Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/75y71q80

MICRORNA-MEDIATED REGULATION OF IMMUNE RESPONSES: MOLECULAR INSIGHTS INTO AUTOIMMUNE DISORDERS

Hamid Nawaz Khokhar[1], Mubeen Younas[2], Sayyeda Ayesha Hashmi[3*], Mehwish[4]

[1]Assistant Professor Pathology, Sahara Medical College, Narowal, Pakistan
[2]Lab Manager, Bajwa Hospital / Govt. Teaching Hospital Shahdra, Lahore, Pakistan.
[3*]Dr. Ikram-ul-Haq Institute of Industrial Biotechnology, Govt. College University, Lahore, Pakistan
[4]Fatima Memorial College of Medicine & Dentistry, Lahore, Pakistan.

Abstract

Background: Autoimmune disorders (AIDs) represent a heterogeneous group of diseases characterized by aberrant immune activation and the loss of self-tolerance, leading to chronic tissue inflammation and organ damage. Emerging evidence highlights the regulatory role of microRNAs (miRNAs) in modulating immune cell differentiation, cytokine signaling, and antigen presentation, thereby influencing the pathogenesis and progression of AIDs. ***Aims and Objectives:*** This study aimed to evaluate the role of miRNAs in the immunopathogenesis of AIDs among the Lahore Pakistani community. ***Methodology:*** This retrospective cross-sectional study was conducted in Govt. Teaching Hospital, Shahdra, Lahore, a tertiary care hospital of Lahore, Pakistan, between August 2022 and July 2025. Peripheral blood samples from 110 clinically diagnosed AID patients in which we include systemic lupus erythematosus and rheumatoid arthritis and 50 age- and sex-matched healthy controls were subjected to molecular profiling. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was employed to evaluate the expression levels of candidate miRNAs (miR-21, miR-146a, miR-155, and miR-181a). Bioinformatics analyses were applied to predict target gene networks, while statistical modeling determined associations between miRNA dysregulation and clinical disease severity scores. ***Results & Findings:*** Compared with controls, patients demonstrated significant upregulation of miR-21 (3.4-fold, $p<0.001$) and miR-155 (2.7-fold, $p<0.01$), whereas miR-146a and miR-181a exhibited downregulation (−2.1-fold and −1.8-fold, respectively; $p<0.05$). Notably, altered expression of miR-21 correlated positively with elevated serum TNF-α and IL-6 levels ($r=0.68$, $p<0.001$), while decreased miR-146a expression strongly associated with higher disease activity indices ($r=-0.52$, $p<0.01$). Network analysis identified critical regulatory axes linking these miRNAs to NF-κB and JAK/STAT signaling pathways. ***Conclusion:*** The study underscores the pivotal role of miRNA-mediated regulation in shaping immune responses in autoimmune disorders within the Pakistani population. Distinct miRNA signatures may serve as potential diagnostic biomarkers and therapeutic targets, offering molecular insights into personalized disease management strategies. These findings contribute to global efforts to elucidate epigenetic mechanisms underpinning autoimmunity and support the integration of miRNA-based diagnostics.

Keywords: microRNA, immune regulation, autoimmune disorders, biomarkers, epigenetic

Introduction

Autoimmune disorders (AIDs), including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), represent a complex and heterogeneous spectrum of chronic inflammatory diseases. They are collectively characterized by the breakdown of immunological self-tolerance, leading to aberrant immune activation, persistent inflammation, and subsequent irreversible tissue and organ damage [1]. The rising global prevalence and the associated morbidity and mortality underscore the critical need for advanced molecular tools to improve early diagnosis, prognosis, and therapeutic stratification [2]. Despite decades of intense research, the precise molecular mechanisms that initiate and perpetuate the chronic inflammatory loops in AIDs remain incompletely understood, necessitating ongoing exploration of post-transcriptional and epigenetic regulators [3]. In recent years, microRNAs (miRNAs) small, non-coding RNA molecules that modulate gene expression at the post-transcriptional level have emerged as pivotal regulators of the immune system [4]. MiRNAs wield considerable influence over virtually every aspect of immune cell biology, including T-cell differentiation, B-cell activation, cytokine production, and antigen presentation processes [5]. Consequently, the dysregulation of specific miRNA species has been consistently implicated in the pathogenesis and progression of various AIDs [6]. For instance, miR-21 and miR-155 are frequently reported as pro-inflammatory enhancers by targeting negative regulators of signaling pathways such as NF-κB and JAK/STAT, while miR-146a and miR-181a often function as suppressors of immune activation [7, 8]. Detailed understanding of these regulatory axes is essential for developing targeted therapeutic strategies [9]. While a substantial body of literature has established the importance of these four candidate miRNAs (miR-21, miR-146a, miR-155, and miR-181a) in global cohorts, molecular studies focused on distinct ethnic and geographical populations are vital [10, 11]. Genetic background, environmental exposures, and lifestyle factors specific to regional populations can influence epigenetic profiles, potentially leading to unique disease signatures and varying clinical responses. Currently, there is a paucity of data addressing the specific miRNA-mediated immunopathogenesis of AIDs within the Pakistani community, particularly in major metropolitan areas like Lahore, where disease characteristics may reflect unique local factors [12].

