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Assessment of plasma microRNA potentials as a non-invasive biomarker in patients with axial spondyloarthropathy

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Abstract. – OBJECTIVE: It is assumed that abnormally expressed MicroRNAs (miRNAs) may be present in the plasma of patients with radiographic axial spondyloarthropathy (rad-AxSpA). Thus, the present study was conducted with the aim of investigating the expression profile of miRNAs in patients with rad-AxSpA.

PATIENTS AND METHODS: A total of 15 patients diagnosed with rad-AxSpA according to the Assessment of the SpondyloArthritis International Society (ASAS) classification criteria and nine healthy controls matched for age and gender were included in the study. Demographic data was collected, and disease activity was evaluated using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). Peripheral blood samples were collected, and miRNAs were extracted. The expression of microRNAs was analyzed using quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) by the miScript miRNA PCR Array Human Inflammatory Response and Autoimmunity.

RESULTS: A total of 84 miRNA profiles were evaluated, and expressions in the study and control groups were compared. When compared to the control group, 6 miRNAs (miR-125b-5p, miR-144-3p, miR-19a-3p, miR-20a-5p, miR-29c-3p, miR-30b-5p) were detected to be upregulated, and 42 miRNAs were detected to be downregulated in the rad-AxSpA group. A p-value < 0.05 was accepted as statistically significant. A significant association was found between miR-145-5p and BASDAI (p = 0.04941). MiR-144-3p, miR-302b-3p, miR-381-3p, miR-497-5p, miR-511-5p, and miR-9-5p were found to be significantly upregulated in the HLA-B27+ patients (p = 0.03063).

CONCLUSIONS: Abnormal miRNA expressions were detected in the plasma of the patients with rad-AxSpA. It was concluded that comprehensive studies should be continued to define these miRNAs as diagnostic biomarkers for rad-AxSpA in order to detect its association with Ankylosing Spondylitis disease activity.

Key Words:
Axial spondyloarthropathy, MiRNA, Biomarker.

Introduction

Spondyloarthropathy (SpA) is a chronic inflammatory disease that has a strong correlation with HLA-B27 and may influence the axial and peripheral joints[1]. The main challenges in the management of SpA include not clearly understanding the pathogenesis of the disease and not being able to use the disease activity-related biomarkers for the prediction of patient's response to therapy. Despite the close relationship between HLA-B27 and SpA, this cannot explain more than 30% of the genetic risks of SpA[2]. So, other genetic disorders that have not yet been explored may have the potential to influence SpA pathogenesis.

MicroRNAs (miRNAs) are non-coding RNAs that regulate the activity of target mRNAs. MiRNAs are the molecules that regulate cellular processes like apoptosis, cellular differentiation, and immune functions and that are stable in human plasma[3]. Abnormal expressions of circulating miRNAs (upregulation or downregulation) have emerged as potential biomarkers for the pathogenesis and activity of various disorders, like cardiovascular disorders, sepsis, and liver damage[4-7].

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Some researches[8-10] have indicated that miRNAs play an important role also in the regulation of immune cellular development. For example, the expression profile of circulating miRNAs has been reported to have the potential to detect systemic rheumatic diseases, like Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), and Multiple Sclerosis (MS).

However, comprehensive studies investigating miRNAs in patients with axial SpA (AxSpA) are not currently available. Such patients may have abnormally expressing miRNAs reflecting the inflammation. These irregular miRNAs may be detected through the miRNA expression profile; however, the expression profile of circulating miRNAs has not been completely investigated in AxSpA, and its diagnostic potential has not been evaluated comprehensively. Therefore, the aim of this study was to investigate the potential of miRNAs as novel non-invasive biomarkers in patients with radiographic axial SpA (rad-AxSpA) and their relationship with disease activity.

Patients and Methods

A total of 15 patients with rad-AxSPA who were admitted to the Physical Medicine and Rehabilitation Outpatient Clinic of Inonu University and met the diagnostic criteria from the Assessment of the Spondyloarthritis International Society (ASAS) (study group) and nine healthy volunteers matched for age and gender (control group) were included in the study. Clinical and demographic characteristics, like age, gender, disease duration, the use of biologic treatment, and HLA B27 positivity, were recorded.

C-reactive protein (CRP) levels were measured using a standard method. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores were calculated following a musculoskeletal system examination. Patients with BASDAI scores greater than 3.5 were accepted as the active group of Ankylosing Spondylitis.

