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MicroRNA-146a and MiR-155 Expression in Toxoplasmosis Patients with Rheumatoid Arthritis

Huda Dhaher Al-Marsomy[1], Zainab Abed Muhsin AL-Haboobi[2], Qudus WamidhJamal[1]

[1]Al-Nahtrain, College of Medicine, Department of Microbiology, Baghdad, Iraq.
[2]AL-Furat Al-Awsat Technical University/ Karbala Technical Institute, Karbala, Iraq
Corresponding author: Huda Dhaher Al-Marsomy, **Mobile** :(964)07703440493,
Email: Hudaalmarsome@yahoo.com. https://orcid.org/0000-0002-3069-8820

ABSTRACT

Introduction: *Toxoplasma gondii* is a protozoan parasite that lives intracellular and has been linked to autoimmune disease. It also can modulate immune responses to varying degrees.

Rheumatoid arthritis, sometimes known simply as RA, is a prevalent autoimmune disease that is distinguished by its multiple pathogenic pathways, which ultimately lead to persistent inflammation and damage to the joints. MicroRNAs play a crucial role in immune response regulation and can be used as novel biomarkers to improve diagnosis, treatment, and disease states.

Objective: The purpose of this study was to investigate the levels of expression of the microRNAs miR-146a and miR-155 as potential biomarkers for the diagnosis of rheumatoid arthritis (RA) in individuals who were infected with toxoplasmosis.

Patients and Methods: The study was conducted on 50 Rheumatoid Arthritis patients positive for toxoplasmosis as diagnosed by ELISA (divided into two groups according to treatment response) and 50 age and sex-matched healthy subjects as a control group. Purified RNA was done, then real-time PCR was carried out using specific primers for MicroRNA-146a and MiR-155.

Results: A significant up-regulation in both Micro-RNA in the studied groups when compared with the control group. A high level of miR-146 expression was associated with low expression for miR-155 observed in the negative response group for treatment when compared with the positive response group.

Conclusion: the miR-146 and mir-155 could be used as biomarkers for host response during the biological treatment.

Keywords: MicroRNA, Toxoplasmosis, Rheumatoid arthritis

INTRODUCTION

Toxoplasmosis is a common disease caused by *Toxoplasma gondii*, a coccidian intracellular parasite. Multiple entry points exist for the parasite to infect humans, the oocyst stage (consumption of contaminated food or water), the bradyzoite stage (eating raw or undercooked meat), and transmission vertically to the fetus from the infected mother[(1).]

Toxoplasmosis in immunocompetent persons is typically asymptomatic, but congenitally infected infants and immunocompromised patients might develop serious sequelae from the infection. like polymyositis, myocarditis, and polyarthritis in the knee and hand [(2).] Because of the unclear effects of the parasite on its host's immunity, its role in the pathogenesis of autoimmune diseases has not been well identified. Some experiments have suggested the protective effect of parasitic infection on the susceptibility to autoimmune diseases, others showed that it may contribute to the development of autoimmune diseases. [(3,4)].

Rheumatoid arthritis (RA) is a chronic type of autoimmune arthritis that affects life quality and mortality. Pathogenesis of the disease results from the interaction between genetics and environmental factors leading to a breakdown of the patient's immune tolerance [(5)].

Infection with *T. gondii* has been reported to be quite common in RA patients, particularly during periods of immunosuppression following tumor necrosis factor medication. *T. gondii* infection, on the other hand, may reduce the severity of arthritis by delaying its development in IL-1 receptor antagonist defective animals via a *T. gondii*-derived Th1 immune response against Th17 cell-mediated arthritis [(6,7)].

MicroRNAs (miRNAs) are small non-coding RNAs. Studies that link host miRNA regulation to infectious illnesses and resistance to or susceptibility to infection emphasize the importance of miRNAs in inflammation and immunological responses [(8)].

In 2010, the first study on MicroRNA and Toxoplasmosis was published, which linked the miRNA profile of primary human foreskin fibroblasts cells (HFF) to *T. gondii* infection. Two clusters of microRNAs, miR-17-92 and miR-106b-25, were shown to be upregulated in infected cells in this investigation [(9)].

Another study published in 2014 found that miR-1792 can regulate apoptosis by inhibiting BIM, a pro-apoptotic protein This apoptosis-inhibiting mechanism is a well-known pathway for T. gondii to bypass an immune attack [(10)].

