Biologics: Targets and Therapy

Dovepress
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ORIGINAL RESEARCH

Expression of lncRNA NEAT1, miR-21, and IL17 in Rheumatoid Arthritis Patients

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Background: Rheumatoid arthritis (RA) is a relatively frequent autoimmune disorder with individual and socioeconomic burden, particularly if diagnosed late. Therefore, identifying novel biomarkers for RA that assist in early diagnosis and managing plan is essential. Long noncoding RNA nuclear paraspeckle assembly transcript 1 (NEAT1), micro-RNA 21 (miR-21) and interleukin 17 (IL17) have emerging roles in the pathogenesis of numerous inflammatory conditions. The present research aims to evaluate NEAT1, miR-21 and IL17 roles in RA manifestations and activity and the possibility of utilizing them as biomarkers or therapeutic targets for the disease. Therefore, expression levels of NEAT1, miR-21 and IL17 in sera of 100 RA cases, and 100 age and sex-matched healthy controls were compared. A subsequent analysis was conducted to examine the correlation of their levels to various RA manifestations and disease activity.

Results: Both NEAT1 and IL17 were significantly up regulated, while miR-21 was significantly down regulated in cases compared to controls. NEAT1 demonstrated a significant positive correlation with tender and swollen joint counts and with the overall DAS-28 score. A significant negative correlation was noted between miR-21 and RA disease duration.

Conclusion: NEAT1, miR-21, and IL17 have differential levels in patients with RA where NEAT1 and IL17 have up regulation, while miR-21 has down regulation. NEAT1 has a significant correlation with RA disease activity. We recommend further research to determine if they could be useful as future biomarkers for RA.

Keywords: rheumatoid arthritis, long noncoding RNAs, NEAT1, miR-21, IL17

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune condition presenting with articular and some extra-articular manifestations. RA is a form of chronic inflammatory conditions that may be a consequence of many interactions, particularly between environmental and genetic factors.[1] Classically, small peripheral joints are the first and most affected by RA, but large joints can be affected as well. Neglected untreated cases with persistent inflammation of joints can result in bone erosions and joint destruction.[2] RA may be triggered by many environmental determinants in genetically susceptible individuals.[3]

Pathogenesis of RA could be associated with epigenetic dysregulation in which long noncoding ribonucleic acids (lncRNAs) are highly involved. They can control the transcription factors expression and activation at different levels including transcription, translation, or post-translational levels. In addition, lncRNAs can regulate the biological functions of the cells, they are also important in the development and pathogenesis of several autoimmune conditions including RA.[4] The lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) is a variant of lncRNAs, located on chromosome 11q13.1. It has 6 transcripts, whose length is at least 200 nucleotides. NEAT1 can influence the autoimmune processes, and its disturbance may contribute to autoimmune disease pathogenesis.[5]

Received: 30 January 2025
Accepted: 10 April 2025
Published: 15 April 2025

NEAT1 was investigated in RA patients with significant elevation of its levels in peripheral blood mononuclear cells (PBMC).[6] It was found to stimulate the fibroblast like synoviocytes (FLS) and enhance the proinflammatory cytokines in RA.[5] Similarly, it was involved in other autoimmune diseases like systemic lupus erythematosus (SLE).[7]

NEAT1 is capable to affect many microRNAs, including (miR)-21, during the inflammatory process.[8] Along the last few years, it was obvious that modifications of the microRNAs (miRNAs) expression levels can give rise to multiple autoimmune diseases including RA.[9] MiRNAs are small, non-coding RNAs that incorporate 20 to 22 nucleotides targeting mRNAs through complementary structures.[10] MiRNAs regulate posttranscriptional genetic function by several modes as translational suppression and degradation of mRNA.[11] miRNAs may contribute to various pathological procedures like carcinogenesis and autoimmunity.[12]

MiR-21 was found to participate in the development and progression of autoinflammatory conditions in addition to T-cell differentiation and autoimmune disorders.[13] MiR-21 may act as a diagnostic biomarker for RA.[3] Its modulation may decrease the production of matrix metaloprotses (1,3and13) so may inhibit the invasiveness ability of the FLS. Consequently, the modification of miR-21 might comprise a new strategy in RA management.[14]

