Original Article

The Role of Retinol-Binding Protein 4 in Women Infected with *Toxoplasma gondii*

Rajaa Abd Ali[1], Azhar Hatif Al-Kuraishi[1], Fatin Shallal Farhan[2]

Departments of [1]Microbiology and [2]Obstetrics and Gynecology, College of Medicine, Al-Mustansiriya University, Baghdad, Iraq

Abstract

Background: Ocular toxoplasmosis mainly affected people in the second to fifth decades, as a result of acute or reactivate congenital or postnatally acquired infection. Retinol binding protein 4 (RBP4) is a protein transporter for retinol (Vitamin A) from the liver to peripheral tissue. Because of its function in vision RBP4 has become a target for ophthalmology research. **Objectives:** Identification of the role of RBP4 parameter in women infected with acute toxoplasmosis. **Methodology:** One hundred and fifteen blood samples were collected from aborted women, who were suspected of having toxoplasmosis for the period October 2020 to March 2021; in addition, 25 samples were collected from apparently healthy women to use as a healthy control group. All sera were investigated for the presence of anti-*Toxoplasma* IgM, IgA and IgG antibodies, all IgG-positive samples (50 samples) were neglected. IgM and IgA-positive samples (40 serum samples) out of 115 samples were used as toxoplasmosis case group (acute toxoplasmosis), while the negative IgM, IgA, and IgG aborted women samples were used as aborted nontoxoplasmic control group (25 serum samples). The samples from healthy women that gave negative results for the three antibodies were used as the healthy control group. **Results:** The RBP4 serum level was reflected a significant decrease 1.84 ± 0.44 ng/ml in aborted toxoplasmic cases when compared with aborted nontoxoplasmic and healthy control groups; 2.85 ± 0.60, 3.90 ± 0.62 ng/ml, respectively. **Conclusion:** There was a highly significant decrease in RBP4 serum levels in acute toxoplasmosis-infected women. This decreasing may be one of the indirect effects of *Toxoplasma gondii* infection on their hosts. Furthermore, this level can be used as aid test for the detection for toxoplasmosis.

Keywords: IgA, IgM, retinol-binding protein 4, *Toxoplasma gondii* infection

Introduction

Toxoplasma gondii is unique parasite among the apicomplexan group because it can invade and multiply in any nucleated cells of virtually all warm-blooded animals, especially, macrophages, muscle cells, epithelial cells, and neurons.[1] Approximately, after 1–3 weeks of infection, the antibodies start to evolve when the tachyzoite begins to localize in cardiac, skeletal muscles, retina, and parenchyma of the brain, as tissue cysts, and disappear from visceral tissues.[2] Wilder was demonstrated *T. gondii* in adult eyes in 1952.[3] Uveitis is a major cause and can explain more than 60% of chorioretinitis cases in several countries.[4] Ocular toxoplasmosis mainly affected people in the second to fifth decades, as a result of acute or reactivate congenitally or postnatally acquired infection.[5] The clinical image and ocular toxoplasmosis damage depend on the patient's age, location, size, and severity of retinochoroiditis inflammation. Ocular symptoms are dependent on the position of a retinal lesion and include reduced vision, floats, vision blurred, eye redness, and photophobia.[6]

Retinol-binding protein 4 (RBP4) is a protein transporter for retinol (Vitamin A) from the liver to peripheral tissue. RBP4

Address for correspondence: Ms. Rajaa Abd Ali,
Department of Microbiology, College of Medicine, Al-Mustansiriya University, Bagdad, Iraq.
E-mail: rajaaabeali7@gmail.com
Asst. Prof. Dr. Azhar Hatif Al-Kuraishi,
Department of Microbiology,
College of Medicine, Al-Mustansiriya University, Bagdad, Iraq.
E-mail: azharhatif033@gmail.com
Asst. Prof. Dr. Fatin Shallal Farhan,
Department of Obstetrics and Gynecology,
College of Medicine, Al-Mustansiriya University, Bagdad, Iraq.
E-mail: Fatin.shallal@uomustansiriyah.edu.iq

Submitted: 25-Aug-2021 **Revised:** 13-Sep-2021 **Accepted:** 13-Sep-2021 **Published:** 30-Jun-2022

Access this article online

Quick Response Code:

Website: http://www.mmjonweb.org

DOI: 10.4103/mj.mj_20_21

How to cite this article: Ali RA, Al-Kuraishi AH, Farhan FS. The role of retinol-binding protein 4 in women infected with *Toxoplasma gondii*. Mustansiriya Med J 2022;21:37-40.

is composed principally by hepatocytes as well as, is produced in a smaller amount by adipocytes of the adipose tissue and serves as a signal to surrounding cells when plasma glucose is low.[7]. The RBP-retinol complex interacts with transthyretin in plasma, which inhibits its loss via the glomeruli in the kidney.[8] Previous studies have shown that RBP4 is implicated in many human conditions, including ocular disease and impaired vision.[9] Because of its function in vision RBP4 has become a target for ophthalmology research.[10]

Hence, both the infection with toxoplasmosis and deficiency in RBP4 has an effect on eyesight. Therefore, this study was built to clarify the role of RBP4 on toxoplasmosis on one hand, and whether RBP4 level affected (elevation or decline) during toxoplasmosis on the other hand.

Methodology

One hundred and fifteen blood samples were collected from aborted women, who were suspected of having toxoplasmosis for the period from October 2020 to March 2021, who had attended at the Obstetrics and Gynecology Department of Al-Yarmouk Teaching Hospital, Kamal Al-Samaraie Hospital, and Private Laboratories in Baghdad City. In addition, 25 samples were collected from apparently-healthy women to use as healthy control group, and hence that the total number of samples included in the study is one hundred and forty samples. All sera were investigated for the presence of anti-*Toxoplasma* IgM and IgG antibodies by (Bio-Medical Com., Ltd, Beijing, China) Rapid Test-Cassette, as well as investigating them also with anti-*Toxoplasma* IgM, IgG ELISA assay (Foresight. Com., USA) and anti-*Toxoplasma* IgA ELISA assay (Mybiosource. Com., USA). All positive IgG-*Toxoplasma* antibodies were neglected (50 samples) because the study was limited only to a recent infection with toxoplasmosis. IgM- and IgA-positive samples (40 samples) were used as toxoplasmosis case group, while the negative IgM, IgG, and IgA aborted women samples (25 samples) were used as aborted control group. In addition, 25, samples from healthy women that give negative results for these tests were used as healthy control group. Then, the RBP4 level was measured using an ELISA kit (Elabscience. Com., USA), which based on sandwich enzyme-linked immune-sorbent assay technology, in each group.

Finally, statistical analysis of data was carried out using SPSS-27. Receiver Operating Characteristic (ROC) curve was applied to determine the application of any parameter as a diagnostic or disease screening tool, and ability to select a Cut-off value that is optimum sensitive and specific for diagnosis.

Results

For toxoplasmosis cases, 40 (34.78%) serum samples out of 115 samples gave results with anti-*Toxoplasma* IgM, while only 9 (22.5%) cases out of 40 samples was positive for anti-*Toxoplasma* IgA [Table 1]. There was a highly significant decrease in RBP4 level 1.84 ± 0.44 ng/ml ($P < 0.001$) in toxoplasmosis cases, when compared with aborted and healthy control groups; 2.85 ± 0.60, 3.90 ± 0.62 ng/ml, respectively, as shown in Figures 1 and 2.

Table 1: The Types of anti-*Toxoplasma gondii* antibodies which detected by ELISA

	Toxoplasmosis cases ($n=40$), *n* (%)	Aborted control ($n=25$), *n* (%)	Healthy control ($n=25$), *n* (%)
ELISA IgM			
Negative (<0.9)	-	25 (100)	25 (100)
Positive (≥0.9)	40 (100)	-	
ELISA IgA			
Negative (<0.7)	31 (77.5)	25 (100)	25 (100)
Positive (≥0.7)	9 (22.5)	-	-

The resulting ROC curve and its functional area under the curve, had shown that ELISA IgM still the best tool for the diagnosis of toxoplasmosis then another tool (RBP4) was provided from the current study as simple analytical forms working parameter for prognosis [Table 2 and Figure 3].

A comparison of each test showed that cut-off value suggested for ELISA IgM is >0.895 (100% sensitivity and 100% specificity), while RBP4 a cut-off value was <2.78 for 100% sensitivity and 100% specificity [Table 3].

