Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 2, 239-247

MicroRNA-146a Gene Expression as a Potential Biomarker for Rheumatoid Arthritis in a Sample of Iraqi Patients

[1]Luma Y. Aldouri, [1]Mohammed I. Nader, [2]Mohammed H. Munshed Al-Osami

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad
[2]Department of Medicine, College of Medicine, University of Baghdad

Received: September 25, 2023 / Accepted: October 22, 2023 / Published: October 30, 2024

Abstract: MicroRNAs (miRNAs), a type of small non-coding RNAs, play a crucial role in controlling post-transcriptional gene expression, suggesting their potential involvement in autoimmune disorders. The objective of the current study was to assess the expression levels of miR-146a as potential indicators for diagnosing rheumatoid arthritis (RA) and to examine their correlation with disease activity. A total of one hundred blood samples divided into 50 RA patients and 50 healthy individuals were obtained. The medical histories of all participants were collected, and they were provided with a comprehensive clinicopathological assessment. Disease activity for the group of patients was evaluated using the Disease Activity Score 28 (DAS28). The RNA content of blood samples was extracted, followed by cDNA synthesis. Subsequently, the levels of miR-146a transcripts were measured using Real-time PCR. Significant statistical differences were seen between patients and healthy controls in terms of miR-146a relative expression, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and anti-cyclic citrullinated peptide (anti-CCP) levels ($p < 0.01$). The miR-146a expression level, with a fold change of 4.87, exhibited a substantial upregulation in the whole blood samples obtained from patients diagnosed with RA compared to those obtained from healthy controls. Furthermore, a positive correlation was seen between the miR-146a expression level and the levels of ESR, DAS28, CRP, and anti-CCP in the RA patients. In diagnosing RA, MiR-146a had superior diagnostic capabilities, exhibiting the most significant levels of sensitivity and specificity at 74% and 87%, respectively. This was further supported by an area under the curve (AUC) value of 0.90, obtained at a cut-off value of P2.55. In comparison, the diagnostic performance of anti-CCP was not as favorable. The diagnostic test demonstrated a sensitivity of 52%, specificity of 100%, and AUC of 0.81 when using a cut-off value of P 122.5U/ml. In summary, the increased expression of miR-146a in the entirety of blood samples from individuals with RA may serve as a possible indicator of disease activity and severity in those with established RA.

Keywords: Rheumatoid Arthritis, miR-146a expression, ESR, CRP, anti-CCP, and DAS28.

Corresponding author: (Email: lumayaldouri2@gmail.com).

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by a persistent inflammatory response that can result in detrimental effects on both joints and extra-articular organs, such as the heart, kidneys, lungs, digestive system, eyes, skin, and neurological system (1). RA is a multifactorial autoimmune disease that impacts around 1-2% of the global population. The prevalence of this condition is higher in women than men, particularly among individuals aged 40 to 60 years (2). Several genetic and environmental factors are related to the

decrease or increase in the risk of RA (3). In genetically susceptible populations, environmental elements cause complex post-translational changes, which initiate a pathological activation of the immune system, ultimately resulting in the clinical manifestation of the disease (4). The clinical presentation of symmetrical joint involvement encompasses symptoms such as arthralgia, edema, erythema, and impaired range of motion (5). MicroRNAs (miRNAs) are a subclass of noncoding RNAs widely believed to serve as diagnostic and prognostic biomarkers for many diseases. They may be found in menstrual blood, saliva, semen, vaginal secretions, and other biological fluids. They regulate most biological processes, including chromosome architecture, cell division, apoptosis, stress tolerance, and stem cell maintenance (6). Two members of the miR-146 family are miR-146a and miR-146b. These two miRNAs, which are expressed on chromosomes 5 and 10, exhibit an extensive number of structural similarities and only differ in mature sequence at the 3′ end by two nucleotides (7). The microRNA-146a has garnered significant interest in autoimmune disorders. The study conducted by (8) identified many target mRNAs implicated in inflammatory signaling, including Interleukin-1 receptor-associated kinase 1 (IRAK-1), IRAK-2, and tumor necrosis factor receptor-associated factor 6 (TRAF-6), that can be bound by mature miRNA-146a. However, it is essential to note that there is no pathognomonic laboratory test for rheumatoid arthritis, which makes the diagnosis of this disease challenging in the early stages. A comprehensive clinical approach is required to make the diagnosis and prevent debilitating joint damage (9). The prevention of common injuries and the achievement of improved long-term outcomes necessitate both early diagnosis and early and successful management. Hence, it is imperative to have dependable biomarkers in conjunction with outcome metrics. (10). However, there are few reports on the association between the quantity of miR-146a present in the whole blood of patients with RA and the level of disease activity. This study aimed to assess the potential use of miR-146a as a biomarker for evaluating RA onset, severity, and progression in patients with established disease. The study focused on examining the expression levels of miR-146a in the blood.