Active RA can result due to the imbalance between the pro-inflammatory Th17 cells, which produce interleukin 17 (IL17), and the anti-inflammatory Treg cells, thus highlighting the great functions of the mentioned cells in RA pathogenesis.[15] Th17 cells (through producing IL17) have a major role in defensing against microorganisms; however they also have a critical effect in autoimmune disorders especially RA.[16] Th17 over-expression together with the reduction in Treg cells levels in PBMC in rheumatoid patients was found to be accompanied with elevation of pro-inflammatory cytokines, mainly IL17. At the same time, miR-21 levels were decreased in rheumatoid patients, so miR-21 is supposed to have a role in Th17/Treg cells imbalance in RA patients.[17] MiR-21 has been proved to be Maresin 1 downstream microRNA, increased by Maresin 1, modifying the balance between Treg and Th17 cells so can influence rheumatoid development.[18]

Chen et al, had found that the PVT1/PTEN/IL17 axis has been decreased aiming at improving wound healing through changing miR-21 levels.[19] On the other hand, miR-21 production in Th17 cells had been up regulated, plus miR-21–lacking (Mir21–/–) mice exhibited deficiency in Th17 cell differentiation.[20]

Accordingly, we can hypothesize that NEAT1, miR-21 and IL17 could play a role in the pathogenesis and activity of rheumatoid arthritis.

Patients and Methods

The current study has involved 100 adult RA patients who had followed up in the Department of Rheumatology and Rehabilitation, Cairo University Hospitals. This study also included 100 healthy persons served as controls. Both groups were matched in both sex and age. Patients have been diagnosed using the RA classification criteria of the American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) for the year 2010.[21] Our work included detailed history taking, full clinical examination, and ordinary lab investigations. Rheumatoid factor (RF) together with anti-cyclic citrullinated peptide (anti-CCP) was recorded from patients' files. X-rays both hands were done for all patients. The treatment received by the patients was recorded. Disease Activity Score 28-Erythrocyte Sedimentation Rate (DAS 28-ESR) was used to assess disease activity.[22] Sera from patients and controls were extracted for testing of NEAT1, miR-21 and IL17 levels. Juvenile patients, pregnant females, patients with other autoimmune disease, chronic diseases or cancer were excluded from the study. Written consents were signed by all participants before starting work. All participants were informed about the purpose of the study in accordance with the Declaration of Helsinki.[23] The study was approved by the Scientific Research Ethics Committee of the Faculty of Medicine, Cairo University, with number (N-214-2023).

Fold Changes of NEAT-1 and miR-21 in Serum

Total RNA was extracted from serum (200 ul) utilizing Qiagen miRNeasy Serum/Plasma extraction kit as instructed by the manufacturer. Extracted RNA yield and purity were measured by a nanodrop (Bioanalyzer Agilent RNA 6000 bioassay). Samples with A260/280 ratio between 1.8 and 2.2 were further used for reverse transcription (RT).

Reverse transcription (RT) was employed on 100 ng of total RNA in a 20 µL RT reaction mixtures using miScript II RT kit (Cat.no. 218160) (Qiagen) following the manufacturer's protocol.

Using GAPDH as an internal control gene, the expression profiles of NEAT1was determined by qPCR. The GAPDH gene has been validated previously for use as an excellent internal control for normalizing lncRNAs. The qPCR assay was conducted using the Maxima SYBR Green PCR kit (ThermoFischer, USA) (Catalog numberK0221) following the manufacturer's recommendations. Melting curve analysis was also performed to ensure the absence of primer dimers.

Using the Rotorgene Q system (Qiagen), real-time PCR was implemented in 20 µL reaction mixtures. MiR-21 expression was done using RT-qPCR. The miScript II RT kit (Qiagen) was used for reverse-transcribing 0.1 µg of total RNA in a 20 µL final volume following the instructions of the manufacturer. The RT was executed using thermal parameters: 60 min at 37 °C and 5 min at 95 °C. For qPCR, the miScript SYBR Green PCR kit (Qiagen) was employed to prepare a total of 20 µL reaction mixtures of the cDNA with the ready-made miScript reverse (Universal) primer and specific forward primers for hsa-miR-21 and SNORD68 (internal control).

Our research group and other reports have previously validated SNORD68 as an internal control for miRNA normalization.[24–26] As SNORD68 expression is stable and consistent across both disease cells and normal cells, it can be used as a reliable reference for estimating relative miRNA levels especially that there is no known control miRNA in serum.

