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Abstract

Serum microRNAs (miRNAs) in patients suffering from ankylosing spondylitis (AS) have rarely been studied. This study aims to identify the Ankylosing spondylitis-specific miRNAs in the sera of AS patients to correlate miRNA-214 expression levels at early and late stages of the disease.

Serum miRNA-214 as a Novel Biomarker for Early-Stage Ankylosing Spondylitis

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This prospective case-control study included 150 participants. The classification was based on the interval between the first spondylo-arthropathic symptom and diagnosis of AS. Disease activity was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score with C-reactive protein (ASDAS-CRP). The patients were then classified into early and late-diagnosis groups using the axial spondyloarthritis classification criteria set by ASAS. Fifty had early-stage AS, fifty had late-stage AS, and fifty individuals were healthy. The patient's sex, age, family history, and disease duration were recorded. 3 ml of venous blood was drawn from each individual to extract RNA and measure the expression of microRNA-214 by RT-PCR.

In screening for groups, the levels of miRNA-214 expression were compared between AS patients and a healthy control group. The patient group's mean level of miRNA-214 was noticeably higher. In particular, the control group's mean miRNA-214 level was 1.204 ± 0.12, while the patients were 2.412 ± 0.20. This study also shows that miRNA-214 levels were significantly higher in early-stage patients than in late-stage patients ($p = 0.0086$). Finally, serum miRNA-214 expression levels were validated in AS patients by qPCR.
Elevated miRNA-214 expression provides valuable insights into the molecular underpinnings of AS. These findings suggest that miRNA-214 is a significant biomarker for early detection and for differentiating disease stages, which is crucial for improving patient prognosis and preventing irreversible structural damage.

Keywords: Ankylosing Spondylitis (AS), miRNA-214, BASDAI, ASDAS-CRP.

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory Rheumatism that mostly affects the axial skeleton. The disease involves a progressive inflammatory process that leads to ossification of ligamentous structures, resulting in significant physical disability and reduced quality of life (1).

MicroRNA (miRNA) is an evolutionarily well-conserved noncoding RNA oligonucleotide. They act as crucial regulators of post-transcriptional gene expression (2). Over 1000 miRNAs are encoded in the human genome, affecting about 60% of all protein-coding genes. (3). MiRNAs are reported to be present in human serum. Hence, the potential of using these serum miRs as biomarkers for disease differentiation warrants exploration (4). miR-214 has emerged as a promising non-invasive biomarker for early AS. miR-214 plays a dual role in regulating both inflammation and the abnormal bone formation characteristic of (5).

microRNA-214 (miR-214) acts as a biological regulator of bone metabolism. Its levels fluctuate depending on whether the disease is in an inflammatory state—miR-214 Levels Early vs. Late Stage (6,7). In early AS, the body is often in a high state of inflammation, driven largely by IL-17A. Studies have shown that IL-17A stimulates miR-214 production. High levels of miR-214 inhibit bone-forming cells (osteoblasts) and are transferred to bone-eating cells (osteoclasts) to ramp up their activity. This explains why many patients experience osteoporosis early in the disease course (8).

As the disease progresses to the late stage, the focus shifts to pathological new bone formation (ankylosis). At the site of the spinal ligaments, miR-214 is often downregulated. miR-214 normally acts as an inhibitor of BMP-2 (a potent bone-growth protein). When miR-214 levels drop, the BMP-2 signaling pathway goes into overdrive, causing fibroblasts to transform into bone-forming cells. This is what creates the "bamboo spine" appearance on X-rays (9). The aim of this study is to identify ankylosing spondylitis-specific miRNA in sera of AS patients and correlate the miRNA-214 expression levels at an early and late stage of the disease.

Materials & Methods

Study Design

This prospective case-control study enrolled 150 participants: 100 patients diagnosed with AS, and 50 healthy controls. The diagnosis of AS was made according to either the new or modified criteria for diagnosis of AS, or according to the axial spondyloarthritis classification criteria set by ASAS. This qualification was conducted at the Rheumatology and Rehabilitation Consultation Clinic at the Medical City-Baghdad/Iraq, between October 2024 and February 2025. The patient has been interviewed to collect demographic and clinical data; investigations have been performed, and consent was obtained from all patients enrolled in the study.

