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Implicated role of miRNA-4512 and miRNA-124-3p in pathogenesis of systemic lupus erythematous.

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Abstract

Systemic lupus erythematous (SLE) is complex systemic autoimmune disease described by the production of antibodies directed against many auto-antigens, resulting in immune-mediated tissue destruction. Whereas, the exact etiology of systemic lupus erythematosus (SLE) remains unidentified, numerous genetic, environmental, and immunological factors might be have implicated role in pathogenesis of SLE. This study amid to demonstrate implicated role of miRNA-4512 and miRNA-124-3p in pathogenesis of systemic lupus erythematous. This study has employed on clinically certain 50 SLE subjects with different ages, their ages reached from 15 to 55 years old attended to rheumatology clinic of Al-Diwaniyah Teaching Hospital, Marjan Teaching Hospital, Imam Sadiq Hospital, and Imam Ali Hospital during the period from January 2023 to the end of September 2023. EDTA blood samples were used for identification of miRNA-4512 and miRNA-124-3p by real time qPCR technique. The results of miRNA-4512 indicated high significant (P value <0.0001) between SLE patients and control groups. Whereas, results of miRNA124-3p between SLE patients and healthy groups were significant (P value < 0.001) .Both miRNA-4512 and miRNA-124-3p have implicated role in pathogenesis of systemic lupus erythematous.

Keyword. Systemic lupus erythematous, miRNA-4512, miRNA-124-3p.

دورmiRNA-4512 and miRNA-124-3pفي امراضية داء الذؤاب الاحمراري الجهازي

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الخلاصة

داء الذؤاب الاحمراري الجهازي هو مرض مناعي ذاتي جهازي معقد يوصف بإنتاج أجسام مضادة موجهة ضد العديد من المستضدات الذاتية، مما يؤدي إلى تدمير الأنسجة بوساطة المناعة. في حين أن المسببات الدقيقة لداء الذؤاب الاحمراري الجهازي لا تزال غير معروفة، فقد يكون للعديد من العوامل الوراثية والبيئية والمناعية دور في التسبب في. المرض. هدفت هذه الدراسة إلى إثبات دور لـ -miRNA 4512 و miRNA-124-3p في التسبب في داء الذؤاب الاحمراري الجهازي. اجريت هذه الدراسة سريريا على 50 مريضا من داء الذؤاب الاحمراري الجهازي بأعمار مختلفة تراوحت أعمارهم بين 15 إلى 55 سنة راجعوا عيادة الروماتيزم في مستشفى الديوانية التعليمي، مستشفى مرجان التعليمي، مستشفى الامام الصادق، ومستشفى الامام علي خلال الفترة من يناير 2023 حتى نهاية سبتمبر 2023. تم استخدام عينات الدم EDTA لتحديد miRNA-4512 وmiRNA-124-3p بواسطة تقنية real time qPCR . أشارت نتائج miRNA-4512 إلى ارتفاع معنوي (قيمة P <0.0001) بين مرضى الذؤاب الاحمراري الجهازي ومجموعات السيطرة. حيث أن نتائج miRNA124-3p بين الذؤاب الاحمراري الجهازي والمجموعات الصحية كانت معنوية (قيمة P <0.001).كل من miRNA-4512 وmiRNA-124-3p لهما دور كبير في التسبب في داء الذؤاب الاحمراري الجهازي.

