies such as cancer patients, are most susceptible to infections⁽⁸⁾. The dense granule antigen 7 of *T. gondii* (TgGRA7) is an important component of the parasitophorous vacuole (PV) and the PV membrane surrounding the tachyzoites, as well as the cyst wall of the bradyzoites⁽⁹⁾. The accuracy of TgGRA7 as a serodiagnostic marker for *T. gondii* infection has been validated by indirect ELISA (IELISA)⁽¹⁰⁾. The aim of this study was to evaluate Recombinant dense granular protein(GRA7) and *Toxoplasma gondii* IgM and IgG in serum of breast cancer women and thalassemia patients.

Materials and Methods

Fifty blood samples were obtained from women with breast cancer diagnosed admitted to the Oncology Center, while an additional fifty samples were acquired from thalassemia patients in the Hematology center. The blood samples were correctly stored and analyzed for *Toxoplasma gondii* GRA7, IgM, and IgG, Briefly 3ml of venous blood was withdrawn from each patient using sterile syringes and was evacuated in a plain tube. Then blood samples was centrifuged at 4000 rpm for five minute to collect serum which was examined using Enzyme-linked immunosorbent assay (ELISA) technique, the samples were placed in a eppendorf tube and marked by number with all profile patient and stored at (-20 °C). IgM and IgG antibodies against *Toxoplasma* have been determined and diagnosed by the ELISA apparatus, according the protocol specified in the kit. While the recombinant dense granular

protein (GRA7) was identified by using the sandwich ELISA technique according to international and local researches⁽¹¹⁾.

Statistical analysis

Statistical analysis was conducted utilizing SPSS version 23 (SPSS, IBM Corporation, Chicago, USA). Categorical variables were given as frequencies and percentages, and a correlation test was employed. Quantitative data were presented as mean \pm standard deviation, along by their 95% confidence range. A NOVA was employed to compare the means of variable values, with a P value of <0.05 being statistically significant.

Results

Figure (1) show the seropositivity of diagnosed breast cancer women with *T. gondii*, it was found that 14 out of 50 (28%) tested positive for (GRA7, IgG, and IgM); 10 out of 50 (20%) were positive for GRA7 (Recombinant dense granular protein); 10 out of 50 (20%) were positive for IgM; and 2 out of 50 (4%) was positive for both IgM and GRA7 by ELISA technique.

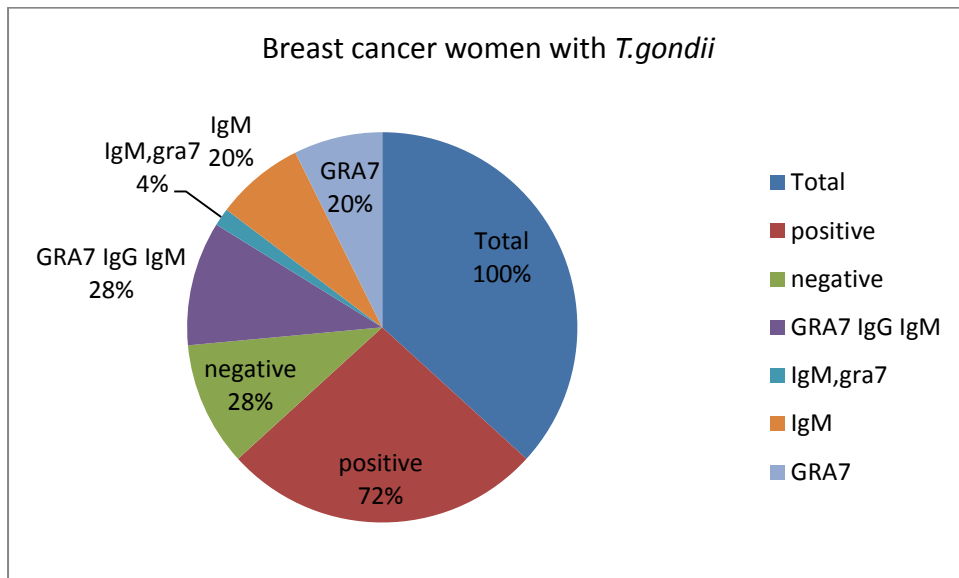
Figure(2) shows the percentage of breast cancer women with toxoplasmosis according to chemotherapy the highest percentage of infection with toxoplasmosis was 22 patients (61.1%) among 36 breast cancer women infected with *T.gondii* under chemotherapy, while 14 patients (38.9%) among 50 breast cancer women infected with *T.gondii* after chemotherapy.