Materials and methods

Study design

The present study employed an observational case-control design, conducted during a period spanning from July to November 2022. Each case provided a thorough medical history, which included demographic data such as name, age, gender, occupation, and address. Information regarding family history of RA, current pregnancy status, and history of concurrent disorders such as diabetes mellitus, hypertension, hyperlipidemia, and other relevant conditions was also recorded. Simultaneously, the DAS28 encompasses a range of values from 0 to 9.4. This score is derived by considering various factors, including assessing sore joints, swollen joints, overall health perception, and laboratory measurement of acute inflammation known as ESR. It is a metric employed by rheumatologists to evaluate disease activity.

Patients and controls

The study comprised a sample of one hundred individuals from Iraq, categorized into two groups: patients

and controls. The patient group consisted of 50 individuals, eight males and forty-two females recruited from the Rheumatology Unit of Baghdad Teaching Hospital. These individuals, aged between 26 and 70, were included in the study after being diagnosed with RA based on the ACR/EULAR classification criteria outlined by Aletaha et al. (11). The patient group was further separated based on their DAS28 score into three subgroups: low disease activity (DAS28 ≤ 3.1), moderate disease activity (3.1 < DAS28 ≤ 5.1), and severe disease activity (DAS28 > 5.1) and Fifty healthy individuals (10 males and 40 females) were obtained from the National Blood Transfusion Center and curious with ages ranging from 28 to 65 years old and with no autoimmune disorders and familial history of autoimmunity, immunodeficiency, and malignancy and whose age and sex were matched with those in the patient's group. The study excluded individuals with other chronic debilitating diseases, chronic disorders, or pregnancy.

Sample collection

From everyone participate enrolled in this study, 5ml of blood was drawn by venipuncture using disposable syringes. Each sample was divided and keep in two tube as follows: first tube contain sodium citrate for ESR analysis. second one was placed in gel tubes for 30 minutes at room temperature, and then centrifuged for 10 minutes in order to obtain serum that divided into aliquots (250 µl) for molecular and Serological study analysis.

Evaluating the MIR-146A gene's expression

A) MicroRNA extraction

RNA was extracted from serum samples according to the protocol of TRIzol™ Reagent. The serum 300 µl was added immediately to 500 µl of TriQuickReagent (SolarBio, China), Lyse cells in sample by vortex several times, let in RT for 10 min. 0.2 mL of chloroform add for aqueous phase containing of RNA, 0.5 mL of isopropanol was added for RNA precipitated as white gel-like pellet, 0.5mL of 70% ethanol was added for RNA washing, finally Pellet was rehydrated in 50µl of Nuclease Free Water then incubated in a water bath at 55-60°C for 10-15 minutes.

B) Reverse transcription

Total RNA was reversely transcribed to complementary DNA (cDNA) using Add Script Reverse Transcriptase kit (add bio, Korea). The procedure was carried out in a reaction volume of 20 µl according to the manufacturer's instructions. All RNA species were subjected to conversion into complementary DNA (cDNA). Thermal cycler steps of conditions cDNA Reverse Transcription described in Table (1).