الكلمة المفتاحية. داء الذؤاب الاحمراري الجهازي .miRNA-4512, miRNA-124-3p

Introduction

Systemic lupus erythematosus (SLE) a chronic autoimmune disease that mainly affects various systems and is marked by many autoantibodies in the blood; it typically affects women who are fertile **(1)**. According to epidemiological surveys, the yearly global incidence of SLE is 5.14 (1.40–15.13) cases per 100,000 people, whereas the prevalence varies from 0 to 241 cases per 100,000 people. Still, there are notable differences in the worldwide epidemiological statistics for SLE because of differences in study designs, environmental exposures, and populations examined **(2)**. Although the precise etiological process of systemic lupus erythematosus (SLE) is still unknown, infections, environmental triggers, and genetic factors are generally accepted to be involved. These elements have the potential to cause abnormal immune cell activation, which can result in an increase in autoantibodies, a decrease in immune tolerance to self-antigens, and problems with immune complex clearance and deposition **(3).** These outcomes can cause long-lasting inflammatory responses and harm to several organs, including the kidneys, hematological system, and nervous system **(4)**. Upon diagnosis, joint issues are present in over 75% of patients and are often the initial indications of lupus. According to established classification standards, the onset of synovitis in two or more joints, together with accompanying edema, pain, or effusion, and at least thirty minutes of stiffness in the morning, is classified as lupus-related joint involvement. Advances in musculoskeletal ultrasonography (US) and magnetic

resonance imaging (MRI) have cast doubt on past beliefs that individuals with SLE only have nonerosive arthritis by demonstrating a higher prevalence of chronic synovitis and erosions than previously believed **(5)**. The intricate abnormal interaction among the innate and adaptive immune systems leads to the overproduction of cytokines, including complement activation, immune complex deposition, inflammation, and tissue damage. Furthermore, SLE is linked to a significant dysregulation of B cells, DCs, NK cells, T cells, macrophages, and T lymphocytes **(6)**. Also the crucial appreciation and signaling role that TLRs show in host protection processes make them an significant period of PRR and a vital family of innate immune protein. TLRs are essential intermediaries of the immune response to a range of pathogens and are expressed in innate immune cells such as dendritic cells (DCs), macrophages, monocytes, neutrophils, T and B cells, as well as nonimmune cells such as fibroblast cells and epithelial cells, endothelial cells **(7).** Because of TNF-α's broad role as a pro-inflammatory agent, biologics that inhibit related cytokine pathways and TNF-α itself have been used to treat a variety of inflammatory and autoimmune illnesses. This indicates that TNF-α is used to treat a wide range of illnesses. It was once thought that these cytokines had minimal impact on the adaptive immune system **(8).** MicroRNAs, or miRNAs, are being more and more clearly shown to be significant participants in the immunological-pathophysiology of SLE. With a length of 18 to 25, small non-coding RNAs known as miRNAs regulate gene expression in a number of ways. Aberrant miRNA expression influences immunological illnesses, such as autoimmune and auto-inflammatory diseases **(9)**. Serositis, musculoskeletal manifestations, and cutaneous manifestations are often lesser illness symptoms that may wax and wane in intensity with disease activity. Nonsteroidal anti-inflammatory medicines (NSAIDS) are frequently used to treat this, along with low-potency immunosuppressive treatments other than hydroxychloroquine and/or brief doses of corticosteroids **(10)**. This study aims to detect significant role of miRNA-4512 and miRNA-124-3p in pathogenesis of SLE disease.

Materials and methods

SLE patients

Fifty patients with Systemic Lupus Erythematous (SLE) were included in the patient groups; there were five men and forty-five females, ranging in age from 15 to 55. When attending the rheumatology clinic at Al-Diwaniyah Teaching Hospital, Marjan Teaching Hospital, Imam Sadiq Hospital, and Imam Ali

Hospital, patients with a clinical diagnosis were dependent upon a professional physician. Samples were gathered between January and September of 2023 .

Control Group

A control group of individuals in apparent good health was chosen at random from the relatives of patients; this group did not have systemic lupus erythematous (SLE) or any other chronic condition. 50 individuals, 5 men and 45 females, aged 15 to 60, matched in terms of age, sex, and number with SLE patients.

Subject Samples

Blood samples were collected under sterile environment, after identifying of vein, draw (2ml) from venous blood, (2ml) in another EDTA tube for extraction of miRNA-4512 and miRNA-124-3p (after treated with 400mic of triZol solution for each 600mic of blood **(11)**. And the sample freezing until use it for miRNA extraction**.**

Primer design

The qPCR Primers for miRNA-4512 and miRNA-124-3p were designed in this study by using NCBI-Database to select miRNA sequence and using miRNA Primer Design Tool. Whereas, qPCR for Housekeeping gene (U6) were designed in this study by using NCBI-Database Table (1).