Figure(3) shows the percentage of toxoplasmosis infection among 36 breast cancer women infected with *T.gondii* was 34 patients (94.4%) in the urban area, while 2 patient (5.6%) was infected with *T.gondii* in the rural area. This indicates that the percentage of breast cancer women with toxoplasmosis varied by residence.

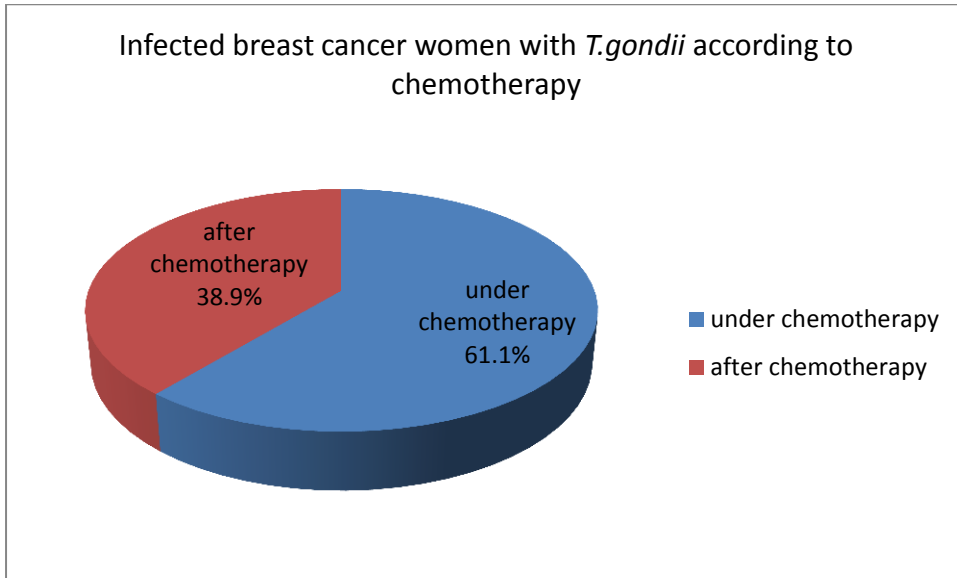
Figure (4) displays the seropositivity of diagnosed thalassemia patients to *T.gondii*, revealing that 2 out of 50 (4%) tested positive for (GRA7, IgG, and IgM); 8 out of 50 (16%) were positive for GRA7 (Recombinant dense granular protein); 6 out of 50 (12%) were positive for IgM by ELISA technique.

Figure (5) show the distribution of *T.gondii* among thalassemia patients according to breeding domestic animals the highest percentage of infection with toxoplasmosis was 12 patients (75%) among 16 thalassemia patients infected with