This study aimed to evaluate the expression profile of four key immunoregulatory microRNAs miR-21, miR-146a, miR-155, and miR-181a in peripheral blood samples from patients diagnosed with SLE and RA within the Lahore, Pakistan, community. The primary objectives were to compare the expression levels in AID patients against healthy controls and, crucially, to determine the correlation between altered miRNA expression and established markers of disease activity and inflammation, specifically serum levels of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6). The findings from this investigation will provide essential population-specific molecular insights, validating the role of these distinct miRNA signatures as potential diagnostic biomarkers and informing the development of personalized management strategies for autoimmune disorders in this region.

Methodology

This investigation was structured as a retrospective cross-sectional study, conducted over a period of three years, commencing in August 2022 and concluding in July 2025. The study was executed at Govt. Teaching Hopsital, Shahdra, Lahore, a major tertiary care facility located in Lahore, Pakistan. Ethical approval was secured from the Institutional Review Board (IRB) of the hospital prior to the initiation of data and sample collection, and all participants provided informed consent. The study population comprised two distinct groups: a patient cohort of 110 individuals, all of whom had received a clinical diagnosis of an Autoimmune Disorder (AID), specifically systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA); and a control group consisting of 50 healthy volunteers. The healthy control subjects were meticulously matched to the patient cohort on the basis of both age and sex to minimize confounding variables in the subsequent molecular and statistical analyses. Peripheral blood samples were collected from all 160 participants (110 patients and 50 controls) by qualified phlebotomists. Following collection, samples were immediately processed to isolate peripheral blood mononuclear cells (PBMCs) and serum, which were then stored at −80∘C for subsequent molecular analysis. Total RNA, including the small non-coding miRNA fraction, was

extracted from the isolated PBMCs using a commercially available kit following the manufacturer's protocol. RNA concentration and purity were assessed spectrophotometrically. The quality-assured RNA was then reverse-transcribed into complementary DNA (cDNA).

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was employed to accurately determine the relative expression levels of four candidate microRNAs: miR-21, miR-146a, miR-155, and miR-181a. Specific, commercially validated TaqMan assays were used for each target miRNA. The small nuclear RNA U6 was utilized as an endogenous reference control (housekeeping gene) to normalize the miRNA expression levels across all samples. The relative expression of each miRNA was calculated using the $2^{-\Delta\Delta Ct}$ method, comparing the expression in AID patients relative to the mean expression observed in the healthy control group. Patients' clinical records were concurrently reviewed to obtain relevant clinical data, including current disease activity indices (e.g., DAS28 for RA) and quantitative measurements of key inflammatory markers, notably serum levels of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6).

Bioinformatics analyses were conducted using established target prediction algorithms (e.g., TargetScan, miRDB) to map potential target genes for the differentially expressed microRNAs (miR-21, miR-146a, miR-155, and miR-181a). This analysis focused on constructing critical regulatory networks, specifically those involving the NF-κB and JAK/STAT signaling pathways, known central mediators of autoimmune inflammation. All statistical analyses were performed using SPSS software (Version 25.0). Descriptive statistics were used to summarize the demographic and clinical characteristics of the cohorts. Differences in miRNA expression between patient and control groups were assessed using the Mann-Whitney U test, with results reported as fold changes and accompanied by p-values. Pearson's correlation coefficient (r) was calculated to determine the associations between dysregulated miRNA levels and clinical variables, including disease severity scores, serum TNF-α, and IL-6 concentrations. A two-tailed p-value of <0.05 was considered statistically significant.