The control group comprised 50 healthy individuals with no history of autoimmune, inflammatory, or bone-related diseases. Controls were age and sex-matched to the patient group to ensure comparability.

Inclusion criteria:

Patients aged 18-65 years who met the modified New York criteria for AS or the Assessment of

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Spondylo-Arthritis International Society (ASAS) classification criteria for axial spondyloarthritis were eligible, with a disease duration of ≥6 months and a stable treatment regimen for ≥3 months before enrollment.

Exclusion Criteria

Encompassed active infections within 4 weeks, malignancies, pregnancy or lactation, concurrent autoimmune diseases, recent vaccination (within 4 weeks), and use of investigational drugs within 3 months. Patients with significant hepatic (ALT/AST >2× upper limit of normal) or renal dysfunction (eGFR <60 mL/min/1.73m²) were also excluded.

Clinical Assessment

Comprehensive clinical evaluation included demographic data collection, detailed medical history, and physical examination. Disease activity was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score with C-reactive protein (ASDAS-CRP).

Laboratory Investigations

3 ml of peripheral blood samples were collected in the morning, between 08:00 and 10:00 AM, after fasting overnight for 12 hours to minimize circadian variations. Samples were centrifuged at 3,000 rpm for 15 minutes at 4°C within two hours of collection, and aliquots were stored at -80°C until batch. Using miScript systems-based kits, reverse transcription was performed, followed by qPCR for miR expression analysis on the cDNA template using predefined miScript PCR primers (Qiagen). A relative quantification was performed using the miScript system (Qiagen) with miR-specific primers for qPCR. Comparisons from one lab to another are somewhat difficult as no accepted normalizer for circulating miRNA exists, for which reason a synthetic spike-in of cel-miR-39-3p was introduced during the RNA extraction for technical normalization of Qpcr using published protocols. The conversion of Cycle threshold values to copy number was based on a standard curve established by the chemical spike-in control. Subsequently, the copy number was normalized to the recovery ratios of cel-miR-39-3p spiking for accurate quantification. All clinical findings were verified by rheumatologists. Clinical data included age, sex, duration of the disease, and family history of AS.

Early and late-stage ankylosing spondylitis

Age at disease onset was defined as the date of the first appearance of AS-related symptoms. These symptoms included Inflammatory Back Pain (IBP), peripheral symptoms, such as arthritis or enthesitis, and uveitis. IBP was defined according to the ASAS criteria. Diagnostic delay was defined as the duration (years) between symptom onset and the time of diagnosis. Assessments of patients' disease status were made using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score with C-reactive protein (ASDAS-CRP). Inflammatory markers, including serum C-reactive protein (CRP) in mg/l and the erythrocyte sedimentation rate (ESR) in mm/h, were recorded in Table 1. The patients were then classified into early and late-stage diagnosis groups based on the median interval of the diagnostic delay. We compared multiple clinical parameters between the early and late diagnosis groups. To identify factors related to the delayed diagnosis of AS.

Statistical analysis

Comparisons between study groups were conducted using t-tests, and differences in means

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between the two groups were statistically calculated (at a significance level of $P \leq 0.05$). Additionally, the Statistical Package for the Social Sciences (SPSS) software—2019 version—was used to analyze differences (distinctions) among the various groups regarding the study indicators.

Table 1: Comparison of the factors between groups of patients with AS, early, and late stages

Factors	Early stage < 5 *years* (n=50)	late stage > 5 *years* (n=50)	P value
BASDAI	5.83 ±0.22	5.33 ±0.23	0.128
ASDAS-CRP	2.3 ± 0.8	1.9 ± 0.7	0.152
ESR (mm/h)	17.82 ±1.69	20.76 ±1.89	0.250
CRP (mg/l)	5.40 ±0.45	5.04 ±0.42	0.506

Results:

Participants were male, accounting for 74% of the sample, while females accounted for 26%. The highest percentage was in the 30-40-year age group (43%), and the lowest in the age group over-50 age group (11%). The distribution across age groups was statistically significant ($p < 0.01$). The findings indicated that 70% have a family history. The duration of the condition varied among participants. The largest group had the condition for less than 5 years (58%), followed by those with 5-10 years (24%) Table 2.