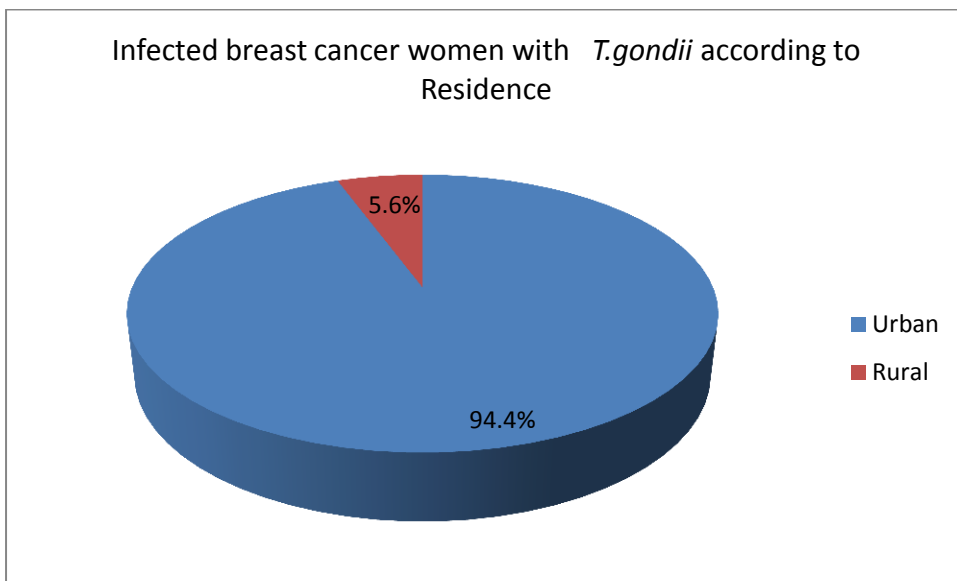
T.gondii was among pet owners, while the lowest percentage was 4 patients (25%) among non-pet owners.



Figure(1): Distribution of *T.gondii* among breast cancer women



Figure(2): shows the percentage of breast cancer women with toxoplasmosis according to chemotherapy



Figure(3): shows the percentage of breast cancer women with toxoplasmosis according to Residence