We employed the Rotorgene Q real-time system (Qiagen) with the following PCR thermal conditions: 15 min at 95 °C, 40 cycles of 15s at 94 °C followed by 30s at 55 °C and 30s at 70 °C.

The fold change (=relative quantitation in patients' samples to control, ie, the amount of gene expression in patients in relation to controls) was calculated for the expression of studied genes using the formula $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct$ patient – ΔCt control group.

Quantitation of IL-17 in Serum

The IL-17 levels in the serum were calculated using an Enzyme-Linked Immunosorbent Assay (ELISA) kit of Bioassay Technology Laboratory (Zhejiang, China) as recommended by the manufacturer. When adding the serum sample, the IL-17 existing become attached to the human IL-17 antibodies already coating the plate. Subsequently, biotinylated human IL-17 antibodies are added and become attached to IL-17 of the sample.

Statistical Analysis

We represented data as the mean (± standard deviation) or median (25 and 75 interquartile range) for parametric data as appropriate, and as frequency (%) for categorized data. The analysis was performed using version 22 of the Statistical Package for Social Sciences (SPSS) (IBM SPSS, IBM Corp., Armonk, N.Y., USA). The Shapiro–Wilk Test assessed the normality of data distributions. As the data were abnormally distributed, analysis employed Kruskal Wallis test then Mann–Whitney test for parametric data and Pearson Chi-Square test for categorized data. Correlation was made using Spearman test. P-values below 0.05 are indicative of statistically significant results.

Results

This study involved one hundred rheumatoid patients, and one hundred sex and age matched normal persons served as controls.

Regarding demographic data, the mean of the age of patients was 36.89±7.32 ranging from 20 to 65 years, whereas the mean age of controls was 35.12±4.24 with range of (30–50) years. Females represented 84% of the cases, while males represented 16%, whereas in the control group 82% were females and 18% were males. There was no significant difference between patients and controls per age and sex (p=0.116 and 0.462 respectively).

Regarding clinical features of the patients (illustrated in Table 1), the median of disease duration has been 6 years, arthritis was found in 77 patients, while 23 patients had deformities. Rheumatoid factor was positive in 70%, while anti-CCP was positive in 62% of patients.

On comparing levels of NEAT1, miR-21, IL-17 in patients and control groups, serum levels of miR-21 were significantly lower in patients than in controls ($p < 0.0001$), while the serum levels of NEAT1 together with IL17 were statistically significant higher in patients than in controls ($p < 0.0001$) (Table 2) (Figure 1).

Table 1 Clinical Characteristics of Patients

Characteristics	**Number and Percentage (n= 100)**
Arthritis	77 (77.0%)
Deformity	23 (23.0%)
Fever	16 (16.0%)
Rheumatoid nodules	15 (15.0%)
Extra-articular manifestations	19 (19.0%)
Anti-CCP positive	62 (62.0%)
RF positive	70 (70.0%)
ANA positive	10 (10.0%)
Disease activity	
Low	7 (7.0%)
Moderate	39 (39.0%)
High	45 (45.0%)
Remission	9 (9.0%)
Plain X ray findings	
Narrowing	100 (100.0%)
Erosion	23 (23.0%)
Osteopenia	77 (77.0%)
Treatment	
MTX	81 (81.0%)
Hydroxychloroquine	28 (28.0%)
Leflunomide	10 (10.0%)
Steroids	22 (22%)
Characteristics	**Median (25–75 percentile)**
Disease duration (years)	6 (2–10)
Morning stiffness(MS)duration (min.)	20 (0.25–45)
Tender joint count (TJC)	3 (1–9.75)
Swollen joint count (SJC)	4 (1–10)
DAS28 score	4.72 (3.57–6.07)
Creatinine	0.81 (0.67–100)
ALT	26.5 (14.25–35.75)
AST	19 (14–28)
ESR	39 (20–53.75)

(*Continued*)

Table 1 (Continued).

Characteristics	Median (25–75 percentile)
Hemoglobin	12.05 (10.73–12.93)
TLC	7 (5.8–9.3)
Platelets	284 (217–341)
CRP (mg/dl)	8 (5–20.25)

Abbreviations: Anti ccp, anti cyclic citrullinated protein antibodies; RF, rheumatoid factor; ANA, antinuclear antibodies; MTX, methotrexate; DAS, disease activity score; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ESR, erythrocyte sedimentation rate; TLC, total leukocyte count; CRP, c reactive protein.