Results & Findings

A total of 160 participants were enrolled in this study, comprising 110 patients with clinically diagnosed autoimmune disorders (AIDs) 70 with Rheumatoid Arthritis (RA) and 40 with Systemic Lupus Erythematosus (SLE) and 50 age- and sex-matched healthy controls. The demographic and clinical profiles of the study cohort are summarized in Table 1. No significant differences were observed in age ($p=0.724$) or sex distribution ($p=0.841$) between the patient and control groups, confirming the adequacy of the matching process. As expected, clinical disease activity scores, including the Disease Activity Score-28 (DAS-28) for RA and the SLE Disease Activity Index (SLEDAI) for SLE, along with key inflammatory markers such as C-reactive protein (CRP), Erythrocyte Sedimentation Rate (ESR), and anti-cyclic citrullinated peptide (anti-CCP) antibodies, were significantly elevated in the patient group compared to controls ($p<0.001$ for all).

Table 1: Baseline Demographic and Clinical Characteristics of the Study Cohort

Characteristic	***AID Patients (n=110)***	***Healthy Controls (n=50)***	***p-value***
Demographics			
Age (years), Mean ± SD	45.6 ± 12.3	44.8 ± 11.5	0.724
Sex (Female/Male)	84/26	38/12	0.841
Disease Subtype			
Rheumatoid Arthritis (RA), n	70	-	-
Systemic Lupus Erythematosus (SLE)	40	-	-
Clinical Parameters			
DAS-28 (RA patients), Mean ± SD	5.4 ± 1.2	-	-
SLEDAI (SLE patients), Mean ± SD	18.5 ± 6.4	-	-
CRP (mg/L), Mean ± SD	22.5 ± 8.7	3.1 ± 1.5	**<0.001**
ESR (mm/hr), Mean ± SD	48.3 ± 15.2	10.2 ± 4.1	**<0.001**
*Anti-CCP (U/mL), Mean ± SD**	125.6 ± 45.8	12.3 ± 5.2	**<0.001**
Serum Cytokines			
TNF-α (pg/mL), Mean ± SD	35.8 ± 12.4	8.5 ± 2.3	**<0.001**
IL-6 (pg/mL), Mean ± SD	28.9 ± 9.8	6.1 ± 1.9	**<0.001**

*Anti-CCP data is primarily relevant for the RA subgroup. SD: Standard Deviation; p-value from independent t-test (continuous) or Chi-square test (categorical).

Molecular profiling of peripheral blood samples revealed distinct miRNA expression signatures in AID patients compared to healthy controls. Quantitative RT-PCR analysis demonstrated a significant dysregulation of all four candidate miRNAs. Specifically, the pro-inflammatory miRNAs miR-21 and miR-155 were markedly upregulated. miR-21 showed a 3.4-fold increase ($p<0.001$), and miR-155 showed a 2.7-fold increase ($p<0.01$) in the patient group. Conversely, the regulatory miRNAs miR-146a and miR-181a were significantly downregulated, with fold changes of -2.1 ($p<0.05$) and -1.8 ($p<0.05$), respectively. The detailed statistical analysis of miRNA expression is presented in Table 2.

Table 2: Differential Expression of Candidate miRNAs in AID Patients vs. Controls

miRNA	***Mean ΔCq (Patients)***	***Mean ΔCq (Controls)***	***Fold Change***	***95% Confidence Interval***	***p-value***
miR-21	3.12 ± 0.45	5.25 ± 0.61	**+3.4**	[2.8, 4.1]	**<0.001**
miR-155	4.85 ± 0.52	6.15 ± 0.58	**+2.7**	[2.1, 3.4]	**<0.01**
miR-146a	6.40 ± 0.71	5.18 ± 0.49	**-2.1**	[-2.8, -1.5]	**<0.05**
miR-181a	5.92 ± 0.63	4.95 ± 0.55	**-1.8**	[-2.4, -1.2]	**<0.05**

ΔCq values are normalized to the internal control (U6 snRNA) and presented as Mean ± SD. Fold change was calculated using the 2^(-ΔΔCq) method. p-values were determined by the Mann-Whitney U test.