Primer	**Sequence 5 '- -3'**		**product**	**Annealing tm**
miR-4512	F	5-AGAATGCTGAGCAGGGATTAGC-3	649	60
	R	5-GCTTCCGGTGGCTCTTGTTA-3		
miRNA-124-3p	F	5-CCGTCTTTCTCCCACGAAC-3	765	57.3
	R	5-CATTGTTCGCCGGATTTGTC-3		
U6	F	GTTTTGTAGTTTTTGGAGTTAGTGT		

		TGTGT		
	R	CTCAACCTACAATCAAAAACAACA CAAACA		

Table 1. Primer Sequence with their product size and annealing temperature

miRNA extraction

The freezing EDTA tube sample leave at room temperature until it is completely thawed. Then shacking the sample by vortex, and all the steps of miRNA extraction were according to kit Promega/ USA.

Preparation reaction mix for one-Step RT-qPCR

The components of one-Step RT-qPCR were added and mix by standard concentrations according to instructions of kit Promega/USA. The components of mixture were summarized in table 2.

Table 2. Concentrations mixture components of one-Step RT-qPCR

Components	Volume per 20µl Reaction	Final Concentration in Reaction
GoTaq® qPCR Master Mix, 2X	10µl	1X
Forward Primer, 10X	2µl	50–300nM
Reverse Primer, 10X	2µl	50–300nM
GoScript™ RT Mix for 1-Step RT-qPCR, 50X or Nuclease-Free Water for Minus-RT Control	0.4µl	1X
RNA Template (500fg–100ng) or Nuclease-Free Water for NoTemplate Control	4µl	variable
Optional: MgCl2, 25mM	11.2	≥2mM
Optional: CXR Reference Dye, 30µM	2.1	≥33nM
Nuclease-Free Water	to 20µl	—

One step Real time qPCR procedure

Schedule a real-time instruments for standard or fast mode one-step RT-qPCR were demonstrate in (Table 3). And all steps of Real time qPCR were according to instructions of Promega/ USA.

Table 3. Program of general thermocycler

Stage	Cycles	Program in Standard or Fast Mode
Reverse transcription	1	≥37°C for 15 minutes
RT inactivation/Hot-start activation	1	95°C for 10 minutes
3-Step qPCR: a. Denature b. Anneal/Collect data c. Extend	40	95°C for 10 seconds 60°C for 30 seconds 72°C for 30 seconds
Dissociation	1	60–95°C

Ethical Approval

The Ethical consent was done through the following

A- Ethical commission in health management.

B- Verbal approval from patients and healthy groups involve in this work.

C- University committee for electronic Turnitin plagiarism

Statistical Analysis

Statistical study was achieved by using statistical package for the social sciences (SPSS) version 23. Categorical difference was offered as frequencies and percentages. Continuous differences were existing as (Means ± SD). Student T test was utilized to compare means among two groups. Mann Whitney Tests were utilized to compare two groups when different was abnormally distributed. (P value of ≤ 0.05) was reflected as significant.

Results

Result of miRNA-4512 in SLE patients and control group

The results of miRNA-4512 between SLE patients and healthy groups were high significant (P value < **0.0001**). The mean level in SLE patients (**12.56 ± 5.37**) was more than mean level of control groups (**1.00 ± 0.31**). and all results were summarized in Table (4) and Figure (1).