Table 2 miR-21, NEAT-1, and IL-17 in RA Patients and Control (Data Expressed in Median (25–75 Percentile))

Characteristics	Control (n= 100)	Patients (n= 100)	Significance
miR-21	1 (1–1)	0.26 (0.08–0.67)	***p* <0.0001**
NEAT-1	1 (1–1)	13.29 (4.58–27.95)	***p* <0.0001**
IL-17	38.18 (34.91–45.22)	122.15 (111.25–145.48)	***p* <0.0001**

Note: P-value in bold font means significant (ie <0.05).
Abbreviations: mir21, micro RNA21; NEAT1, nuclear paraspeckle assembly transcript 1; IL17, interleukin 17.

Correlations between clinical features of patients with NEAT-1, miR-21, and IL-17 (Table 3) revealed a negative clinical correlation between miR-21 and disease duration (p=0.016), positive clinical correlation between NEAT 1 and swollen and tender joint counts, in addition to DAS 28 ($p < 0.0001$) (Figure 2).

There were no significant differences between seropositive and seronegative patients as regards NEAT1, miR-21 and IL17 levels (Table 4). Patients who demonstrated bone erosions in hand X-rays had significantly higher levels of NEAT1 and lower levels of miR-21 compared to those without erosions ($p < 0.002$ and 0.0001, respectively) (Table 5).

Correlations between levels of NEAT1, miR-21 and IL17 across various disease activity groups (measured by DAS 28-ESR) revealed that NEAT-1 was significantly elevated in the high disease activity group compared to low activity and remission group ($p < 0.0001$) and moderate activity group ($p = 0.006$). NEAT1 was also positively correlated with overall disease activity ($p<0.0001$). MiR-21 was significantly elevated in the high disease activity group versus group with low

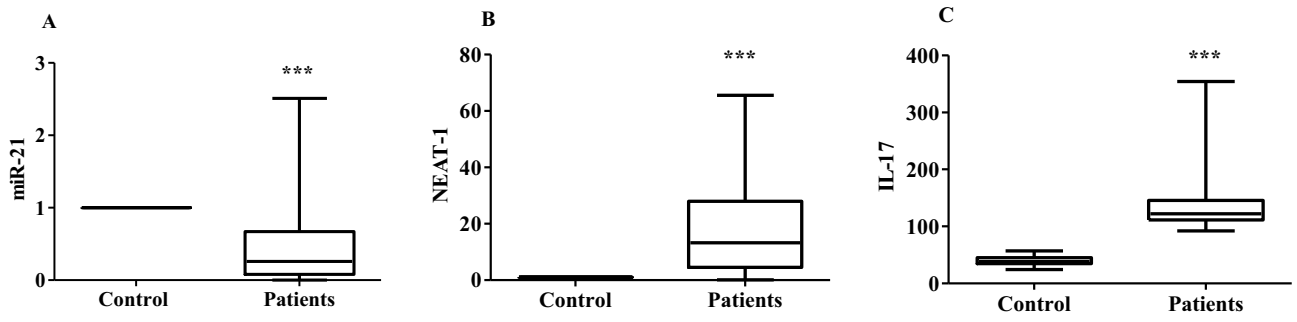

Figure 1 The comparison between patients and controls regarding miR21, NEAT1, and IL17.
Notes: (**A**) Comparison between patients and controls regarding miR-21, (**B**) Comparison between patients and controls regarding NEAT1, (**C**) Comparison between patients and controls regarding IL-17. ***P < 0.001.

https://doi.org/10.2147/BTT.S519558

Table 3 Correlations Between Clinical Characteristics of Patients with miR-21, NEAT-1 and IL-17

Characteristics	miR-21		NEAT-1		IL-17	
	r	*P*-value	r	*P*-value	r	*P*-value
Disease duration (years)	−0.240	**0.016**	0.043	0.670	−0.092	0.365
Tender joint count (TJC)	0.178	0.076	0.376	**0.0001**	0.067	0.507
Swollen joint count (SJC)	0.130	0.197	0.354	**0.0001**	0.048	0.632
DAS28 score	0.220	0. 28	0.412	**0.0001**	0.132	0.192
ESR	0.175	0.081	0.120	0.235	0.061	0.545
CRP	0.076	0.454	0.059	0.561	0.083	0.413

Notes: Correlation was made by Spearman test. P-value in bold font means significant (ie <0.05).
Abbreviations: mir21, micro RNA21; NEAT1, nuclear paraspeckle assembly transcript 1; IL17, interleukin 17; DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, c reactive protein.

activity and remission (p = 0.023) but did not demonstrate a significant correlation with the overall disease activity (p=0.06). IL17 showed non-significant correlations with all disease activity groups and overall disease activity (Table 6). Correlations between NEAT 1, miR-21 and IL17 with other disease characteristics revealed non-significant results.