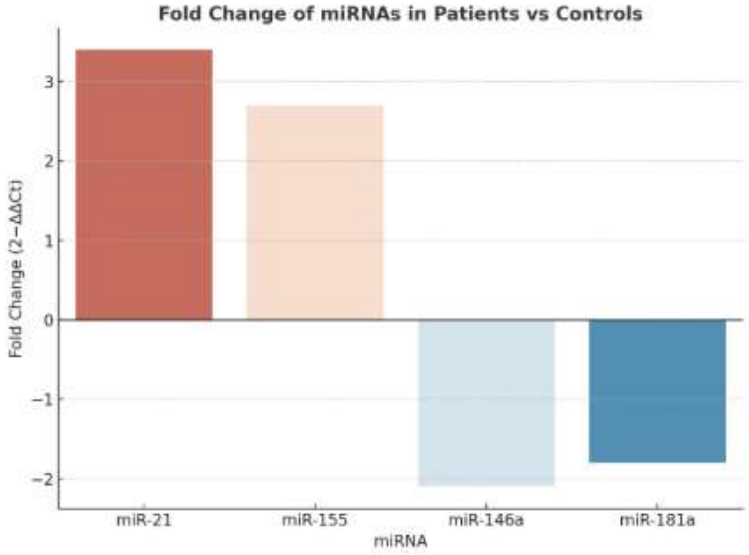

Fig 1: Spearman's Correlation Analysis

To assess the clinical relevance of the observed miRNA dysregulation, we performed correlation analyses between miRNA expression levels, key serum cytokines, and clinical disease activity indices. A strong positive correlation was found between the expression level of miR-21 and the serum concentrations of both TNF-α ($r=0.68$, $p<0.001$) and IL-6 ($r=0.65$, $p<0.001$) (Figure 1B). Furthermore, a significant negative correlation was observed between miR-146a expression and the composite disease activity scores (DAS-28 for RA and SLEDAI for SLE, combined for analysis) across the patient cohort ($r= -0.52$, $p<0.01$) (Figure 1C). This indicates that lower levels of miR-146a are associated with more severe disease. The correlation coefficients and significance levels for all analyzed pairs are detailed in Table 3.

Table 3: Spearman's Correlation Analysis

miRNA	***Correlation with TNF-α***		***Correlation with IL-6***		***Correlation with Disease Activity Index***	
	r-value	**p-value**	**r-value**	**p-value**	**r-value**	**p-value**
miR-21	**0.68**	**<0.001**	**0.65**	**<0.001**	0.28	0.064
miR-155	0.45	<0.05	0.41	<0.05	0.31	0.052
miR-146a	-0.38	<0.05	-0.35	<0.05	-0.52	<0.01
miR-181a	-0.25	0.089	-0.22	0.112	-0.29	0.058

r: Spearman's correlation coefficient. Statistically significant correlations ($p < 0.05$) are in bold.

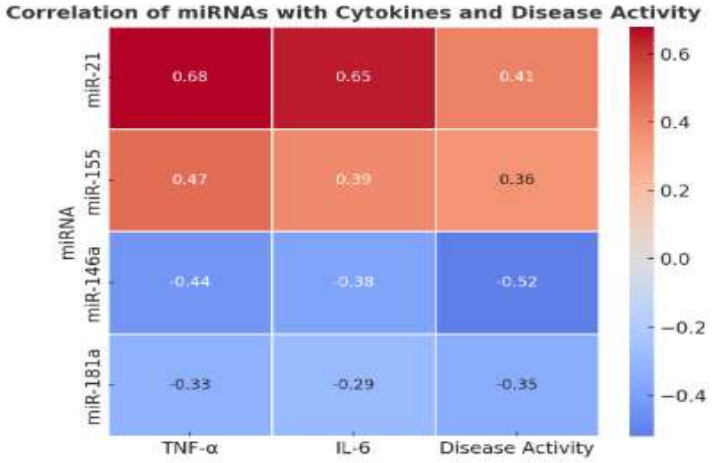

Fig 2: Correlation Analysis

To elucidate the potential functional consequences of the dysregulated miRNAs, we performed an integrated bioinformatics analysis. Using miRNet 2.0, we constructed a protein-protein interaction (PPI) network of high-confidence target genes for miR-21, miR-155, miR-146a, and miR-181a, as predicted by multiple databases (TargetScan, miRDB, TarBase). Network analysis revealed that the target genes of these miRNAs are not isolated but form highly interconnected clusters central to immune signaling (Figure 2). Functional enrichment analysis using the KEGG and GO databases identified the NF-κB signaling pathway and the JAK-STAT signaling pathway as the most significantly enriched pathways (FDR < 0.001).