Table 4. The results of miRNA-4512 between SLE patients and healthy group

Parameters	Patients Mean±S.D	Control Mean±S.D	T test	P= value
Mi4512	12.56 ± 5.37	1.00 ± 0.31	15.71	<0.0001*

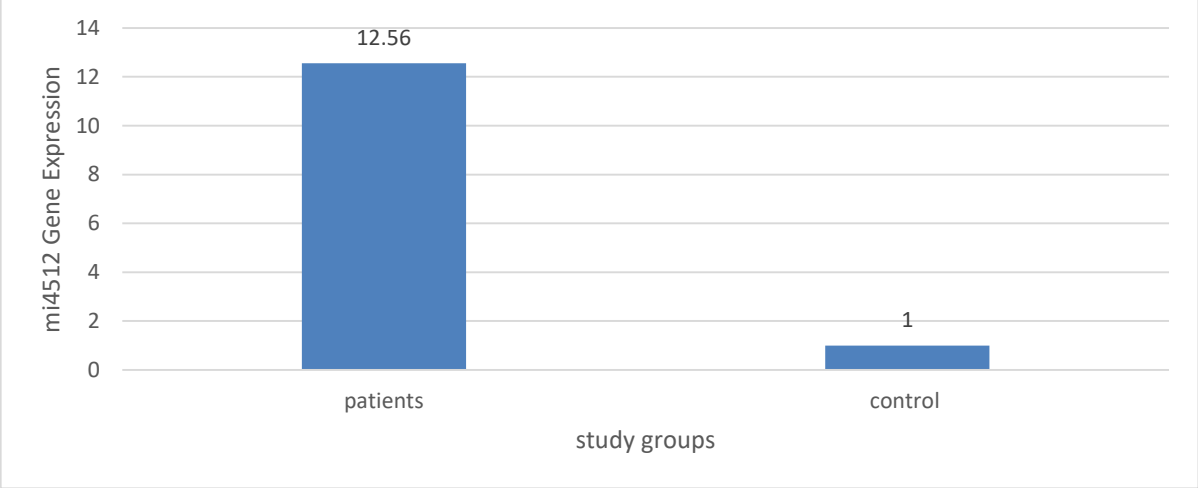

Figure 1. The results of miRNA-4512 between SLE patients and healthy group

Results of miRNA-124-3p in SLE patients and control group

The results of miRNA124-3p between SLE patients and healthy control groups were significant (P value > 0.001). The mean level in SLE (**16.21 ± 3.40)** patients was more than mean level of control groups (**1.13 ± 0.43),** all results miRNA-124-3p were summarized (Table 5) and Figure 2.

Table 5. Result of miRNA124-3p to SLE patients and healthy group

Parameters	Patients Mean±S.D	Control Mean±S.D	T test	P= value
miRNA124	16.21 ± 3.40	1.13 ± 0.43	31.07	0.001*