Table (1): Program PCR converted RNA to cDNA

Step	Temperature (^{o}C)	Time (min)	No. of cycles
Annealing	25	10	1
Extension	50	60	1
Enzyme inactivation	80	5	1
Hold	4 ∞		

C) Quantitative real time PCR

Following the conversion of RNA into cDNA, real time PCR reaction was used and the template this time is cDNA, RNA detection using RNA-specific primers as Table (2). The component is composed of the reaction mix with their quantity as

mentioned in Table (3) and Real Time PCR Program thermal cycling conditions for miR-146a, is shown in (Table 4). The expression of the *U6snRNA* was employed as an endogenous control for the purpose of normalizing the data. The fold change in *miR-146a* expression between the patient and the healthy group was determined by calculating the comparative relative quantification (RQ) level using the $2^{-\Delta\Delta Ct}$ method.

Table (2): Primers that use for gene expression of *miR-146a* and *U6* genes.

Primer	Sequence (5′→3′ direction)	primer size bp	Tm °C	Reference
***miR-146a* (Gene Expression)**				
Forward	GGGTGAGAACTGAATTCCA	19	58	(12)
Reverse	CAGTGCGTGTCGTGGAGT	18	62	
U6 snRNA				
***U6* F.P.**	CTCGCTTCGGCAGCACA	17	60	13)(
***U6* R.P.**	AACGCTTCACGAATTTGCGT	20	60	

Table (3): Volumes and concentrations of RT-PCR reaction mix.

Components	20 µl
Luna® Universal qPCR Master Mix	10
Nuclease free water	6
Forward Primer (10 µM)	1
Reverse Primer (10 µM)	1
Cdna	2

Table (4): program for RT-PCR cycling.

Step	Temperature °C	Time m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:20	45
Annealing	60	00:30	
Extension	95	00:20	1

Laboratory investigations

Estimation of Erythrocyte sedimentation rate (ESR)

Erythrocyte sedimentation rate was estimated in mm/1st hour using Westergren method

Estimation of C-reactive protein (CRP), Rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP)

Serum samples were performed using the Sandwich enzyme linked immunosrbent assay (ELISA) kits (Dialab , China) and (SunLong Biotech, China) in accordance with the manufacturer's protocols to determine the (RF Cat. No R97422), (CRP Cat.No SL0881Hu and ACCP Cat.No SL01Hu).

Statistical analysis

The Statistical Analysis System (SAS, 2018) software was employed to identify the impact of various factors on study parameters. The quantitative variables were characterised using statistical measures such as the mean, standard deviation (SD), and range. The Least Significant Difference (LSD) test, which is a component of the Analysis of Variance (ANOVA), was employed to determine the statistical significance of the differences between means. An estimation of the correlation coefficient between the variables in this study is requested. The Pearson correlation coefficient test is a statistical measure used to assess the strength and direction of the linear relationship between two

variables, denoted as X and Y. It yields a numerical value ranging from -1 to +1, where a value of +1 indicates a perfect positive correlation, 0 indicates no correlation, and -1 indicates a perfect negative correlation. A receiver operating characteristic (ROC) curve analysis was conducted in order to evaluate the diagnostic accuracy of miR-146a. The Receiver Operating Characteristic (ROC) Curve is computed through the utilization of binary logistic regression. The estimation of the specificity and sensitivity of RA diagnosis was conducted by calculating the area under the receiver operating characteristic (ROC) curve (AUC) and determining the corresponding 95% confidence interval (CI).

Results and discussion

Distribution of rheumatoid arthritis patients and control group according to age

The age of RA patients ranged from 26 to 70 years with mean±SD of 45.22 ±12.90 years and from 28 to 65 years with mean±SD of 45.64 ±12.15in the control group. With respect to age, there was no statistically significant difference between both the control and patient groups. This was done to ensure an objective and scientific comparison between the patients with RA and the control group, as presented in (Table 5).