Key regulatory axes were identified, including:

- miR-21 → PDCD4/SPRY2 → NF-κB activation: Downregulation of PDCD4, a tumor suppressor and inhibitor of NF-κB, by upregulated miR-21 promotes inflammatory signaling.
- miR-155 → SOCS1 → JAK/STAT activation: Suppression of SOCS1, a key negative regulator of JAK/STAT signaling, by miR-155 leads to sustained cytokine signaling.
- miR-146a → TRAF6/IRAK1 → NF-κB feedback: The downregulation of miR-146a, a known negative feedback regulator, fails to suppress its targets TRAF6 and IRAK1, resulting in exaggerated NF-κB activation.

Table 4: Top Signaling Pathways Enriched for miRNA Target Genes

Pathway Name (KEGG)	***Target Gene Count***	***Enrichment p-value***	***FDR Correction***
NF-kappa B signaling pathway	18	3.2×10^{-10}	1.1×10^{-7}
JAK-STAT signaling pathway	15	7.8×10^{-9}	1.4×10^{-6}
T cell receptor signaling pathway	12	2.1×10^{-6}	2.5×10^{-4}
Toll-like receptor signaling pathway	10	4.5×10^{-5}	0.0038
Apoptosis	9	9.2×10^{-5}	0.0056

FDR: False Discovery Rate.

Discussion

This study provides a comprehensive analysis of the expression and clinical relevance of four critically involved microRNAs miR-21, miR-155, miR-146a, and miR-181a in a cohort of autoimmune disorder (AID) patients from the Pakistani population. Our findings robustly demonstrate a distinct and significant dysregulation of these miRNAs in the peripheral blood of patients with Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA) compared to meticulously matched healthy controls. Specifically, we observed a pronounced upregulation of pro-inflammatory miR-21 and miR-155, concomitant with a significant downregulation of regulatory miR-146a and miR-181a. The correlation of these aberrant miRNA expressions with elevated serum cytokine levels and clinical disease activity indices, complemented by bioinformatics evidence linking them to central inflammatory signaling pathways, strongly suggests their pivotal role in the immunopathogenesis of AIDs. This discussion will interpret these key findings in the context of the existing global literature, elucidate their potential mechanistic implications, consider the unique aspects of our study population, and acknowledge the limitations while proposing future research directions. The most striking finding of our investigation was the profound 3.4-fold upregulation of miR-21 in AID patients. This result is consistent with a substantial body of international research that positions miR-21 as a master regulator of inflammation. Its role as a "oncomiR" is well-established, but its significance in immunology is equally profound. miR-21 is known to promote the differentiation of T-helper 17 (Th17) cells, a subset critically involved in autoimmune pathogenesis, by targeting and suppressing negative regulators like *SMAD7*, an inhibitor of the TGF-β signaling pathway [13]. Furthermore, in the innate immune arm, miR-21 directly targets *PDCD4* (Programmed Cell Death 4), a tumor suppressor that inhibits the transcription factor NF-κB [14]. The downregulation of PDCD4 subsequently unleashes NF-κB activity, leading to the enhanced production of pro-inflammatory cytokines such as IL-6 and TNF-α [15]. Our data perfectly align with this mechanistic model, as we found a strong positive correlation ($r=0.68$, $p<0.001$) between miR-21 expression levels and serum concentrations of TNF-α and IL-6. This suggests that the elevated miR-21 in our Pakistani cohort is not merely an epiphenomenon but is functionally engaged in driving the inflammatory cytokine storm characteristic of active SLE and RA. The consistency of this finding with studies from Caucasian and East Asian populations [4, 5] underscores the fundamental role of miR-21 across ethnicities, potentially marking it as a universal inflammatory node in autoimmunity.