Discussion

To our knowledge, this is the first study in the world to measure RBP4 serum levels in women with toxoplasmosis. The results of the current study showed that there is a highly significant decrease in this parameter (1.84 at $P < 0.001$) in women with this disease compared with control groups. To clarify the role of *T. gondii* in changing the levels of RBP4 in the body, compared to the control groups, this can be explained by the estimation of vitamin A and its relationship to each of them. RBP4 has a key role in the transport of Vitamin A, which is an important, fat-soluble nutrient, for embryogenesis, also, plays an important role in vision, cellular immunity function, reproduction and proliferation, regulation, and differentiation of cells.[11] Several studies have investigated the relationship between RBP4 and Vitamin A.[12-14] An *in vivo* study,[15] which revealed that RBP4 required for hepatic retinol mobilization and that mobilization is important for ocular and visual function development. Another study[16] showed that steatohepatitis in mice severely effects Vitamin A metabolism in the liver, resulting in decreased retinol due to the accumulation of retinyl esters in healthy hepatocytes and hepatic RBP4 levels are also decreased. Thus, steatohepatitis does not result in true Vitamin A deficiency as indicated by previous studies, but it leads to hepatic retinol deficiency due to metabolic changes. Another study showed that RBP4 normally supplies retinol to peripheral tissues, including the placenta and fetal eye, from liver reserves, therefore, Vitamin A deficiency increases the risk of congenital eye abnormalities and blindness during pregnancy.[17] A studies

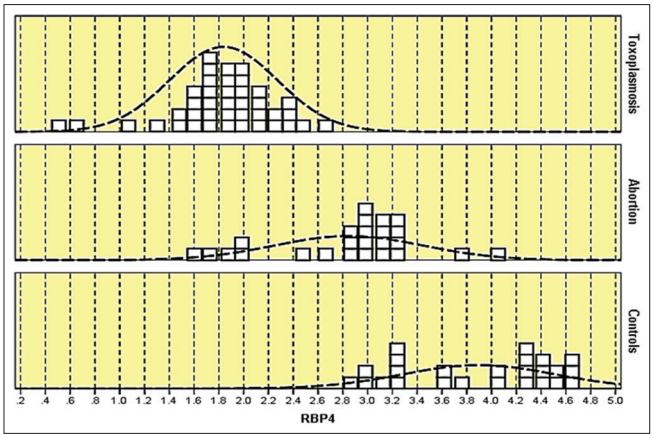

Figure 1: The distributions of retinol binding protein 4 values of women infected with *Toxoplasma gondii* compared with control groups

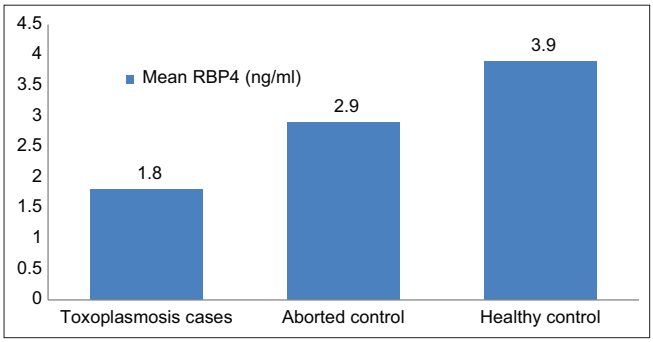

Figure 2: The retinol binding protein 4 levels of women infected with *Toxoplasma gondii* compared with control groups