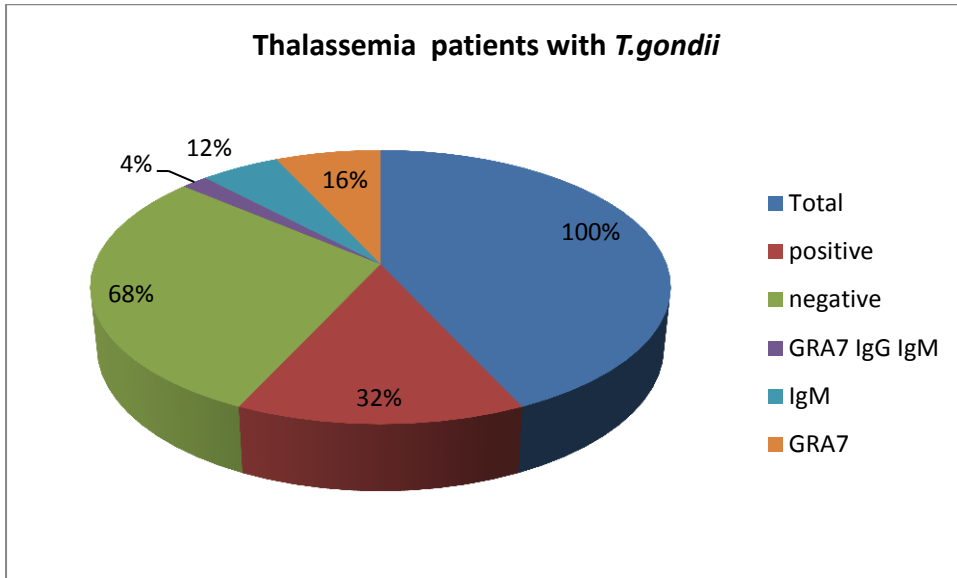


Figure (4) : Distribution of *T.gondii* among thalassemia patients

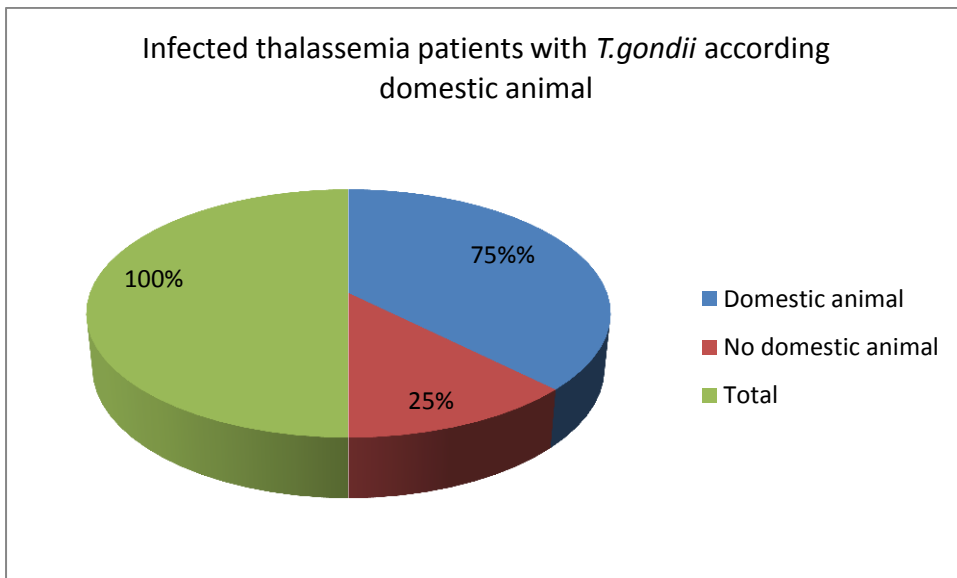


Figure (5) show the distribution of *T.gondii* among thalassemia according to domestic animals

Discussion

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects nearly all mammals, including humans. Although most infections are clinically asymptomatic, they may result in severe clinical manifestations and potentially death

in immunocompromised patients⁽¹²⁾ . It is a big disaster that the cancer patients were seropositive for *T. gondii* test. Cancer may promote serious opportunistic parasitic infections during anticancer treatment⁽¹³⁾ . The immune system plays an essential role in regulating and eliminating parasite diseases⁽¹⁴⁾ .

The results of this study in **Figure(1)** showed that it was found that (28%) tested positive for (GRA7, IgG, and IgM); (20%) were positive for GRA7 (Recombinant dense granular protein); (20%) were positive for IgM; and (4%) was positive for both IgM and GRA7 by ELISA technique, compared to healthy people that show no *T.gondii* infection present with a significant difference between them ($p < 0.05$). TgGRA7 is prevalent on the surface of host cells, within the lumen and membrane of the parasitophorous vacuole, and in the host cell cytoplasm. It elicits an adequate antibody response during the acute and early stages of infection⁽¹⁵⁾ . The findings of our investigation indicate that the prevalence of toxoplasmosis was 20% as determined by GRA7 , 20% by IgM and 28% by all(GRA7,IgM,IgG). This our results indicates an acute infection in women with breast cancer; it agrees with the findings of other studies⁽¹⁶⁾ . also TgGRA7 considered a good diagnostic biomarker for *T.gondii* in many global studies by sandwich ELIAS⁽¹⁷⁾ . Additionally, GRA7 serves as an effective ELISA-based serodiagnostic marker for human toxoplasmosis, with a specificity of 98–100% and a sensitivity of 81–98.9%^(18,19) . Also our study recorded infection by *T.gondii* among breast cancer women according to laboratory tests made by ELISA technique , as a result of impaired immune response following immunosuppressive therapy ,lead to increase chance of toxoplasmosis ,so the results of our study agree with a previous studies^(20,21,22) . The results of our study revealed that the breast cancer women consider dangerous weakness of the immune system so must be away from source of *T.gondii* infection .

Data recognized in **Figure(2)** show that, the frequency of *T. gondii* was higher in under chemotherapy patients was (61.1%) than patients after chemotherapy was (38.9%), so this mean weakness immune systems in patients with cancer under chemotherapy , which may facilitate the reactivation of previous chronic toxoplasmosis or even increase the potential risk of acquiring new infections⁽²³⁾ . The results our current study are similar to other studies that conducted by several researchers^(24,25,26) .