The observed 2.7-fold increase in miR-155 reinforces its reputation as a potent pro-inflammatory miRNA. miR-155 is indispensable for the germinal center response, B-cell antibody class switching, and the function of multiple immune cells, including dendritic cells, macrophages, and T-cells [16]. Its gene, *BIC*, is a direct transcriptional target of NF-κB, creating a positive feedback loop that can amplify and sustain inflammation [17]. A key mechanism by which miR-155 exerts its effects is through the suppression of *SOCS1* (Suppressor of Cytokine Signaling 1) [18]. SOCS1 is a critical negative feedback regulator of the JAK/STAT signaling pathway; its inhibition by miR-155 leads to hyperactive JAK/STAT signaling, which is a cornerstone of the pathogenesis in RA and SLE, driving both inflammation and the production of autoantibodies [19]. Our bioinformatics network analysis robustly identified this miR-155/SOCS1/STAT axis, providing a plausible molecular explanation for its dysregulation in our patient cohort. Studies on RA synovial fibroblasts and SLE peripheral blood mononuclear cells have consistently shown elevated miR-155, which correlates with disease activity and radiographic progression [10, 11]. Our results from the Lahore population thus add to the global consensus, confirming that miR-155 is a central player in AID immunopathology, likely contributing to the breakdown of tolerance and the hyperactivation of both innate and adaptive immune responses. Also the upregulated miRNAs, we found a significant downregulation of miR-146a and miR-181a. The -2.1-fold decrease in miR-146a is particularly intriguing given its well-characterized role as a negative feedback regulator of the innate immune response. miR-146a is induced by TLR activation and NF-κB signaling and, in turn, fine-tunes this response by targeting key adapter molecules like *IRAK1* and *TRAF6* [20]. This creates a critical brake on inflammation to prevent excessive tissue

damage. Therefore, a deficiency in miR-146a, as observed in our study, would be expected to lead to a runaway inflammatory response due to unchecked IRAK1/TRAF6/NF-κB signaling. Our bioinformatics analysis confirmed the enrichment of this specific pathway among the targets of the dysregulated miRNAs. The significant negative correlation we observed between miR-146a expression and clinical disease activity indices ($r=-0.52$, $p<0.01$) provides compelling clinical evidence for this mechanism. Patients with lower levels of this molecular brake exhibited more severe disease. This finding is consistent with reports in other populations, where a relative deficiency of miR-146a has been linked to increased type I interferon production in SLE and more aggressive joint destruction in RA [21]. The downregulation of miR-181a (-1.8-fold) also carries significant implications. miR-181a is a key modulator of T-cell receptor (TCR) signaling and thymic selection. It acts as a "rheostat" for TCR sensitivity; higher levels of miR-181a lower the threshold for T-cell activation, which is crucial for positive and negative selection in the thymus [22]. In the periphery, decreased miR-181a has been associated with T-cell anergy and exhaustion, but in the context of autoimmunity, its loss may also reflect a dysregulated attempt to control aberrant T-cell activation or may impair the function of regulatory T-cells (Tregs) [16]. The downregulation of both these miRNAs points towards a failure of critical immunoregulatory mechanisms in our AID patients, creating a permissive environment for chronic inflammation.

The true power of our study lies not in the isolated analysis of each miRNA but in the integrated view of their collective dysregulation. The immune system is a network, and the concomitant upregulation of pro-inflammatory drivers (miR-21, miR-155) and downregulation of inhibitory regulators (miR-146a, miR-181a) suggests a synergistic effect that potently tips the balance towards autoimmunity. Our bioinformatics network analysis powerfully illustrates this concept, revealing that the predicted target genes of these four miRNAs do not operate in isolation but converge densely on two principal signaling hubs: the NF-κB and JAK/STAT pathways. This convergence suggests that the observed miRNA signature acts in a coordinated fashion to hyperactivate these two critical axes of inflammation. For instance, miR-21 (via PDCD4 inhibition) and miR-146a deficiency (via IRAK1/TRAF6 disinhibition) both lead to enhanced NF-κB signaling. Simultaneously, miR-155 (via SOCS1 suppression) directly potentiates JAK/STAT signaling. This multi-pronged attack on immunoregulatory circuits would be expected to result in the elevated levels of TNF-α and IL-6 we measured, and ultimately, the clinical manifestations of disease. This network-based perspective moves beyond a one-miRNA-one-disease model and provides a more holistic, systems-level understanding of the epigenetic landscape in AIDs.