It has been suggested that microRNAs may be expressed abnormally at various phases of rheumatoid arthritis development, allowing miRs to be used to monitor disease severity and better understand its etiology [(11)]. MiR-146 and miR-155 levels are observed to be higher in rheumatoid arthritis peripheral blood

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mononuclear cells. MiR-146a, in particular, has been linked to disease activity [12], whereas miR-155 is necessary for human myeloid cell proinflammatory activation and antigen-induced inflammatory arthritis [13].

Therefore, the study aimed to evaluate the levels of miRNA -146 and miRNA-155 in Rheumatoid Arthritis patients under treatment and infected with Toxoplasmosis, and the possibility to use them as diagnostic biomarkers.

PATIENTS AND METHODS

Twenty-five RA patients who had a previous diagnosis of toxoplasmosis were included in the research, and their medical records were obtained from the Unit of Rheumatology at Baghdad Teaching Hospital between November 2018 and August 2019. The samples were taken from blood left over in the lab who were already diagnosed with RA.

Biological Parameters

When identified with an immunoturbidimetric assay, RF values >30 IU/mL were considered positive (Quantia-RF kit, Abbot Laboratories, Chicago-IL, US). Anti-cyclic citrullinated peptide (Anti-CCP) antibody levels were determined using the EDIATM anti-CCP-kit (Euro Diagnostica, Malmö, Sweden). Positive anti-CCP titers were assessed at concentrations of >5 U/mL.

The seropositivity for *T. gondii* (IgM and IgG) antibody was measured by sandwich ELISA Kits (Foresight, USA).

Isolation of microRNAs from serum

To separate serum, whole blood was obtained from participants via direct venous puncture into gel tubes. First, the blood was processed by centrifuging at 4,500 rpm for 15 min. Then 400 µl of serum was transferred to a tube containing 600 µl of Trizol and stored at −30°C. Next, RNA was purified by the traditional organic method using chloroform as the manufacturing protocol of Thenrmo Fisher Scientific Company. Finally, purified RNA samples were quantified using a Quantus fluorometer (Promega) and stored at −30°C.

Utilizing Real-Time Polymerase Chain Reaction for Quantitative Analysis

The amount of RNA solution that was utilized to do the reverse transcription was kept constant. GoScript (Promega) reverse transcription system was used to generate cDNA utilizing miR-specific RT primers. The Veriti PCR System (Thermo-Scientific) reaction conditions were 16°C for 30 minutes, 42°C for 30 minutes, 85°C for 5 minutes, and hold at 4°C. Specific forward primer and universal reverse primer prepared for the study by ASCo were used for real-time PCR on a MIC qPCR Cycler (BioMolecular System, Australia) at 95°C for 5 min, then 40 cycles of 95°C for the 20s and 60°C for 20s. Learning Center **(Table 1)** with GoTaq qPCR system (Promega). To identify which is the right miRNA as an endogenous reference for this investigation, Best Keeper software was employed. All the data were normalized to RNU43. miR expression levels were determined by the $2^{-\Delta\Delta CT}$ method after all Ct values were uploaded to the Excel spreadsheet.

Table (1): Sequences of microRNA and reference gene primers

Gene	Primer	Sequence (5′ 3′)
miR-146a	RT	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGC ACCAGAGCCAAC CTGAAG
	Forward	GTTTGGCCTCTGAAATTCAGTT
miR-155	RT	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGC ACCAGAGCCAAC TGTTAA
	Forward	GTTTGGCTCCTACATATTAGCA
RNU-43	RT	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGA GCCAACAATCAG
	Forward	GTGAACTTATTGACGGGCG
	Universal reverse	GTGCAGGGTCCGAGGT

Ethical consideration:

The study was approved by the Ethics Board of Al-Nahtrain University, and ethical approval, as well as informed consent, were obtained from each participant in this study following the declaration of Helsinki.

Statistical analysis

ANOVA test was done using Excel. F-test and P-value were calculated after data entry. Mean and SD was also calculated to define the variation between patient and
control groups.