According to the laboratory investigation of our results in **Figure(3)** show higher prevalence of toxoplasmosis in urban area than rural area , This may be due to the small sample size of our sample; this is a possibility. Another possibility is that there might be occult infection in rural areas that are misdiagnosed, or perhaps the cultural level plays a role in the interest, as urban people are more aware, so our study are similar with the study conducted in Baghdad city⁽²⁷⁾.But ,also there are several studies which disagreement with previous studies, demonstrated the presence of domestic animals and the contamination of water sources and vegetables with the infectious stage of the parasite, which is influenced by the presence of animals in rural areas, have shown that infection is more prevalent than in urban areas for a variety of reasons. ^(28,29) .

Our results in Figure (4) show the seropositivity of thalassemia patients with *T.gondii* by ELISA technique for Rcombinant dense granular protein (GRA7) and IgG,IgM . Serodiagnosis of toxoplasmosis using GRA7 considered golden test to detect *T.gondii* especially in early stage other studies were similar to our study and also in chronic infection ⁽³⁰⁾ . Determination of toxoplasmosis in thalassemia patients in our study using IgM and IgG ELISA techniques, are identical to other studies that were conducted by a variety of researchers^(31,32) . It has been demonstrated that blood transfusion significantly contributes to the transmission of toxoplasmosis in thalassemia patients; hence, screening for toxoplasmosis in blood donors is essential⁽³³⁾ .

data documented in Figure (5)show the prevalence of toxoplasmosis among thalassemia patients were among pet owner these our results are identical to other studies that conducted by several researchers^(34,35) .But other study were in contrast with our study^(36,37,38) .

The primary source of toxoplasmosis infection is domestic animals, which are the principal vectors of disease transmission by direct contact with infected hosts. This

transmits via the discharge of contaminated feces, which can contaminate water and food in its infectious state⁽³⁹⁾.

CONCLUSION

The Recombinant dense granular protein considered as gold test for diagnosis of *T.gondii* more accurate than IgM and IgG antibodies because appear in the first time of infection .

References

1. Dardona Z, Amane M, Boussaa S. Toxoplasmosis related psychological, behavioral, neurological, and hormonal changes: a literature review. Eur J Med Health Sci. 2023;5(5): 128-44.
2. Adem D, Ame M. Toxoplasmosis and its significance in public health: a review. Journal of Biomedical and Biological Sciences. 2023;2(1):1-20.
3. Abd Ellah OH, Mobarak AH, Wahman MM, Abd El Azeem E, Elkady AM. Seroprevalence of Toxoplasma Gondii among Cancer patient in Qena Governate. SVU-International Journal of Medical Sciences. 2024;7(1):245-50.
4. Roller S, Angulo-Tisoc JM, Pacheco JI, Jimenez J, Vargas-Calla A, Morales-Cauti SM, Lopez-Urbina MT, Gonzalez AE, Gomez-Puerta LA. Molecular detection of Toxoplasma gondii in domestic and wild guinea pigs (*Cavia spp.*) from the Marangani