Table 2: Distribution of the sample study according to sex, Age, Family history, and Duration of disease

Factor		No Total =100	%	P-value
Gender	Male	74	74.00	<0.001
	Female	26	26.00	
Age groups (year)	<30 yr.	27	27.00	<0.001
	30-40 yr.	43	43.00	
	41-50	19	19.00	
	>50 yr.	11	11.00	
Family history	Yes	70	70.00	<0.001
	No	30	30.00	
Duration	<5 yr.	58	58.00	<0.001
	5-10 yr.	24	24.00	
	11-15 yr.	10	10.00	
	>15 yr.	8	8.00	

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Level of miRNA-214 expression in the AS and control

Compared to the control group, the patient group's mean miRNA-214 level was noticeably higher ($p<0.001$). In particular, the control group's mean miRNA-214 level was 1.204 ±0.12, while the patients' was 2.412 ±0.20. The results of a T-test analysis showed a statistically significant difference between the two groups, with a T-test value of 0.591 and a $P\leq0.01$. This finding raises the possibility of a link between ankylosing spondylitis and increased levels of miRNA-214, which could indicate a function for the protein as a biomarker or a role in the pathophysiology of the illness, as shown in Table 3.

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Table 3: Comparison between patients and control groups in miRNA214 expression

Group	Means ±SE of miRNA214
Patients	2.412 ±0.20
Control	1.204 ±0.12
T-test	0.591 **
P-value	<0.001

Level of miRNA-214 expression in early and late stages of AS patients

The study also shows that the level of miRNA-214 was significantly higher in patients with early-stage disease than in those with late stage disease with mean values of 2.939±0.37 and 1.886±0.12, respectively ($p=0.0086$). This investigation suggests that miRNA-214 may be a biomarker for differentiating between the early and late phases of AS, as shown in Table 4)

Table 4: Comparison between patients with late disease **and** early disease **groups in miRNA214 expression**

Group	Means ±SE of miRNA214
Patients/ Late disease	1.886 ±0.12
Patients/ early disease	2.939 ±0.37
T-test	0.779 **
P-value	0.0086

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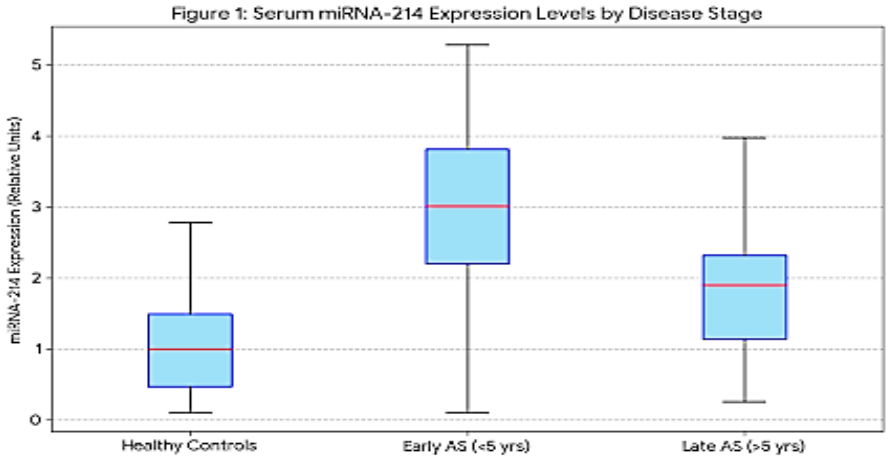

Figure 1: Box plot showing miRNA-214 expression across Controls, Early, and Late AS

Discussion

The significantly higher levels of miRNA214 in AS patients suggest its potential role as a diagnostic biomarker. Because AS is often difficult to diagnose in its early stages (sometimes requiring years to confirm via X-ray), identifying molecular signatures such as miRNA214 is a major focus of current rheumatology research (10).