Table (5): Characteristics of the studied participants

Characteristics	Patient group mean ± S.D	Control group mean ± S.D	t-test	p-value
Age (years)	45.22 ±12.90	45.64 ±12.15	4.97 NS	0.86
NS, non-significant				

Comparison between RA patient and healthy control groups as regards ESR, RF, CRP and anti-CCP.

In our study, RA patients had elevated levels of ESR, RF, CRP and (anti-CCP) biomarkers were (40.94 ±8.60mm/h, 45.74 ±8.76IU/ml, 11.38 ±1.06mg/dl and 122.49 ±41.67 IU /ml), respectively. While the control were(14.34 ±3.89 mm/h, 27.12±5.06 IU/ml, 6.49 ±1.96mg/dl and 81.97 ±22.56 IU /ml). (Table 6) shows a significant statistical difference and high disparity in the clinical and laboratory attributes of patients with RA compared to the control group. Specifically, there are significant variations observed in parameters such as ESR, RF, CRP, and anti-CCP levels ($p < 0.001$). These increases in ESR, CRP, ACCP and RF levels are due to the production of inflammatory cytokines and immune complexes that are characteristic of autoimmune diseases. Our findings are consistent as well similar to with finding of (14, 15).

Table (6): Comparison between RA patient and healthy control groups as regards ESR, RF, CRP and anti-CCP

Parameters	RA Mean± SD	Control Mean± SD	Independent *t*-test	
			t-test	p-value
ESR (mm/hour)	40.94 ±8.60	14.34 ±3.89	2.65 **	0.001**
CRP (mg/dl)	11.38 ±1.06	6.49 ±1.96	0.62**	0.001**
RF (U/ml)	45.74 ±8.76	27.12 ±5.06	2.84*	0.001**
Anti-CCP (U/ml)	122.49 ±41.67	81.97 ±22.56	13.29**	0.001**
****: Significant (p<0.01)**				

MiR-146a expression level in sample by one step RT-qPCR

In the present study, a significant up regulation of miR-146a gene expression was observed in the whole blood samples obtained from patients with RA compared to the control group. RA patients exhibited a fold change of 4.87, while the controls showed a fold change of 1.0 (P≤0.01) (Table 7). According to Nakasa et al. (16), there is an observed upregulation of miR-146a expression in both synovium and whole blood of patients with active RA, indicating a heightened disease activity. The observed increase in miR-146a expression in patients with RA can be attributed to the activation of the NF-κB-dependent pathway, which leads to the transcriptional upregulation of *miR-146a*. This activation occurs in response to various pro-inflammatory immune mediators, including lipopolysaccharide (LPS), interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), and latent membrane protein 1 (LMP1), all of which are known to be elevated in RA patients (17). El-bakry et al. (18) reported a statistically significant difference in miR-146a relative expression between patients and healthy controls. Rezaeepoor et al. (19) conducted a study in which they identified several microRNAs (miR-155, miR-150, miR-146a, miR-146b, miR-125a-5p, and miR-223) that exhibited increased expression levels in both groups of RA patients, namely those with poor and reasonable response to therapy when compared to a healthy control group.

Table (7): MiR-146a normalized gene expression levels in the different studied groups (2^-ΔΔCt)

Group	Means Ct of MiR-146a	Means Ct of U6	ΔCt	ΔΔCt	$2^{-\Delta\Delta CT}$	experimental / Control group	Fold of gene expression	p-value
Patients	30.00	27.37	2.62	-2.27	4.84	4.84/0.99	4.87	0.001**
Control	31.29	26.38	4.90	0.00	0.99	0.99/0.99	1.0	

Correlation analysis

Moreover, *MiR-146a* expression levels showed significant positive correlations with those of ESR (r =0.34and p=0.01), Anti-CCP antibody (r=0.29 and p=0.001) CRP (rho=0.31 and p=0.03), and DAS28 (r=0.30 and p=0.03).However, *miR-146a* expression levels showed no statistically significant correlation with any of the RF levels (rho=0.14 and p=0.30), age (rho=0.05 and p=0.74), (Table 8).