- district in Cuzco, Peru. *Veterinary Parasitology: Regional Studies and Reports*. 2024;52:101038.
5. Zhu Y, Yang X, Chen M, Hu Y, Chang Y, Wu X. Research Progress on the Association between Schizophrenia and *Toxoplasma gondii* Infection. *Biomedical and Environmental Sciences*. 2024;37(6):647-660.
 6. Jaiswal AK, Kumar P, Agrawal V, Singh A, Singh SK. Parasites of the musculoskeletal system. In *Organ-Specific Parasitic Diseases of Dogs and Cats 2023* Jan 1 (pp. 265-295). Academic Press.
 7. Wei HX, He C, Yang PL, Lindsay DS, Peng HJ. Relationship between cat contact and infection by *Toxoplasma gondii* in humans: a meta-analysis. *Comparative Parasitology*. 2016;83(1):11-9.
 8. Khan IA, Ouellette C, Chen K, Moretto M. *Toxoplasma*: immunity and pathogenesis. *Current clinical microbiology reports*. 2019;6:44-50.
 9. Nadipuram SM, Kim EW, Vashisht AA, Lin AH, Bell HN, Coppens I, Wohlschlegel JA, Bradley PJ. In vivo biotinylation of the *Toxoplasma* parasitophorous vacuole reveals novel dense granule proteins important for parasite growth and pathogenesis. *MBio*. 2016;7(4):10-128.
 10. Dupadahalli K, Yunus MH, Othman N, Osman S. Recombinant proteins from new constructs of SAG1 and GRA7 sequences and their usefulness to detect acute toxoplasmosis. *Tropical biomedicine*. 2012;29(1):129-37.
 11. Al-Jumaily EF, Mahdi AA, Jumma IM. Study the purified cell wall mannoproteins *Candida albicans* CA18 as immunomodulators on vaccination of mice. *Asian Journal of Pharmaceutical Science & Technology* .2015;5(3):118-122
 12. Daher D, Shaghilil A, Sobh E, Hamie M, Hassan ME, Moumneh MB, Itani S, El Hajj R, Tawk L, El Sabban M, El Hajj H. Comprehensive overview of *Toxoplasma gondii*-induced and associated diseases. *Pathogens*. 2021;10(11):1351.
 13. Ding H, Wu S, Jin Z, Zheng B, Hu Y, He K, Lu S, Zhuo X. Anti-Tumor effect of parasitic protozoans. *Bioengineering*. 2022;9(8):395.

14. Rossi M, Fasel N. How to master the host immune system? *Leishmania* parasites have the solutions!. *International immunology*. 2018;30(3):103-11.
15. Abaza S. Invasion and egress cascade in intracellular protozoa: Part 2 (*T. gondii*). *Parasitologists United Journal*. 2021;14(2):107-11.
16. Arab-Mazar Z, Fallahi S, Koochaki A, Haghighi A, Tabaei SJ. Immunodiagnosis and molecular validation of *Toxoplasma gondii*-recombinant dense granular (GRA) 7 protein for the detection of toxoplasmosis in patients with cancer. *Microbiological research*. 2016;183:53-9.
17. Ybañez RH, Kyan H, Nishikawa Y. Detection of antibodies against *Toxoplasma gondii* in cats using an immunochromatographic test based on GRA7 antigen. *Journal of Veterinary Medical Science*. 2020;82(4):441-5.
18. Ybañez RH, Nishikawa Y. Comparative Performance of Recombinant GRA6, GRA7, and GRA14 for the Serodetection of *T. gondii* Infection and Analysis of IgG Subclasses in Human Sera from the Philippines. *Pathogens*. 2022;11(2):277.
- 19.. Kotresha D, Poonam D, Muhammad Hafiznur Y, "Saadatnia G" , Nurulhasanah O, Sabariah O, et al. Recombinant proteins from new constructs of SAG1 and GRA7 sequences and their usefulness to detect acute toxoplasmosis. *Trop Biomed*. 2012; 29:129–37.
20. Kalantari N, Ghaffari S, Bayani M, Elmi MM, Moslemi D, Nikbakhsh N, Ghavipankeh F. Preliminary study on association between toxoplasmosis and breast cancer in Iran. *Asian Pacific Journal of Tropical Biomedicine*. 2015;5(1):44-7.
21. Haghbin M, Maani S, Bagherzadeh MA, Bazmjoo A, Shakeri H, Taghipour A, Falahi S, Kenarkoobi A, Badri M, Abdoli A. Latent Toxoplasmosis among Breast Cancer Patients in Jahrom, South of Iran. *International Journal of Breast Cancer*. 2023;2023(1):4792260.
22. Assim MM, Saheb EJ. The association of severe toxoplasmosis and some cytokine levels in breast cancer patients. *Iraqi Journal of Science*. 2018:1189-94.