On correlating the three parameters (miR-21, NEAT1 and IL17) with each other, we found a significant positive correlation of IL17 with miR-21 (*P=0.036*).

Discussion

Successive data has proposed an emerging role for lncRNAs and miRNAs in the pathogenetic mechanisms of some disorders and inflammatory processes involving carcinomas, cardiovascular illnesses and autoimmune arthritides.[10,27] RA is considered a relatively frequent autoimmune disease with well-defined genetic risk factors and substantial individual and socioeconomic burden, particularly if diagnosed late.[28,29] Therefore, there is an essential need for identifying novel biomarkers for RA that assist in early diagnosis and measuring the activity, and guide the treatment plan.

In the current research, we aimed to study serum levels of lncRNA NEAT1, miR-21 and IL17 in RA in comparison to healthy controls and their relations to disease characteristics. Serum was the medium of choice owing to its numerous advantages, it is easily collected and stored in a simple non-invasive technique, and it needs only clotting of blood then

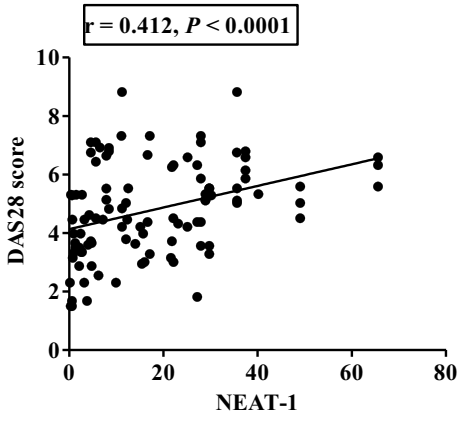

Figure 2 Correlation between NEAT-1 and DAS-28.
Abbreviation: r, Correlation coefficient.

Table 4 Levels of miR-21, NEAT-1, and IL-17 According to RF and Anti-CCP Status

Parameters	RF		Anti-CCP	
	Negative	**Positive**	**Negative**	**Positive**
miR-21	0.15 (0.05–0.57)	0.32 (0.09–0.72)	0.49 (0.07–0.77)	0.17 (0.08–0.62)
P-value	–	0.232		0.211
NEAT-1	13.91 (3.08–27.95)	13.29 (4.58–28.94)	9.82 (4.44–27.38)	15.27 (4.49–28.94)
P-value	–	0.425		0.704
IL-17	129.75 (111.83–162.70)	121.60 (111.18–140.03)	130.20 (113.38–172.35)	120.80 (110.65–138.53)
P-value	–	0.233		0.097

Abbreviations: RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptide antibodies; mir21, micro RNA21; NEAT1, nuclear paraspeckle assembly transcript 1; IL17, interleukin 17.

Table 5 miR-21, NEAT-1, and IL-17 in RA Patients without and with Erosion

Parameters	Non-Erosive (n= 77)	Erosive (n= 23)	p-value
miR-21	0.45 (0.12–0.75)	0.08 (0.05–0.17)	**<0.0001**
NEAT-1	11.08 (2.97–27.57)	27.19 (12.08–29.75)	**0.002**
IL-17 (pg/mL)	124.70 (111.95–155.30)	117.90 (111.10–132.10)	0.144

Note: P-value in bold font means significant (ie <0.05).
Abbreviations: mir21, micro RNA21; NEAT1, nuclear paraspeckle assembly transcript 1; IL17, interleukin 17.