Table (8): Correlation analysis of the miRNA expression levels and clinicopathological properties (ESR, RF, CRP anti-CCP) of the RA patients

		ESR	RF	Anti-CCP	CRP	DAS28	Age
***miR-146a* folding**	**R**	0.34*	0.14 NS	0.29*	0.31*	0.30*	0.11
	Sig	0.01	0.30	0.03	0.03	0.03	0.41
	*** Correlation is significant at the 0.01 level (2-tailed)**						

Abou-zeid *et al.* (20) ,found notable positive correlations between the expression levels of *miR-146a* and disease activity, as measured by the DAS-28 and ESR values.Divergent results were seen in reference to the association between the expression level of *miR-146a* and the advancement of the disease. The findings of our investigation unveiled a noteworthy correlation between the expression levels of *miR-146a* and the degree of disease activity. The observation of a linear rise in miR-146a expression across different patient subgroups serves as clear evidence. In particular,

the expression of *miR-146a* was shown to be significantly elevated in RA patients exhibiting the most severe disease activity, whereas it was observed to be notably reduced in individuals with low-grade disease activity. The findings suggest that *miR-146a* has the potential to serve as a predictive biomarker for RA, as demonstrated in (Table 9).

Table (9): The association between the level of miR-146a expression and the course of the disease.

Disease Activity Score 28	miR-146a Mean± SD
Low (4)	2.00 ±0.65
Moderate (18)	3.82 ±0.47
High (28)	4.12 ±0.53
LSD value	2.371 NS
P-value	0.283
NS: Non-Significant.	

Accuracy of *miR-146a* in diagnosing a compared to anti-CCP

The review study has determined that the overall specificity and sensitivity of a single miRNA as a biomarker for RA is generally limited. Therefore, utilizing a set of several miRNAs or combining miRNAs with other parameters is recommended for improved diagnostic accuracy. Identifying anti-CCP antibodies has emerged as a diagnostic marker for RA, offering a valuable tool for accurate diagnosis. A ROC curve was generated to assess and evaluate the diagnostic performance of *miR-146a* relative expression and anti-CCP in diagnosing RA, as presented in (Table 10). In the present investigation, it was observed that *miR-146a* exhibited higher diagnostic accuracy than anti-CCP in identifying patients with RA. The AUC for *miR-146a* was determined to be 0.992, with a cut-off value of P2.16 folds. The sensitivity and specificity of *miR-146a* were 96% and 100%, respectively (Figure 1).

Table (10): Performance characteristics of *Mir-146a* expression and anti-CCP in diagnosing RA.

Parameters	AUC	Explanation	P value	The best Cutoff	Sensitivity %	Specificity %
ACCP	0.81	Very good	0.001	122.5000	52	100
MiR-146a	0.90	Excellent	0.001	2.550	74	87

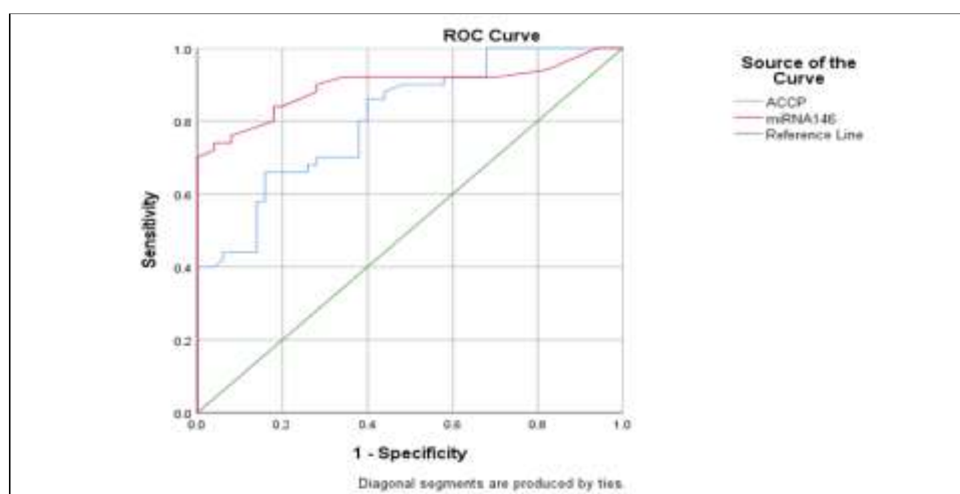

Figure (1): Combined ROC curve analysis of miR-146a and ACCP.