In Ankylosing Spondylitis, the primary pathological issues are chronic inflammation and syndesmophyte formation (new bone growth that fuses the spine). miRNA214 is known to regulate genes controlling how bone-building cells function, and the Wnt/β-catenin signaling pathway, a key pathway that, when dysregulated, leads to the spinal fusion seen in AS (11). Kinne et al. reported that various miRNAs, including the miR-200 family and miR-214, are differentially expressed in the serum of AS patients. Their findings support the idea that these molecules circulate at higher levels during active disease states (12). Chen et al. (2019).

Studies of bone metabolism have identified miRNA-214 as a master regulator of bone cell cross-talk. Specifically, it has been shown to inhibit ATF4, a protein essential for bone formation. High miRNA214 may be a compensatory mechanism or a direct driver of the imbalanced bone remodeling seen in the disease (10). Logue et al. (2021). Compared to healthy individuals, AS patients frequently exhibit a "pro-inflammatory miRNA signature (13).

The results indicating a difference in miR-214 expression between early and late-stage Ankylosing Spondylitis (AS) reflect a critical shift in the disease's biology from an active inflammatory state to a structural remodeling state (ankylosis) (14). The late-stage patients likely show significantly lower levels of miR-214 compared to early-stage patients. A landmark study published in the International Journal of Rheumatic Diseases found Kook, Hyun Yi, et al. (2019) that miR-214 is consistently lower in AS patients than in those with Rheumatoid Arthritis (RA) or healthy individuals (15). This suggests that miR-214 is not just a general marker of joint pain but is specific to the unique bone-forming

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pathology of AS. The most significant finding in late-stage AS is the role of miR-214 in syndesmophyte (bone bridge) formation. A study by Ding, Lixiang, et al (2021) proved that miR-214 directly targets and inhibits BMP-2 (Bone Morphogenetic Protein 2) (16). In early AS, Higher miR-214 levels keep BMP-2 in check, preventing rapid bone growth. In Late AS, the loss or downregulation of miR-214 on the BMP-TGFβ axis. This leads to the pathological overgrowth of bone that eventually causes spinal fusion (16).

Conclusion

Elevated levels of miRNA-214 in patients with ankylosing spondylitis provide valuable insights into the molecular underpinnings of the disease. When viewed alongside the aforementioned research on miRNA-214 and bone metabolism, these findings open exciting avenues for future investigation and for understanding and managing ankylosing spondylitis. In this way, early detection and treatment improve patient prognosis and prevent irreversible damage from advanced disease.

Ethics approval

Consent was obtained verbally after all study participants were informed of the procedures. The study protocol, subject information, and the informed consent form were examined and approved by the local college and hospital ethics committee under document number. [IRB: 262, 26/12/2024].

Conflicts of interest: nil

Author contributions

All authors contributed to the collection of data, the preparation of the draft, and the review and approval of the final version of the manuscript.