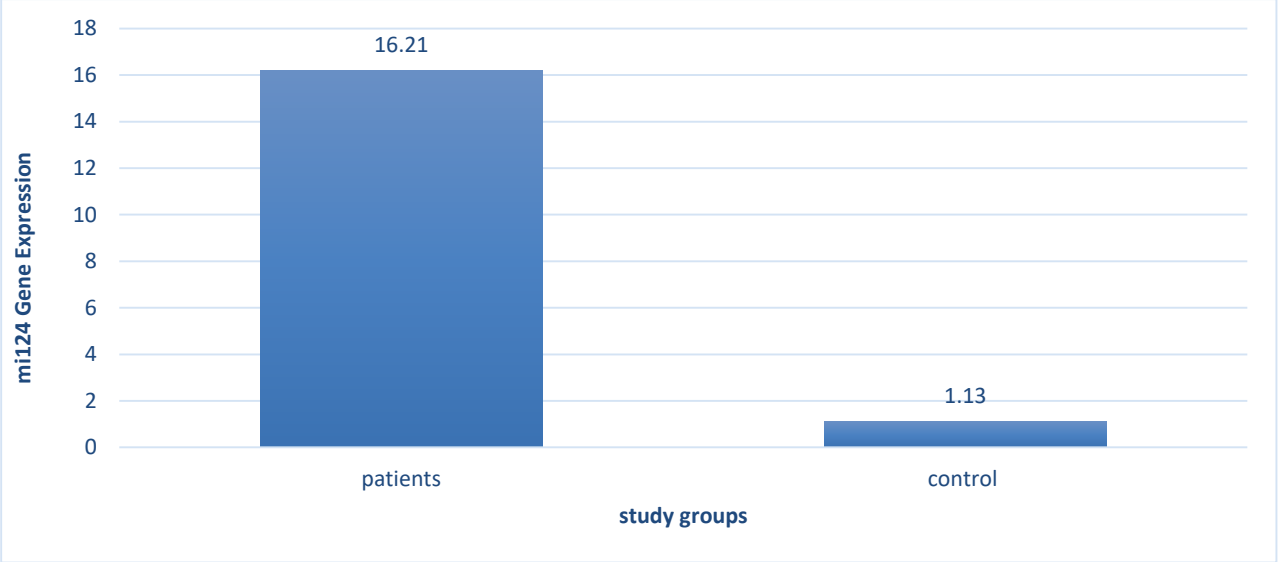

Figure 2.Result of miRNA124-3p to SLE patients and healthy group

Discussion

The result of miRNA-4512 in present study of SLE patients was high significant than healthy control groups. This results were agreement with **(12)** who show miRNA-4512 was potential miRNA implicated in the control of neutrophil stimulation and chemokine-related pathways using combined miRNA and mRNA expression profiling. The expression of MiR-4512 was considerably decreased in SLE patient monocytes and macrophages. MiR-4512 specifically targeted TLR4 and CXCL2 to inhibit the TLR4 pathway. Decreased level of miR-4512 in monocyte and macrophage caused the production of many proinflammatory cytokines in vitro. The production of NETs was considerably increased ($P < 0.05$) by the supernatants miR-4512 antagomir-transfected of monocytes and macrophages **(13)** refer to recent research has demonstrated the tight relationship between miRNA and NET. The discovery of NET-related miRNA vectors and miRNAs in NET-enriched supernatants (NET-miRs) has been made for the first time by Linhares-Laseda *et al.* This opens up the possibility of creating and delivering novel compounds in NETs, as well as a new protein platform. According to their findings, NET plays a new function in cellular communication by helping miRNAs go from neutrophil to nearby cells.

NET functions as a negative feedback loop that lowers hyper-reactivity and preserves the regular control of inflammatory reactions. TLR stimulation and the release of pro-inflammatory cytokines. Research has demonstrated that miRNA-142-3p may also have an impact on these actions, indicating that NET may enable broad regulation of nearby cellular processes via releasing miRNAs **(14**) illustrate according to recent research, the generation of NETs plays a significant role in the pathophysiology of systemic autoimmune disorders. This is in line with the higher expression of proteins linked to NETs that we have seen in SLE patients from both the Han and the Tibetan populations. Exposure to many autoantigens coincides with the production of neural epithelial cells (NETs), which can trigger or worsen autoimmune disorders. In fact, blood samples from SLE patients showed a considerable upregulation of NETs-related markers such cfDNA. Additionally, our research has demonstrated that SLE patients have unusually high levels of serum-related markers (Myeloperoxidase (MPO), cell-free DNA (cfDNA), and NE) **(15)** refer to autoantibodies may develop throughout the pathophysiology of SLE as a result of defective NET breakdown and apoptotic debris clearing. miR-4512 may ultimately prepare neutrophils for NETosis as its down-regulation in monocyte and macrophage stimulates pro-inflammatory cytokines production and CXCL2 expression. MPO and cfDNA, two NETs-relevant markers, had higher levels in the sera of SLE patients. Neutrophils extracted from individuals with SLE have considerably higher amounts of NE, the primary component of NETs. Thus, the pathophysiology of SLE involves either dysregulation of NET release or faulty NET clearance. Thus, the function of miR-4512 in the regulation of innate immunity in SLE suggests that the miR-4512-TLR4-CXCL2 axis might be a novel target for treatment in SLE. Whereas, The results of miRNA124-3p in present study of SLE patients were significant than healthy control groups. This results were agreement with **(16)** who show that PBMCs and blood from SLE patients had substantially greater expression of miR-124-3p and miR-377-3p than the normal control group ($P < 0.05$). ROC curve analysis revealed that plasma miR-124-3p and miR-377-3p had AUCs of 0.714 (95% CI, 0.610 to 0.820, $P < 0.05$) and 0.705 (95% CI, 0.600 to 0.809, $P < 0.05$), respectively, suggesting that they could be useful SLE diagnostic biomarkers. Moreover, miR-124-3p and miR-377-3p had a greater combined diagnostic efficiency than either miR-124-3p or miR-377-3p by themselves. Additionally, compared to PBMCs, the AUCs for miR-124-3p and miR-377-3p in plasma were greater. **(17)** indicated to mechanistic investigations have revealed that the over-expression of TRAF6