RESULTS

The clinical features of the individuals who were enrolled in the research were shown in **(Table 2).**

Table (2): Clinical characteristics of the patients

	RA patient with positive drug response	RA patient with negative drug response
Positive response to treatment	**100%**	**0%**
Toxoplasma IgM	**0%**	**0%**
Toxoplasma IgG	**100%**	**100%**
Positive anti-CCP antibody	**100%**	**93%**
Positive CRP	**100%**	**100%**
ESR	**47.5±5.8**	**73.6±9.8**
Positive rheumatoid factor	**25%**	**56%**
miR146	**8.16±6.7**	**16.1±1.6**
miR155	**7.61±3.7**	**5.10±1.3**

Treatment response was evaluated from 4-6 months and was classified according to the EULAR response criteria, **Table (3).**

Table (3): Treatment response according to EULAR criteria

EULAR response criteria	Interpretation
ΔDAS-28 < 2.0	Responders
ΔDAS-28 >5.0	Non-Responders

In this study, we tried to find the relationship between the control and two groups of RA patients who had a history of toxoplasmosis. The first group was comprised of the patients who had had a positive response to the drug, while the second group had a negative response. In **Table 2**, the clinical characteristics present a significant difference in RF factor between the two patient groups, while the other factors were not significantly different. To determine the stage of toxoplasmosis in RA patients, samples were examined for specific anti-*T. gondii* IgM and IgG antibodies using ELISA testing, and all patients were in the chronic stage.

The results revealed dysregulated expression of two immunomodulatory miRNAs, (miR-146, and miR-155) in rheumatoid arthritis patients with a history of toxoplasmosis. Relative analysis of miR-146a data after normalization with RNU43 showed a significant up-regulation in miR-146a and miR-155 expression levels in RA patients compared to healthy controls (for miR-146: 1.22±0.78, miR-155: 1.63±0.96) ($P<0.05$) (**Figure-1**). Furthermore, negative response drug patients presented a high level of miR-146 expression compared with those who give positive responses to the drug. In contrast, miR-155 was present at a significantly low level of expression in negative response drug patients.

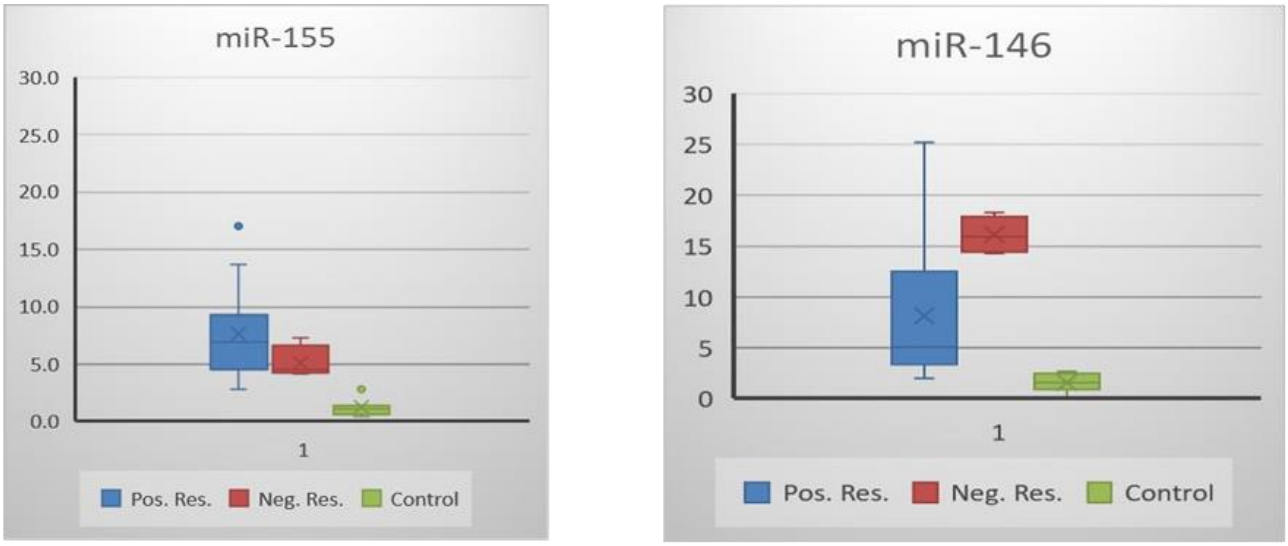

Figure (1): Relative quantification analysis of miR-146 and miR-155 for two groups of patients and control.