Isolation of MiRNA and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Four ml of peripheral blood, which was collected from the patients and controls in EDTA tubes, was transferred to the laboratory. After collection, blood samples were centrifuged at 4°C and 4000 rpm for 15 min, and plasma was stored at -80°C until subsequent assay. miRNAs were purified from plasma using miRNeasy Serum/Plasma kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. c-DNA Synthesis was performed using miScript II RT kit (Qiagen, Frederick, MD, USA) according to the supplier. The expression of 84 miRNAs were analyzed using MIHS-105ZR-24 miScript miRNA PCR Array Human Inflammatory Response and Autoimmunity (Qiagen, Maryland, MD, USA), on a real-time PCR instrument (Rotor-Gene Q, Qiagen, Hilden, Germany). Gene expression profiles were generated in 96-well arrays using the custom miScript miRNA PCR array for the microRNAs according to manufacturer's instruction as follows: 15 min at 95°C for 1 cycle, 15s at 94°C, 30s at 55°C, and 30s at 70°C for 40 cycles using Rotor-Gene Q (Qiagen, Hiden, Germany). The CT values obtained from these studies for the analysis of the 84 miRNAs were recorded in an Excel file as raw data. Real-time PCR data were analyzed with a web-based software (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php). Average delta CT, $2^{-\Delta\Delta CT}$ fold change, *p*-value and fold regulation were calculated with the software. Relative gene expression levels were normalized to housekeeping genes (SNORD61, SNORD68, SNORD72, SNORD95, SNORD96A and RNU6-2) and the fold changes of the target gene(s) expression relative to those of the control group were analyzed by the $2^{-\Delta\Delta CT}$ method.

Inonu University Clinical Research Ethics Committee approval was obtained and this study was supported by Inonu University Scientific Research Projects Unit (BAP ID: 2016/182).

Results

Eighty-four miRNA profiles that were-well characterized in terms of response to inflammation and auto-immunity were evaluated in the present study, and the expressions in the patient (n = 15) and control (n = 9) groups were analyzed and compared. The demographic and characteristic features of the participants are shown in Table I.

MiRNAs with greater than two-fold regulation were accepted as upregulated, while miRNAs with less than two-fold regulation were accepted as downregulated. Additionally, those demonstrating any fold regulation between these values were accepted as having no change in expression.

When compared to the control group, 6 miR-

Table I. Clinical, laboratory and demographic characteristics of patient and control groups.

	Rad-AxSpA, patients (n = 15)	Healty controls (n = 9)	p
Age, years mean ± SD	38.1 ± 9.5	39.7 ± 6.3	0.18
Cinsiyet, male %	12 (85%)	8 (90%)	0.72
Disease duration, years ± SD	10.6± 8.9		
BASDAI score ± SD	3.58 ± 1.54		
Use of biological therapy positivity %	13 (90%)		
CRP, nmol/l positivity, %	10 (70%)		
HLA B 27 positivity %	9 (60%)		

Abbreviations: Rad-AxSpA, radiographic axial spondyloarthritis BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein.

NAs (miR-125b-5p, miR-144-3p, miR-19a-3p, miR-20a-5p, miR-29c-3p, miR-30b-5p) seemed to be upregulated, and 42 miRNAs seemed to be downregulated in the patient group (Table II, Figures 1, 2).

Afterwards, it was aimed to evaluate the relationship between circulating miRNA levels and disease activity parameters. A positive correlation was detected between miR-145-5p expression which was downregulated and BASDAI in rad-AxSpA patients (p=0.04941) (Table III).

When the miRNAs expression profiles were compared between the HLA-B27-positive and -negative patients, miR-144-3p, miR-302b-3p, miR-381-3p, miR-497-5p, miR-511-5p, and miR-9-5p were found to be significantly upregulated in the HLA-B27 positive patients (p = 0.03063) (Table IV).

Discussion

AxSpA is a chronic inflammatory disease that mainly affects the spine and sacroiliac joints[11-14].