Table 6 Levels of miR-21, NEAT-1, and IL-17 in Each DAS28-ESR Activity Class

DAS 28-ESR Activity Classes		miR-21	NEAT1	IL17
a	Remission & low (DAS28 ≤ 3.2) (n= 16)	0.13 (0.04–0.48)	4.27 (0.63–15.86)	124.30 (112.80–145.65)
b	Moderate (DAS28 > 3.2 but ≤ 5.1) (n= 39)	0.14 (0.06–0.63)	12.08 (3.22–23.10)	119.00 (105.70–135.30)
c	High (DAS28 >5.1) (n= 45)	0.48 (0.13–0.74)	27.19 (7.86–35.63)	123.30 (114.65–155.30)
P-value$_{a-b}$		0.481	0.052	0.359
P-value$_{a-c}$		**0.023**	**< 0.0001**	0.793
P-value$_{b-c}$		0.103	**0.006**	0.062
P-value (among all classes a, b, c)		0.060	**< 0.0001**	0.180

Notes: P-value in bold font means significant (ie <0.05). p-value$_{a-b}$: comparison between serum levels of the studied molecules in (low and remission) class and moderate disease activity class. p-value$_{a-c}$: comparison between serum levels of the studied molecules in (low and remission) class and high disease activity class. p-value$_{b-c}$: comparison between serum levels of the studied molecules in moderate class and high disease activity class.
Abbreviations: mir21, micro RNA21; NEAT1, nuclear paraspeckle assembly transcript 1; IL17, interleukin 17.

centrifugation, and no need for anticoagulation in serum preparation. In addition, serum is widely used and trusted in serological and biomarker testing.[30] Serum samples are stable, making it a reliable technique giving relatively accurate results. Serum also comprise components of all body proteins and peptides which could be associated with many pathological disorders and could be used as biomarkers for diseases diagnosis and follow-up.[31] However, serum still has some disadvantages including hemolysis and coagulation interference in addition to decreased purity of the serum and the interference of large proteins and immunoglobulins.[32] These disadvantages are not present in other tissue samples like

peripheral blood cells, however serum in comparison to other tissues, is a simple non-sophisticated technique with less cost and lower incidence of expertise errors.

We observed a significant elevation of NEAT1 levels in sera of RA cases in comparison to healthy individuals. In addition, NEAT1 demonstrated a significant positive correlation with tender and swollen joint count and with overall DAS-28 score, its level was significantly increased in high disease activity group in comparison to the other two groups. NEAT1, also, was significantly greater in RA cases with than without bone erosions. Several studies support our results of increased NEAT1 levels in RA and in other autoimmune diseases. Chatterjee et al revealed elevated levels of NEAT1 in plasma, synovial fluid and PBMCs in rheumatoid patients, and they also found a positive correlations between NEAT1 and DAS28.[33] Similarly, Wang et al demonstrated up regulation of NEAT1 in synovial tissues and FLSs in RA patients in comparison to that in healthy controls.[34] In addition, NEAT1 was up regulated in PBMC from individuals with RA.[35] In a more recent study, NEAT1 was significantly increased in FLSs derived from patients with RA as compared to FLSs from healthy individuals.[36] NEAT1 was also studied in different autoimmune diseases, its levels were elevated in sera of Behcet's disease patients,[37] PBMC of SLE patients[7,38] and in deep skin lesions of psoriasis patients.[39]

However, and in contrary to our results, Cieśla et al revealed declined plasma levels of NEAT1 and other lncRNAs in RA patients.[40.] These controversial results need more studies with a larger number of patients to elucidate the definite role of NEAT1 in RA.

In addition to its role in autoimmune diseases, NEAT1 correlated positively with several types of cancers.[36]

Studies revealed a proinflammatory role of NEAT1 in RA, supporting our observation of its increased serum levels and relations to disease activity. The overexpression of NEAT1 stimulates the differentiation of Th17 cells.[6] Overexpression of NEAT1, additionally, sustains the level of signal transducer and activator of transcription 3 protein (STAT3) and consequently, shifts the immune response to Th17 cells. The knockdown of NEAT1 might have a preventive effect on the development of arthritis as shown in vivo using mice CIA models.[41] Migration and invasion of RA-FLS were found to be stimulated by upregulation of NEAT1 and inhibited by its downregulation.[34] The data from that study concluded that NEAT1 encouraged cell viability, migration, invasiveness, and inflammatory cytokine secretion mainly of TNF-α and MMP-9 secretion from RA-FLS.