DISCUSSION

MiR-146 (miR-146a and miR-146b) and miR-155 have emerged as important immune system regulators. Both of these miRNAs have been linked to a variety of pathologic disorders defined by persistent inflammation, such as rheumatoid arthritis. They are very sensitive to several inflammatory stimuli such as TNF and Toll-like receptor (TLR) ligands in many cell types, especially monocytes/macrophages [(14)].

Tumor necrosis factor-alpha is a pro-inflammatory cytokine that can bind to two different receptors, resulting in different signaling cascades that can lead to an inflammatory reaction [(15)]. High concentrations can cause side effects that results in bone resorption [(16)].

In RA, TNF inhibitors are used to control high levels of these cytokines. In our study, all patients were under biological treatment for at least six months and disease activity was measured depending on ESR, CRP (c_reactive protein), and Anti-CCP. Patients were divided into two groups according to their treatment response. Nemtsova, (2019) mentions that changes in miRNA expression may be related to rheumatoid arthritis treatment [(17)].

All RA patients in this study were *Toxoplasma gondii* seropositive for IgG. In this study, the transcript levels of miR-146 and miR-155 differed across the two patient groups. The low levels of miRNA-155 in the negative group for treatment are consistent with Y. Xu et al (2021) [(18)], which demonstrated that animals lacking miR-155 had a greater propensity to develop chronic *T. gondii* infections than mice with miR-155 intact. MiR-146a was discovered to be activated in the brain during chronic infection oocysts in a different investigation conducted by an independent researcher. [(19)].

In mice that were chronically infected with Type II Toxoplasma, Cannella *et al.*, (2014) [(20)] found that there was an increase in the levels of miR-146a in the central nervous system and that this rise was associated with cyst development. According to Hu *et al*., MiR-146a was also discovered to be activated in the brain during persistent oocyst infection [(19)], also MiR-155 was induced after *T. gondii* infection [(20,21)].

Testa *et al*. (2017) mention that T regulatory lymphocytes (Treg) lose their suppressive effects when miR-146a expression is absent, and this explains the dysregulation of miR146a expression in autoimmune diseases, which is in accordance with our results [(14)].

We used serum to extract MicRNA because it is stable and resistant to RNAse action, freezing and thawing conditions, and extreme pH [(22,21)].

To improve our results, we recommend the study be replicated on a larger number of patients and use the asset of several miRNAs. Also, further research into the parasite load would be useful.

CONCLUSION

The miR-146 and mir-155 could be used as biomarkers for host response during the biological treatment of Toxoplasmosis.

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Author contribution: All of the authors made comparable contributions to the study.

REFERENCES

Wilking H, Thamm M, Stark K, Aebischer T, Seeber F (2016): Prevalence, incidence estimations, and risk factors of Toxoplasma gondii infection in Germany: a representative, cross-sectional, serological study. Scientific reports, 6:22551. https://doi.org/10.1038/srep22551 PMID: 26936108.

1. **Tian A, Gu Y, Zhou N, Cong W, Li G, Elsheikha H *et al.* (2017):** Seroprevalence of Toxoplasma gondii infection in arthritis patients in eastern China. Infectious Diseases of Poverty, 6:6153.
2. **Shapira Y, Agmon-Levin N, Selmi C, PetrõÂkovaÂ J, Barzilai O, Ram M *et al*. (2012):** Prevalence of anti-toxoplasma antibodies in patients with autoimmune diseases. Journal of autoimmunity, 39(1-2):112-116. https://doi.org/10.1016/j.jaut.2012.01.001 PMID: 22297145.
3. **Fischer S, Agmon N, Shapira Y, Katz B, Graell E, Cervera R *et al.* (2013):** *Toxoplasma gondii:* bystander or cofactor in rheumatoid arthritis. Immunologic research, 56(2-3):2837. https://doi. org/10.1007/s12026-013-8402-2
4. **Calabresi E, Petrelli F, Bonifacio A, Puxeddu I (2018):** A. One year in review: Pathogenesis of rheumatoid arthritis. Clinical and experimental rheumatology, 36(2):175–184.
5. **El-Henawy A, Hafez E, Nabih N, Shalaby N, Mashaly M (2017):** Anti*Toxoplasma* antibodies in Egyptian rheumatoid arthritis patients. Rheumatology international, 37(5):785–90. doi: 10.1007/s00296-017-3703-8
6. **Washino T, Moroda M, Iwakura Y, Aosai F (2012):** *Toxoplasma gondii* infection inhibits Th17-mediated spontaneous development of arthritis in interleukin1 receptor antagonist-deficient mice. Infection and Immunity, 80:1437–44.