Table II. Upregulated or down-regulated miRNAs compared to the control group.

miRNA	Fold regulation	miRNA	Fold regulation
Upregulated compared to the control group			
miR-125b-5p	2.59	miR-20a-5p	2.8*
miR-144-3p	2.83	miR-29c-3p	3.17
miR-19a-3p	2.14	miR-30b-5p	3.23
Downregulated compared to the control group			
let-7a-5p*	-5.62	miR-410-3p*	-5.22
let-7b-5p*	-4.38	miR-449a	-2.52
let-7c-5p	-3.63	miR-449b-5p*	-4.4
let-7d-5p	-2.44	miR-497-5p*	-3.25
let-7e-5p	-4.56	miR-511-5p	-2.3
let-7f-5p	-3.53	miR-513b-5p*	-3.66
miR-101-3p*	-2.71	miR-519c-3p*	-3.66
miR-130b-3p	-2.18	miR-519d-3p	-2.98
miR-1324	-2.93	miR-520d-3p	-2.88
miR-145-5p	-3.62	miR-520e*	-3.2
miR-181b-5p	-2.22	miR-524-5p*	-4.9
miR-181c-5p	-2	miR-545-3p*	-3.66
miR-181d-5p	-3.11	miR-548c-3p*	-2.62
miR-202-3p	-2.63	miR-548d-3p	-3.9
miR-20b-5p*	-2.89	miR-590-5p*	-2.38
miR-302a-3p*	-9.02	miR-607*	-3.66
miR-302b-3p*	-6.25	miR-655-3p*	-3.66
miR-302c-3p*	-6.05	miR-656-3p*	-3.85
miR-34a-5p*	-2.92	miR-875-3p	-4.03
miR-34c-5p	-3.07*	miR-9-5p*	-3.26
miR-381-3p	-3.89	miR-98-5p	-3.6

-*0.05 indicates significant.

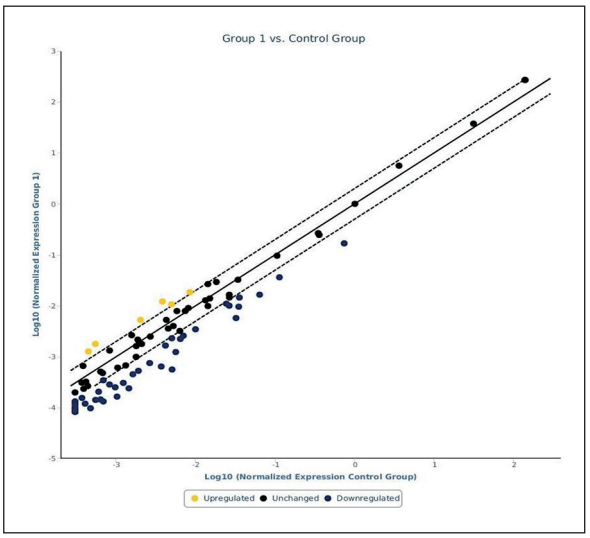

Figure 1. miRNAs whose expression is unchanged, upregulated and down-regulated compared to the control group.

The disease consequently leads to reduced spinal mobility, which may significantly impair daily living activities and quality of life[15-16]. So, early diagnosis is of great importance for the prevention of dysfunction and improvement of prognosis. However, there is a long delay between the onset of symptoms and the duration of diagnosis[17-19].

A significant proportion of miRNAs are detected to be acellular in the blood circulation[20].

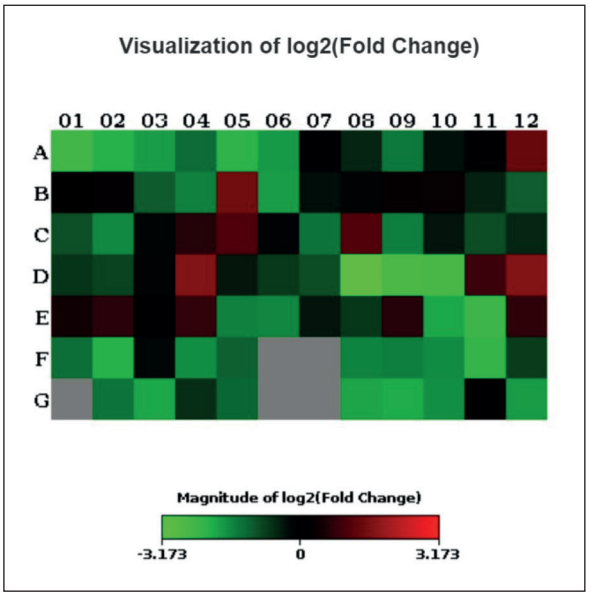

Figure 2. miRNAs fold changes in the patient group. -Reds indicate increased miRNA expression and green decreasing miRNA expression as compared to the control group.

Table III. miRNAs with altered expression in patients with a BASDAI score above 3.5.

miRNA	Fold regulation	*p*
miR-145-5p*	3.1517	0.04941

*Downregulated in the patient group compared with the control group.