23. Ali MI, Abd El Wahab WM, Hamdy DA, Hassan A. Toxoplasma gondii in cancer patients receiving chemotherapy: seroprevalence and interferon gamma level. *J Parasit Dis.* 2019; 43(3):464-471.
24. Al-Taj MA, Alkobati HA. SEROPREVALENCE OF TOXOPLASMA GONDII AMONG CANCER PATIENTS IN AL-AMAL CENTER FOR TREATMENT OF CANCER PATIENTS IN TAIZ CITY, YEMEN. *Electronic Journal of University of Aden for Basic and Applied Sciences.* 2024;5(3):254-62.
25. Hosseini SA, Sharif M, Sarvi S, Janbabai G, Keihanian S, Abediankenari S, Gholami S, Amouei A, Javidnia J, Saberi R, Shekarriz R. Toxoplasmosis among cancer patients undergoing chemotherapy: a population study based on the serological, molecular and epidemiological aspects. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 2021;115(6):677-86.
26. Fadel EF, EL Hady HA, Ahmed AM, Tolba ME. Current Trend of Toxoplasmosis in Cancer Patients, Sohag University Hospitals, Sohag, Egypt. *Sohag Medical Journal.* 2024;28(1.):8-17.
27. ALIbady K, Ali QA. Detection Study: Prevalence of toxoplasmosis in aborted women infected with toxoplasma gondii in Baghdad province, with implications for oncology. *Onkologia i Radioterapia.* 2024;18(2).
28. Mustafa KM, Mohammed AB, Mero WM. Seroprevalence of Toxoplasma gondii Antibodies and Associated Risk Factors Among Women in Zakho City, Iraq. *Cureus.* 2024;16(3).
29. Jayesh ZH, Al-Waaly AB. Epidemiological and frequency rate of Toxoplasma gondii in arthritis patient's. *Al-Qadisiyah Journal of Pure Science.* 2024;29(1):17.
30. Harminarti N, Sari IP, Artama WT, Imran D, Kurniawan A. Comparative GRA6 and GRA7 for their Utility as Genetic Markers in the Genotyping of Cerebral Toxoplasmosis in Cerebrospinal Fluid. *Acta Parasitologica.* 2024;69(3):1555-61.

31. Shihab RN, Al-Aubaidi IK. Toxoplasmosis in thalassemic Iraqi patients: serological and hematological study. *Romanian Journal of Infectious Diseases/Revista Romana de de Boli Infectioase*. 2024;27(2).
32. MOUSA NM, NAHAB HM. Serological detection and hematological changes associated with Toxoplasmosis in thalassemia patients in Al-Samawah province. *International Journal of Pharmaceutical Research*. 2020;12(4):872-6.
33. Gouda MA, Saied SA, Ashry WM, Abd-Eltwab RA, Aldesoky MM, El-Dydamoni OA, Yousef M, El-Derbawy MM. Trends of *Toxoplasma gondii* and common transfusable venereal infections among blood donors in Menoufia Province, Egypt. *Scientific Reports*. 2024;14(1):20920.
34. Barzinij KR. Seroprevalence and risk factors of toxoplasmosis among University of Kirkuk female students. *Annals of Parasitology*. 2021;67(2).
35. Ferra B, Holec-Gąsior L, Grażlewska W. *Toxoplasma gondii* recombinant antigens in the serodiagnosis of toxoplasmosis in domestic and farm animals. *Animals*. 2020;10(8):1245.
36. Wei HX, He C, Yang PL, Lindsay DS, Peng HJ. Relationship between cat contact and infection by *Toxoplasma gondii* in humans: a meta-analysis. *Comparative Parasitology*. 2016;83(1):11-9.
37. Cong W, Elsheikha HM, Zhou N, Peng P, Qin SY, Meng QF, Qian AD. Prevalence of antibodies against *Toxoplasma gondii* in pets and their owners in Shandong province, Eastern China. *BMC infectious diseases*. 2018;18:1-5.
38. Alkubaisi SA, Al-Zubaidy IA. Toxoplasmosis in females from Al-Anbar, Iraq. *Journal of the Faculty of Medicine Baghdad*. 2023;65(1):74-8.
39. Almuzaini AM. Flow of Zoonotic Toxoplasmosis in Food Chain. *Pakistan Veterinary Journal*. 2023;43(1).