7. **Acuña S, Floeter L, Muxel S (2020):**. MicroRNAs: Biological Regulators in Pathogen–Host Interactions. Cells Journal, 9:113. doi:10.3390/cells9010113'
8. **Zeiner G, Norman K, Thomson J, Hammond S, Boothroyd J (2010):** *Toxoplasma gondii* infection specifically increases the levels of key host microRNAs. PLoS one, 5(1): 8742. doi: 10.1371/journal.pone.0008742
9. **Kuo G, Wu C, Yang H (2019):** Mir-17-92 cluster and immunity. Journal of the Formoson Medical Association, 118(1): 2–6.
10. **Bogunia K, Wysoczanska B, Piatek D, Iwaszko M, Ciechomska M, Swierkot J.(2016):** Significance of Polymorphism and Expression of miR-146a and NFkB1 Genetic Variants in Patients with Rheumatoid Arthritis. Archivum Immunologiae Therapiae Experimentalis, 64(1):131–6. doi: 10.1007/s00005-016-0443-5.
11. **Elsayed H, Khater W, Lbrahim A, Hamdy M, Morshedy N (2017):** MicroRNA-146a expression as a potential biomarker for rheumatoid arthritis in Egypt. The Egyptian Journal of Medical Human Genetics, 18(2):173179.
12. **Pashangzadeh S, Motallebnezhad M, Vafashoar F, Khalvandi A, Mojtabavi M (2021):** Implications of the role of miR-155 in the pathogenesis of Autoimmune diseases. Frontiers in Immunology, 12 (5):43-65.
13. **Testa U, Pelosi E, Castelli G, Labbaye C (2017):** miR-146 and miR-155: Two Key Modulators of Immune Response and Tumor Development. Noncoding RNA., 3 (4): 2-22. http://dx.doi.org/10.3390/ncrna3030022
14. **Cao Y, Jansen I, Sprangers S, deVries T, Everts V (2017):** TNF-a has both stimulatory and inhibitory effects on mouse monocyte-derived osteoclastogenesis. Journal of cellular physiology, 232(12):3273-3285. doi: 10.1002/jcp.26024.
15. **Vries T, El Bakkali I, Kamradt T, Schett G, Jansen I, D'Amelio P (2019):** What Are the Peripheral Blood Determinants for Increased Osteoclast Formation in the Various Inflammatory Diseases Associated With Bone Loss? .Frontiers in Immunology, 10:505. doi: 10.3389/fimmu.2019.00505
16. **Nemtsova M, Zaletaev D, Bure I, Mikhaylenko D, Kuznetsova E, Alekseeva E et al. (2019):** Epigenetic Changes in the Pathogenesis of Rheumatoid Arthritis. Frontiers in Genetics, 14(10):570. doi: 10.3389/fgene.2019.00570.
17. **Xu Y, Wu J, Yuan X, Liu W, Pan J, Xu B (2021):** MicroRNA-155 contributes to host immunity against *Toxoplasma gondii*. Parasitology Journal, 28 (5) :83
18. **Hu R, He J , Elsheikha H, Zhang F *et al.* (2018):** Differential brain microRNA expression profiles after acute and chronic infection of mice with *Toxoplasma gondii* oocysts. Microbiology Journal, 9:2316.
19. **Cannella D, Brenier M , Braun L *et al.* (2014):** miR-146a and miR-155 Delineate a microRNA Fingerprint Associated With Toxoplasma Persistence in the Host Brain. Cell Reports, 6: 928. doi: 10.1016/j.celrep.2014.02.002
20. **He J, Ma J, Wang J *et al.* (2016):** Analysis of miRNA expression profiling in mouse spleen affected by acute Toxoplasma gondii infection. Infection and genetics Journal, 37: 137-142. doi: 10.1016/j.meegid.2015.11.005
21. **Glinge C , Clauss S , Boddum K , Jabbari R , Jabbari J , Risgaard B *et al* . (2017):** Stability of Circulating Blood-Based microRNAs - Pre-Analytic Methodological Considerations. Frontiers in Genetics,12(2): 16.