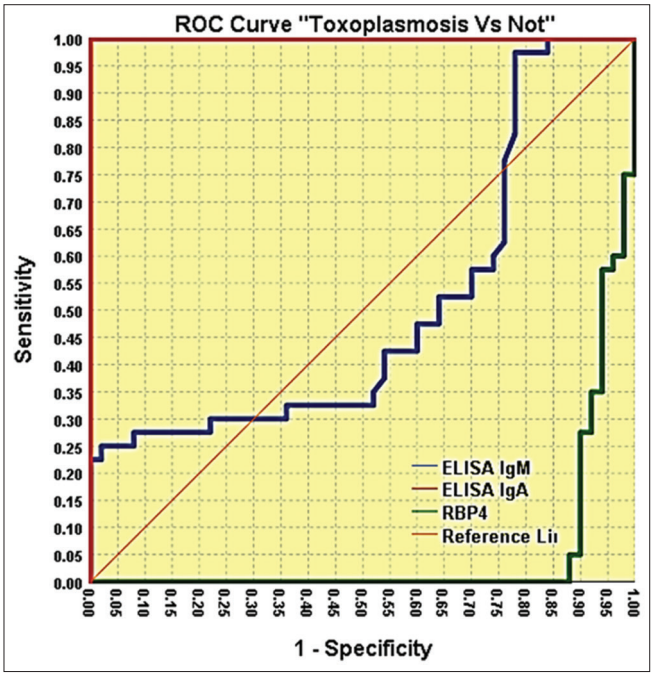

Figure 3: The receiver operating characteristic curve when use to diagnosis of acute toxoplasmosis as compared with nontoxoplasmosis (both aborted and healthy control groups)

Table 2: The receiver operating characteristic analysis area for selected parameters when used to diagnosis of acute toxoplasmosis as compared with nontoxoplasmosis (both aborted and healthy control groups)

Test result variables	AUC	SE	*P*	95% CI (lower bound–upper bound)
ELISA IgM	1.000	0.001	0.0001**	1.000-1.000
ELISA IgA	0.566	0.065	0.284	0.440-0.692
RBP4	0.948	0.024	0.0001**	0.901-0.995

*Significant difference at 0.05 level, **Highly significant difference at 0.01 level. CI: Confidence interval, AUC: Area under the curve, SE: Standard error

Table 3: The cut-off values, sensitivities, and specificities for early detection of acute toxoplasmosis as compared with nontoxoplasmosis (both aborted and healthy control groups)

Variables (cut-off values)	Sensitivity (%)	Specificity (%)
ELISA IgM (≥0.895)	100.0	100.0
ELISA IgA	-	-
RBP4 (≤2.78)	100.0	100.0

conducted by Al-Fartusie *et al.*[18] and Khaleel *et al.*,[19] showed that serum Vitamin A was significantly decreased in infected women with toxoplasmosis compared to those control groups. A recent study showed decrease, but nonsignificant in the level of Vitamin A during the pregnancy period in the placental of women with toxoplasmosis.[20] Therefore, the present finding contributed to the association between circulating RBP4 (carrier of this vitamin) and toxoplasmosis, especially in the acute form. In addition, other evidence that one of the symptoms of toxoplasmosis is a change in the retina that leads to blindness.[21] *In vitro* studies have demonstrated the ability of tachyzoites to cross retinal pigmented epithelial cells with dendritic cells after systemic infection in which Vitamin A metabolism occurs within these cells in addition to controlling intra-retinal homeostasis and immune regulation.[22,23]

It can be concluded that there was a highly significant decrease in RBP4 serum levels in acute toxoplasmosis-infected women. This decreasing may be one of the indirect effects of *T. gondii* infection on their hosts. Furthermore, this level can be used as aid test for the detection of toxoplasmosis.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References
1. Horta MF, Andrade LO, Martins-Duarte ÉS, Castro-Gomes T. Cell invasion by intracellular parasites–the many roads to infection. J Cell Sci 2020;133:jcs232488.
2. Dunay IR, Gajurel K, Dhakal R, Liesenfeld O, Montoya JG. Treatment