The *miR-146a* biomarker demonstrated the highest area under the curve (AUC) value of 0.90, whereas the anti-CCP biomarker exhibited an AUC value of 0.81, which indicates that the miR-146a level was a strong diagnosis biomarker of RA. This result was in accordance with previous studies which elevated expression of miR-146a was detected in synovial fluid, synovial tissue and whole blood in RA patients (21, 22). It is imperative to acknowledge that the investigations above are constrained by a very modest sample size and were carried out on patients from diverse racial and ethnic backgrounds. The study's sample size of fifty participants is acknowledged as a disadvantage due to its small magnitude. Nevertheless, our research findings, which have demonstrated the significance of miR-146a as a biomarker for RA, have the potential to serve as a reference for future comprehensive investigations aimed at gaining a more comprehensive comprehension of the precise involvement of miRNAs in rheumatic conditions. This study aimed to utilize and assess simplified and minimally intrusive sampling techniques to enhance participant adherence.

Conclusion

This study's findings indicate a significant upregulation of *miR-146a* transcript levels observed in the whole blood samples obtained from patients diagnosed with RA, compared to the control group consisting of healthy individuals. Furthermore, there was a significant association between the expression levels of miRNAs and other clinicopathological aspects of the patients, such as ESR, CRP, anti-CCP, and disease activity as measured by DAS28. The diagnostic performance of the tested method exhibited superior results compared to the anti-CCP assay.

References

1. Conforti, A.; Di Cola, I.; Pavlych, V.; Ruscitti, P.; Berardicurti, O. and Ursini, F. (2021). Beyond the joints, the extra-articular manifestations in rheumatoid arthritis. Autoimmunity Reviews, 20(2): 102-735.
2. Al-Saffar, E.A. and Al-Saadi, B.Q. (2022). Study the Association of IRAK1 Gene Polymorphism and Some Immunological Markers with the Risk of Rheumatoid Arthritis Incidence in Sample of Iraqi Patients. Iraqi Journal of Biotechnology, 21(2): 46-60.
3. Scott, D.L.; Wolfe, F. and Huizinga, T.W. (2010). Rheumatoid arthritis. The Lancet, 376(9746): 108-1094.
4. Porter, C. K.; Riddle, M. S.; Laird, R. M.; Loza, M.; Cole, S.; Gariepy, C., *et al*. (2020). Cohort profile of a US military population for evaluating pre-disease and disease serological biomarkers in rheumatoid and reactive arthritis: Rationale, organization, design, and baseline characteristics. Contemporary clinical Trials Communications, 17: 100522.
5. Taha, M.A. and Da'ad, A.H. (2022). Role Investigation of Interleukin-IL-17 rs763780 T/C Gene Polymorphism with Iraqi Rheumatoid Arthritis Patients. Iraqi Journal of Biotechnology, 21(2): 14-103.
6. Shaker, O.G.; El Boghdady, N.A. and El Sayed, A.E. (2018). Association of MiRNA-146a, MiRNA-499, IRAK1 and PADI4 polymorphisms with rheumatoid arthritis in Egyptian population. Cellular Physiology and Biochemistry, 46(6): 49-2239.
7. Wang, H.; Li, X.; Li, T.; Wang, L.; Wu, X.; Liu, J., *et al*. (2019).Multiple roles of microRNA-146a in immune responses and hepatocellular carcinoma. Oncology Letters, 18(5): 42-5033.
8. Saba, R.; Sorensen, D.L. and Booth, S.A. (2014). MicroRNA-146a: a dominant, negative regulator of the innate immune response. Frontiers in immunology, 5:578.
9. Obaed, N.G.; Elsheshtawi, M.; Jones, C.; Kothari, V.; Estica, T. and Manchaca, K. (2023). Functional Quadriplegia as an Initial Presentation of Severe Rheumatoid Arthritis. Cureus, 15(1).
10. Lönnblom, E.; Leu Agelii, M.; Sareila, O.; Cheng, L.; Xu, B. and Viljanen, J. (2023). Autoantibodies to Disease-Related