Acellular circulating miRNAs appear to be a promising disease biomarker[21]. Altered miRNA expression and target gene disorders have been shown to contribute to the pathophysiology of many auto-immune disorders, including rheumatic disorders[9]. Alterations have been detected in the expression of miRNAs in the T cells and peripheral blood mononuclear cells of AxSpA patients[22,23].

In the present study, irregular miRNA expressions were detected in the rad-AxSpA patient group. Of these miRNAs, six (miR-125b-5p, miR-144-3p, miR-19a-3p, miR-20a-5p, miR-29c-3p, miR-30b-5p) were detected to be upregulated, and 42 were detected to be downregulated. miR-145-5p demonstrated a significantly high expression irregularity in the active rad-AxSpA patient group. Moreover, miR-144-3p miR-302b-3p, miR-381-3p, miR-497-5p, miR-511-5p, and miR-9-5p levels had significantly different expression profiles in the HLA-B27-positive patients.

Magrey et al[24] screened 175 miRNAs in the plasma samples of patients with AxSpA and healthy controls and detected that the miRNAs selected in the plasma demonstrated irregular expression: two miRNAs were upregulated (miR-32 and miR-34a), five miRNAs were downregulated (miR-16, miR-150, miR-10b, miR-30a, miR-154), and the miR-32 expression profile was generally high among the HLA-B27-positive patients.

Table IV. miRNAs with variable expression in HLA-B27 positive patients.

miRNA	Fold regulation	*p*
miR-144-3p*	4.1411	0.03063
miR-302b-3p**	3.0071	0.01546
miR-381-3p**	8.4425	0.03196
miR-497-5p**	4.8202	0.01709
miR-511-5p**	5.5804	0.03692
miR-9-5p**	4.7775	0.0358

*Upregulated in the patient group compared with the control group. **Downregulated in the patient group compared with the control group.

Qian et al[25] detected two miRNAs (miR-146a and miR-155) as upregulated in the serum of the patients with AS when compared with controls. Receiver Operating Characteristic (ROC) analysis has revealed that these two miRNAs could serve as novel biomarkers for the diagnosis of AS. MiRNA-155 was observed to be significantly higher in AS patients whose thoracic kyphosis angle was above 70 degrees compared to patients whose thoracic kyphosis angle was below 70 degrees[25].

In another study, miRNAs were isolated from the plasma of patients with progressive spinal disease. MiR-625-3p levels was found to be significantly different between patients with non-radiographic AxSpA (nr-AxSpA) and healthy controls. Moreover, 18 miRNAs were shown to have lower expression levels in the AS patients compared to the healthy controls, of which 14 exhibited lower expression levels among the rad-AS patients when compared to the nr-AxSpA patients. A relationship was not found between miRNA and BASDAI, however, a correlation was found between miRNA and disease activity in the nr-AxSpA patients[26].

Another study that included following 800 miRNAs showed the altered expression of six plasma miRNAs (miR-146a-5p, miR-125a-5p, miR-151a-3p, miR-22-3p, miR-150-5p and miR-451a) in AS patients. It has been demonstrated that there is a relationship between these six miRNAs and potential target proteins related to AS pathophysiology. Additionally, the expression levels of miR-146a-5p, miR-125a-5p, and miR-22-3p were found to be significantly elevated in active AS patients[27].

Conclusions

Abnormal expressions of miRNA were detected in patients with rad-AxSpA in the present study. The expression levels of some miRNAs were significantly higher in the active disease group.

The results of the present study support the role of miRNAs in the pathogenesis of AxSpA and as biomarkers of disease progression. However, additional studies that include larger patient groups are required in order to verify this data, because only a small part of the circulating miRNAs was analyzed, and the functions of many miRNAs are not currently known.

The presence of defined miRNAs in plasma, whether it is associated with disease activity indices and structural bone damage should be evaluated with more comprehensive studies.

All previous studies have indicated that research in this field is still at an early stage. Studies evaluating the expression profile of miRNAs in AxSpA are still lacking, and no definitive conclusion has been reached regarding the diagnostic potential of miRNAs. Further comprehensive studies that evaluate the potential of serum miRNAs could continue to help define the diagnostic biomarkers of AxSpA, understand its pathogenetic mechanisms, improve the therapeutic efficacy of AxSpA treatment, and design new treatment strategies.

This study will contribute to the investigation into the potential of miRNAs as a non-invasive biomarker in AxSpA and their relationship with disease activity.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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