may be able to counteract the effects of miR-124 on apoptosis, cell propagation, and the production of inflammatory influences in human red blood cells. All of these results point to the possibility that downregulated miR-124, which targets TRAF6, is a potential diagnostic sign in human lung nanoparticles and inhibits renal mesangial cell proliferation and inflammation. **(18)** show the miRNAs have long been thought to play a significant roles in immunological tolerance pathways, autoimmunity, and the normal operation of the immune system. Recent searches from animal models and clinical studies suggest that miRNAs are involved in the genesis of a number of autoimmune diseases. There is a strong correlation between the development of certain autoimmune diseases in people and aberrant miRNA expression. miRNAs operate at several checkpoints in peripheral and central lymphoid organs to maintain immunological tolerance and fight autoimmune diseases. identification of biomarkers and creation of miRNA-based treatments for various autoimmune diseases in order to support immunological homeostasis, recent research has identified certain miRNAs that control the ancestry specificity and effector abilities of T helper subsets **(19)** indicated to mechanistic investigations have revealed that the over-expression of TRAF6 may be able to counteract the effects of miR-124 on apoptosis, cell propagation, and the production of inflammatory factors in human red blood cells. All of these results point to the possibility that downregulated miR-124, which targets TRAF6, is a potential diagnostic sign in human lung nanoparticles and inhibits renal mesangial cell proliferation and inflammation **(20)** found approximately 90% of genes are regulated by miRNAs, although making up just 3% of the human DNA. A single miRNA has the ability to affect hundreds or thousands of target genes, meaning that it is involved in the regulation of a substantial portion of the genome. This includes functions like regulating the growth of the immune system's innate and adaptive arms, which are both biologically relevant. With its lack of specificity, the innate immune system protects the body from infections before memory responses can be produced. However, the adaptive immune system provides long-term immunological memory that shields against new antigenic assaults and causes particular reactions to antigenic stimuli. Deregulated miRNA expression can therefore result in major immune system problems.

Conclusion

The miRNA-4512 and miRNA-124-3p have implicated role in progress of systemic lupus erythematous severity, these parameters may serve as diagnostic markers to SLE disease.

References.