REFERENCES

1. M. Ivanova, O. Zimba, I. Dimitrov, A. K. Angelov, and T. Georgiev, "Axial spondyloarthritis: an overview of the disease," *Rheumatol. Int.*, vol. 44, no. 9, pp. 1607–1619, 2024. https://doi.org/10.1007/s00296-024-05601-9
2. J. A. Oo, R. P. Brandes, and M. S. Leisegang, "Long non-coding RNAs: novel regulators of cellular physiology and function," *Pflügers Archiv-European Journal of Physiology*, vol. 474, no. 2, pp. 191–204, 2022. https://doi.org/10.1007/s00424-021-02641-z
3. R. N. Mustafin, "The relationship of transposable elements with non-coding RNAs in the Emergence of Human Proteins and Peptides," *Curr. Proteomics*, vol. 21, no. 3, pp. 140–161, 2024. https://doi.org/10.2174/0115701646319572240805103747
4. R.-J. Zhao, W.-Y. Zhang, and X.-X. Fan, "Circular RNAs: Potential biomarkers and therapeutic targets for autoimmune diseases," *Heliyon*, vol. 10, no. 1, 2024. https://doi.org/10.1016/j.heliyon.2023.e23694
5. K. Mocová, "Screening for circulating biomarkers characterizing early axial spondyloarthritis and progression of the disease," *dspace.cuni.cz.* 2025. https://doi.org/10.26717/bjstr.2018.04.0001098
6. G. Lopalco, A. Cito, F. Iannone, et al. Proft, "Beyond inflammation: the molecular basis of bone remodeling in axial spondyloarthritis and psoriatic arthritis," *Front. Immunol.*, vol. 16, p. 1599995, 2025. https://doi.org/10.3389/fimmu.2025.1599995
7. Sun Y, Kuek V, Liu Y, et al. MiR-214 is an important regulator of the musculoskeletal metabolism and disease. *Journal of Cellular Physiology*. 2019 Jan;234(1):231-45. https://doi.org/10.1002/jcp.26856
8. Z. Liu *et al.*, "miR-214 stimulated by IL-17A regulates bone loss in patients with ankylosing spondylitis," *Rheumatology*, vol. 59, no. 5, pp. 1159–1169, 2020. https://doi.org/10.1093/rheumatology/kez594
9. L. Ding *et al.*, "microRNA-214-3p Suppresses Ankylosing Spondylitis Fibroblast Osteogenesis via BMP–TGF β Axis and BMP2," *Front. Endocrinol. (Lausanne).* vol. 11, p. 609753, 2021. https://doi.org/10.3389/fendo.2020.609753

AIMCJ. (2026),3; (1):103-110
https://doi.org/10.58564/AIMCJ3.2.20226.274

10. Liu Z, Huang F, Luo G, et al. miR-214 stimulated by IL-17A regulates bone loss in patients with ankylosing spondylitis. *Rheumatology*. 2020 May 1;59(5):1159-69. https://doi.org/10.1093/rheumatology/kez594
11. Sun Q, Liu S, Feng J, et al. Current status of MicroRNAs that target the Wnt signaling pathway in regulation of osteogenesis and bone metabolism: a review. Medical Science Monitor: *International Medical Journal of Experimental and Clinical Research*. 2021 Apr 8;27:e929510-1. https://doi.org/10.12659/MSM.929510.
12. Humphries B, Yang C. The microRNA-200 family: small molecules with novel roles in cancer development, progression and therapy. *Oncotarget*. 2015 Jan 30;6(9):6472. https://doi.org/10.18632/oncotarget.3052
13. Li J, Xie X, Liu W, et al. MicroRNAs as biomarkers for the diagnosis of ankylosing spondylitis: a systematic review and meta-analysis. Frontiers in Medicine. 2021 Aug 10;8:701789.. https://doi.org/10.3389/fmed.2021.701789
14. Liao HT, Tsai CY, Lai CC, et al. The potential role of genetics, environmental factors, and gut dysbiosis in the aberrant non-coding RNA expression to mediate inflammation and osteoclastogenic/osteogenic differentiation in ankylosing spondylitis. *Frontiers in Cell and Developmental Biology*. 2022 Jan 20;9:748063. https://doi.org/10.3389/fcell.2021.74806
15. Kook HY, Jin SH, Park PR, et al. Serum miR-214 as a novel biomarker for ankylosing spondylitis. *International Journal of Rheumatic Diseases*. 2019 Jul;22(7):1196-201. https://doi.org/10.1111/1756-185X.13475
16. Ding L, Yin Y, Hou Y, et al. microRNA-214-3p Suppresses Ankylosing Spondylitis Fibroblast Osteogenesis via BMP–TGF β Axis and BMP2. *Frontiers in Endocrinology*. 2021 Apr 15;11:609753. https://doi.org/10.3389/fendo.2020.609753