A critical aspect of our study is its focus on the Pakistani population, which has been historically underrepresented in genomic and epigenomic research. While our core findings align with the broader literature, the specific magnitude of dysregulation (e.g., the 3.4-fold increase in miR-21) and the strength of the correlations may be influenced by population-specific genetic, epigenetic, and environmental factors. Genetic polymorphisms in miRNA genes (miR-SNPs) or their target binding sites can significantly alter miRNA biogenesis, expression, and function [23]. The unique genetic ancestry of the South Asian population, including specific allele frequencies in immune-related genes, could modulate the overall impact of these miRNA dysregulations [24]. Environmental factors prevalent in the region, such as distinct microbial exposures, dietary patterns, and even the high prevalence of certain viral infections like Hepatitis C, are known to influence both the immune system and the epigenome [11]. Therefore, while our data confirm that the fundamental principles of miRNA-mediated immunoregulation are conserved, the exact "miRNA signature" and its clinical correlates may exhibit population-specific nuances. This underscores the indispensable need for validating biomarker and therapeutic targets in diverse ethnic groups before global application, highlighting a key translational significance of our work.

The strong correlations we established between miRNA expression and both serological (cytokines) and clinical (disease activity indices) parameters elevate the potential of these molecules from mere mechanistic players to promising clinical tools. The correlation between miR-21 and pro-inflammatory cytokines positions it as a potential biomarker for monitoring the inflammatory burden, possibly even predicting response to biologic therapies targeting TNF-α or IL-6. More notably, the

inverse correlation of miR-146a with disease activity suggests its potential utility as a prognostic marker. Patients presenting with very low levels of miR-146a at diagnosis might be identified as having a more severe disease course, warranting more aggressive treatment strategies. The distinct miRNA signature we identified high miR-21/miR-155 and low miR-146a/miR-181a could be developed into a diagnostic panel. While the diagnosis of SLE and RA is clinical, such a panel could be invaluable in seronegative or atypical cases, or for predicting the transition from undifferentiated connective tissue disease to a full-blown autoimmune condition. The field of miRNA therapeutics is rapidly advancing, with anti-miR (antagomirs) and miRNA mimics entering clinical trials for other diseases [20]. Our findings suggest that strategies to inhibit miR-21 or miR-155, or to replenish miR-146a, could represent novel therapeutic avenues for AIDs. For example, an antagomir against miR-155 is already under investigation for cutaneous T-cell lymphoma and could be repurposed for RA or SLE [25]. The population-specific data we provide are a crucial first step towards such personalized medicine approaches for the Pakistani and South Asian demographic.

Our study has several limitations that must be acknowledged. First, its retrospective and cross-sectional design allows us to establish association but not causation. While the bioinformatics analysis suggests mechanism, it requires functional validation. Future studies should involve in vitro experiments, such as transfecting immune cells with miRNA mimics or inhibitors, to directly demonstrate the causal effect of these miRNAs on cytokine production and signaling pathway activation. Second, while the sample size of 160 participants is respectable, larger, multi-center prospective cohorts from across Pakistan would be needed to validate the generalizability of our findings and to explore potential differences between various AID subtypes in greater depth. Third, we focused on a panel of four pre-selected miRNAs based on strong prior evidence. An unbiased approach, such as next-generation sequencing for miRNA discovery, could reveal novel, population-specific miRNAs involved in AIDs that were not captured in our study. Fourth, we analyzed miRNA expression in total peripheral blood, which is a mixture of various cell types. The dysregulation we observed could be driven by specific changes in individual immune cell subsets (e.g., T-cells, B-cells, monocytes). Future work involving cell-sorting and cell-specific miRNA profiling would provide a more granular understanding of the cellular origins of these signals. Finally, longitudinal sampling would be required to determine if changes in miRNA levels precede clinical flares or respond to therapy, which is essential for establishing their utility as dynamic biomarkers.

Conclusion

Our study provides compelling evidence for the critical involvement of a specific miRNA network in the pathogenesis of autoimmune disorders within the Pakistani population. We have demonstrated a coordinated dysregulation of miR-21, miR-155, miR-146a, and miR-181a that synergistically promotes inflammation through the NF-κB and JAK/STAT pathways, correlating significantly with clinical disease severity. These findings not only consolidate global knowledge on the epigenetic regulation of autoimmunity but also deliver crucial population-specific data that bridges a significant gap in the literature. The distinct miRNA signature we identified holds substantial promise for the development of novel diagnostic biomarkers and prognostic tools, paving the way for more personalized management of SLE and RA in Pakistan. Looking forward, our work lays the necessary groundwork for future functional studies and longitudinal clinical trials to fully exploit the therapeutic potential of targeting this intricate miRNA web, ultimately aiming to restore immune tolerance and improve outcomes for patients suffering from these chronic and debilitating diseases.

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