1. Pons-Estel, G. J., Ugarte-Gil, M. F., & Alarcón, G. S. Epidemiology of systemic lupus erythematosus. *Expert review of clinical immunology*, (2017). *13*(8), 799-814.
2. Tian, J., Zhang, D., Yao, X., Huang, Y., & Lu, Q. Global epidemiology of systemic lupus erythematosus: a comprehensive systematic analysis and modelling study. *Annals of the Rheumatic Diseases*, (2023). *82*(3), 351-356.
3. Accapezzato, D., Caccavale, R., Paroli, M. P., Gioia, C., Nguyen, B. L., Spadea, L., & Paroli, M. Advances in the pathogenesis and treatment of systemic lupus erythematosus. *International Journal of Molecular Sciences*, (2023). *24*(7), 6578.
4. Crow, M. K. Pathogenesis of systemic lupus erythematosus: risks, mechanisms and therapeutic targets. *Annals of the rheumatic diseases*, (2023). *82*(8), 999-1014.
5. Santacruz, J. C., Mantilla, M. J., Pulido, S., Isaza, J. R., Tuta, E., Agudelo, C. A., ... & Agudelo, C. A Practical Overview of the Articular Manifestations of Systemic Lupus Erythematosus. Cureus, (2023). 15(9).
6. Zheng, M., Zhou, W., Huang, C., Hu, Z., Zhang, B., Lu, Q., & Zhao, M. A single-cell map of peripheral alterations after FMT treatment in patients with systemic lupus erythematosus. Journal of Autoimmunity, (2023). 135, 102989.
7. Jawad, O. S., & Shareef, H. K. (2023). The correlation between TLR9 (rs148805533 Del/Ins) gene polymorphisms and susceptibility to pulmonary tuberculosis. Medical Journal of Babylon, 20(3), 463-468.
8. Abed, A. K., Alkhafaji, Z. A., & Abood, A. J. Serological detection of hepatitis B virus e antigen and TNF-α in a dialysis patient. Medical Journal of Babylon, (2023) ; 20(4), 771-776.
9. Li, W., Liu, S., Chen, Y., Weng, R., Zhang, K., He, X., & He, C. Circulating exosomal microRNAs as biomarkers of systemic lupus erythematosus, (2020). *Clinics*, 75.
10. Vale, E. C. S. D., & Garcia, L. C. Cutaneous lupus erythematosus: a review of etiopathogenic, clinical, diagnostic and therapeutic aspects. Anais Brasileiros de Dermatologia, (2023). 98, 355-372.

11. Katarzyna, P. B., Wiktor, S., Ewa, D., & Piotr, L. Current treatment of systemic lupus erythematosus: a clinician's perspective. *Rheumatology International*, (2023). 1-13.
12. Mestry, C., Ashavaid, T. F., & Shah, S. A. Key methodological challenges in detecting circulating miRNAs in different biofluids. *Annals of Clinical Biochemistry*, (2023). *60*(1).
13. Yang, B., Huang, X., Xu, S., Li, L., Wu, W., Dai, Y., ... & Deng, D. Decreased miR-4512 levels in monocytes and macrophages of individuals with systemic lupus erythematosus contribute to innate immune activation and neutrsophil NETosis by targeting TLR4 and CXCL2. Frontiers in Immunology, (2021). 12, 756825.
14. Linhares-Lacerda, L., Temerozo, J. R., Ribeiro-Alves, M., Azevedo, E. P., Mojoli, A., Nascimento, M. T., ... & Saraiva, E. M. Neutrophil extracellular trap-enriched supernatants carry microRNAs able to modulate TNF-α production by macrophages. *Scientific Reports*, (2020). *10*(1), 2715.
15. Duvvuri, B., & Lood, C. Cell-free DNA as a biomarker in autoimmune rheumatic diseases. Frontiers in immunology, (2019). 10, 442736.
16. Apel, F., Zychlinsky, A., & Kenny, E. F. The role of neutrophil extracellular traps in rheumatic diseases. Nature Reviews Rheumatology, (2018). 14(8), 467-475.
17. Yan, L., Jiang, L., Wang, B., Hu, Q., Deng, S., Huang, J., ... & Chen, W. Novel microRNA biomarkers of systemic lupus erythematosus in plasma: miR-124-3p and miR-377-3p. *Clinical Biochemistry*, (2022). *107*, 55-61.
18. Zhang, L., Zhang, X., & Si, F. MicroRNA-124 represents a novel diagnostic marker in human lupus nephritis and plays an inhibitory effect on the growth and inflammation of renal mesangial cells by targeting TRAF6. *International Journal of Clinical and Experimental Pathology*, (2019). *12*(5), 1578.
19. Zhang, L., Zhang, X., & Si, F. MicroRNA-124 represents a novel diagnostic marker in human lupus nephritis and plays an inhibitory effect on the growth and inflammation of renal mesangial cells by targeting TRAF6. International Journal of Clinical and Experimental Pathology, (2019). 12(5), 1578.
20. Singh, R. P., Massachi, I., Manickavel, S., Singh, S., Rao, N. P., Hasan, S., ... & Rehimi, H. The role of miRNA in inflammation and autoimmunity. Autoimmunity reviews, (2013). 12(12), 1160-1165.