of toxoplasmosis: Historical perspective, animal models, and current clinical practice. Clin Microbiol Revi 2018;31:e00057-17.
3. Monotoya JG, Remington JS. *Toxoplasma gondii.* In: Mandell GL, Bennet lE. Dolin R, editors. Principles and Practice of Infectious Diseases. Vol. 2. 5th ed. London: Churchill Livingstone; 2000. p. 2858-88.
4. Majumder PD, Ghosh A, Biswas J. Infectious uveitis: An enigma. Middle East Afr J Ophthalmol 2017;24:2-10.
5. Wilson CB, Nizet V, Maldonado Y, Remington JS, Klein JO. Remington and Klein's infectious diseases of the fetus and newborn infant. 8th ed. China:Elsevier Health Sciences; 2015. p. 949.
6. Casoy J, Nascimento H, Silva LM, Fernández-Zamora Y, Muccioli C, Dias JR, *et al*. Effectiveness of treatments for ocular toxoplasmosis. Ocul Immunol Inflamm 2020;28:249-55.
7. Karamfilova V, Gateva A, Alexiev A, Zheleva N, Velikova T, Ivanova-Boyanova R, *et al.* The association between retinol-binding protein 4 and prediabetes in obese patients with nonalcoholic fatty liver disease. Arch Physiol Biochem 2019;63:1-6.
8. Zabetian-Targhi F, Mahmoudi MJ, Rezaei N, Mahmoudi M. Retinol binding protein 4 in relation to diet, inflammation, immunity, and cardiovascular diseases. Adv Nutr 2015;6:748-62.
9. Li ZZ, Lu XZ, Liu JB, Chen L. Serum retinol-binding protein 4 levels in patients with diabetic retinopathy. J Int Med Res 2010;38:95-9.
10. Cioffi CL, Dobri N, Freeman EE, Conlon MP, Chen P, Stafford DG, *et al*. Design, synthesis, and evaluation of nonretinoid retinol binding protein 4 antagonists for the potential treatment of atrophic age-related macular degeneration and Stargardt disease. J Med Chem 2014;57:7731-57.
11. Steinhoff JS, Lass A, Schupp M. Biological functions of RBP4 and its relevance for human diseases. Front Physiol 2021;12:659977.
12. Kawaguchi R, Yu J, Honda J, Hu J, Whitelegge J, Ping P, *et al*. A membrane receptor for retinol binding protein mediates cellular uptake of Vitamin A. Science 2007;315:820-5.
13. Hermsdorff HH, Zulet MÁ, Puchau B, Bressan J, Martínez JA. Association of retinol-binding protein-4 with dietary selenium intake and other lifestyle features in young healthy women. Nutrition 2009;25:392-9.
14. Alapatt P, Guo F, Komanetsky SM, Wang S, Cai J, Sargsyan A, *et al*. Liver retinol transporter and receptor for serum retinol-binding protein (RBP4). J. Bio. Chem 2013;288:1250-65.
15. Shen J, Shi D, Suzuki T, Xia Z, Zhang H, Araki K, *et al.* Severe ocular phenotypes in Rbp4-deficient mice in the C57BL/6 genetic background. Lab Invest 2016;96:680-91.
16. Saeed A, Bartuzi P, Heegsma J, Dekker D, Kloosterhuis N, de Bruin A, *et al*. Impaired hepatic Vitamin A metabolism in NAFLD mice leading to Vitamin A accumulation in hepatocytes. Cell Mol Gastroenterol Hepatol 2021;11:309-25.
17. Chou CM, Nelson C, Tarlé SA, Pribila JT, Bardakjian T, Woods S, *et al.* Biochemical basis for dominant inheritance, variable penetrance, and maternal effects in RBP4 congenital eye disease. Cell 2015;161:634-46.
18. Al-Fartusie FS, Marzook AT, Morad TS. Study of some trace elements and antioxidant vitamins in sera of Iraqi women with toxoplasmosis. Al-Mustansiriyah J Sci 2012;23:199-206.
19. Khaleel FM, Hameed AS, Dawood AS. Evaluation of antioxidant (GSH, Vitamin A, E, C) and MDA in Iraqi women with toxoplasmosis. Indian J Forensic Med Toxicol 2020;14:1447.
20. Ibraheem MI, Mustafa LA, Ismail AY. Effect of some biochemical parameters such as antioxidants in placenta of infected pregnant woman with toxoplasmosis. J. Education Sci 2020;29:58-67.
21. Arruda S, Vieira BR, Garcia DM, Araújo M, Simões M, Moreto R, *et al*. Clinical manifestations and visual outcomes associated with ocular toxoplasmosis in a Brazilian population. Sci Rep 2021;11:1-7.
22. Furtado JM, Bharadwaj AS, Ashander LM, Olivas A, Smith JR. Migration of *Toxoplasma gondii*–infected dendritic cells across human retinal vascular endothelium. Invest Ophthalmol Visual Sci 2012;53:6856-62.
23. Greigert V, Bittich-Fahmi F, Pfaff AW. Pathophysiology of ocular toxoplasmosis: Facts and open questions. PLoS Negl Trop Dis 2020;14:e0008905.