On the contrary to NEAT1, the current study revealed down regulation of miR-21 serum levels in RA patients. Moreover, within the RA group, a significant negative correlation was noted between miR-21 and RA disease duration and was even significantly lower in RA cases with than without bone erosions, while it was higher in patients of high disease activity group in comparison to those of remission and low disease activity group. Studies on miR-21 serum levels in RA are scanty, and this field requires more research to explain miR-21 effect in RA. In a recent study, and against our results, miR-21b levels were found to be higher in plasma of seropositive and seronegative RA patients than in healthy controls.[42] Several researches studied miR-21 levels in other tissues: Dong et al, for example, showed a significant lower level of miR-21 in PBMC from RA patients than in healthy controls. They attributed this to the possible impact of miR-21 on the biology of Treg cells, where the levels of miR-21 correlated negatively with the ratio of Th17/Treg cells in RA cases, indicating a shift towards pro-inflammatory arm and confirming that Treg cells are deregulated in RA disease. They concluded that miR-21 might work as a regulator for the T-cells differentiation and might offer a novel therapeutic objective for controlling RA disease.[17] Likewise, Yang et al found miR-21 and miR-125a also decreased in PBMC of RA cases compared to healthy controls.[35] Down regulation of miR-21, in their hypothesis, may hasten the immune response through variable signaling pathways, including phosphate and tension homology located on chromosome 10 and phosphatidylinositol 3 kinase pathways, which both may contribute to the pathogenesis of rheumatoid arthritis.[43] miR-21 was thought to promote NFκB nuclear translocation which activates the NF-κB pathway and increases RA-FLS proliferation.[44] Researchers, also, noticed that exosomal miR-21 released from mesenchymal stem cells (BMSC) in the bone marrow when incorporated into mouse FLSs it suppressed the production of inflammatory cytokines through the miR-21/TET1/KLF4 pathway. Exosomal miR-21 administration in Collagen-Induced Arthritis Affected mice (CIA-mice) appeared to reduce inflammatory-cell infiltration in synovial tissues.[45,46] This observation raises the possibility that miR-21 might work as an inflammatory process-regulator in RA shifting it towards anti-inflammatory response.

Serum miR-21 was also studied in other autoimmune diseases with conflicting results. While it was downregulated in Behcet's disease,[47,48] it was upregulated in ankylosing spondylitis[49] and SLE.[50]

In our study, IL17 expression level was significantly up regulated in sera of RA cases as compared to healthy individuals. Similarly, Al-Saadany et al reported that Th-17 cell proportions and IL-17 levels were higher significantly in RA cases in comparison to controls.[51] In a more recent study, IL-17 serum levels were also significantly higher in RA patients in comparison to healthy control proving its proinflammatory function in RA.[52]

Due to the widespread expression of IL17 receptors, Th17 cells are considered powerful inflammatory mediators that have several roles in many disorders, such as RA, lupus, inflammatory bowel diseases (IBD), psoriasis, and asthma.[53,54]

In RA, IL17 works on osteoblasts and synoviocytes adding to synovitis and joint degradation.[54,55] IL17 causes numerous changes within the RA synovium, distinguished by neoangiogenesis, hyperplasia, and invasion by immune cells. These alterations provoke the devastation of bone and cartilage.[56,57]

However, despite the robust statistical power, we were unable to establish a correlation between this high expression of IL17 in our RA group of patients and the level of disease activity determined by DAS28 –ESR score. On the contrary, Al-Saadany et al demonstrated a statistically significant association between percentages of Th-17 cells and IL-17 levels in their cohort, and both were related significantly with disease activity and MRI scores for joint synovitis and bone erosion.[51]

Limitations of the Study

Many limitations were effacing us while preparing the current study; these limitations included the relatively small sample size, the financial challenges and the time limitations. In addition, there is a notable scarcity of previous research that studied the same molecules in sera of RA patients. We recommend future research with a larger study population and better financial support to confirm our findings and explore the potential for their application.

Conclusion

Our results suggest that NEAT1, miR-21 and IL17 have differential levels in patients with RA where NEAT1 and IL17 have up regulation, while miR-21 has down regulation compared to healthy controls. Moreover, NEAT1 demonstrated a significant positive correlation with tender and swollen joint counts and with the overall DAS-28 score. NEAT1 was also found to be significantly higher in patients who had bone erosions on X- ray hands. These findings indicate that NEAT1 could be beneficial as a future biomarker for RA. MiR-21 demonstrated a negative correlation with disease duration; and its level was lower in patients who had erosions on X-rays. IL17 showed no correlation with disease parameters which might indicate that IL17, being an inflammatory cytokine, is plausibly elevated in RA patients, but is not necessarily a specific biomarker for the disease.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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