Proteins in Joints as Novel Biomarkers for the Diagnosis of Rheumatoid Arthritis. Arthritis and Rheumatology, 75 (7): 1110–1119.

11. Aletaha, D.; Neoga, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham III, C. O., *et al*. (2010). rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Arthritis and Rheumatism, 62(9): 81-2569.
12. Xie, Y. F.; Shu, R.; Jiang, S. Y.; Liu, D. L.; Ni, J. and Zhang, X. L. (2013): MicroRNA-146 inhibits pro-inflammatory cytokine secretion through IL-1 receptor-associated kinase 1 in human gingival fibroblasts. Journal of Inflammation, 10(1): 1-9.
13. Liu, Y.; Xue, M.; Du, S.; Feng, W.; Zhang, K.; Zhang, L., *et al*. (2019). Competitive endogenous RNA is an intrinsic component of EMT regulatory circuits and modulatesEMT. Nature Communications, 10 (1): 1637.
14. Elmalt, H.A.; Ibrahim, A.M. and Girgiss, M.W. (2021). MicroRNA-146a Expression and Serum Interleukin-17 Level as potential biomarkers for Rheumatoid Arthritis. Egyptian Journal of Chemistry, 64(7): 9-3423.
15. Bagheri-Hosseinabadi, Z.; Mirzaei, M.R.; Hajizadeh, M.R.; Asadi, F.; Rezaeian, M. and Abbasifard, M. (2021). Plasma MicroRNAs (miR-146a, miR-103a, and miR-155) as potential biomarkers for rheumatoid arthritis (RA) and disease activity in iranian patients. Mediterranean Journal of Rheumatology, 32(4):324.
16. Nakasa, T.; Miyaki, S.; Okubo, A.; Hashimoto, M.; Nishida, K.; Ochi, M., *et al*. (2008). Expression of microRNA‐146 in rheumatoid arthritis synovial tissue. Arthritis and Rheumatism, 58(5): 92-1284.
17. Taganov, K.D.; Boldin, M.P.; Chang, K.J. and Baltimore, D. (2006). NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences, 103(33): 6-12481.
18. EL-Bakry, R.M.; Fadda, S.M.; Mohamed, R.A. and Abdelaleem, E.A. (2022). Circulating miR-146a-5p in Egyptian patients with Rheumatoid Arthritis. Egyptian Journal of Immunology, 29(1): 3-29.
19. Rezaeepoor, M.; Pourjafar, M.; Tahamoli-Roudsari, A.; Basiri, Z.; Hajilooi, M., *et al*. (2020). Altered expression of microRNAs may predict therapeutic response in rheumatoid arthritis patients. International Immunopharmacology, 83:106-404.
20. Abou-Zeid, A.; Saad, M. and Soliman, E. (2011). MicroRNA 146a expression in rheumatoid arthritis: association with tumor necrosis factor–alpha and disease activity. Genetic Testing and Molecular Biomarkers, 15(11):807-12.
21. Elsayed, H.M.A.; Khater, W.S.; Ibrahim, A.A.; Hamdy, M.S. and Morshedy, N.A. (2017). MicroRNA-146a expression as a potential biomarker for rheumatoid arthritis in Egypt. Egyptian Journal of Medical Human Genetics, 18(2): 9-173.
22. Chen, Z. Z.; Zhang, X. D.; Chen, Y. and Wu, Y. B. (2017). The role of circulating miR-146a in patients with rheumatoid arthritis treated by Tripterygium wilfordii Hook F. Medicine, 96(20).