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Circulating miR-21 Overexpression Correlates with PDCD4 and IL-10 in Systemic Lupus Erythematosus (SLE): A Promising Diagnostic and Prognostic Biomarker

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Abstract

Background: Systemic Lupus Erythematosus (SLE) is a chronic autoimmune condition that affects multiple organs significantly impacts morbidity and mortality. The development of SLE is influenced by genetic predisposition and dysregulated immune response. Our objective was to investigate miR-21, IL-10, and PDCD4 expression in SLE patient plasma and analyze their correlations and potential diagnostic and prognostic values.

Methods: The study included 100 healthy subjects, 50 newly diagnosed (ND), and 50 under-treatment (UT) SLE patients. The patients were observed for 24 weeks to track relapses. miR-21 and PDCD4 gene expression levels were measured using real-time RT-PCR, and IL-10 production was measured using ELISA.

Results: miR-21 and IL-10 expression levels were significantly greater in SLE patients than in healthy subjects, with the highest levels observed in ND patients. PDCD4 expression was also significantly greater in SLE patients than in subjects, with the highest levels observed in UT patients. ROC curve analyses and Cox-Mantel Log-rank tests indicated miR-21, PDCD4, and IL-10 as proper diagnostic and prognostic biomarkers for SLE. The study also revealed a significant positive correlation between miR-21 and PDCD4 and IL-10 levels in SLE patients.

Conclusions: The studies suggest that dysregulation of miR-21, PDCD4, and IL-10 in patients with SLE may contribute to disease development and provides new diagnostic and prognostic markers. Additionally, the observed correlation between miR-21, PDCD4, and IL-10 levels in SLE patients signifies a potential interplay between these molecules.

Keywords: Interleukin-10 (IL-10), Microrna-21 (miR-21), Programmed Cell Death 4 Protein (PDCD4), Systemic Lupus Erythematosus (SLE).

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that can affect multiple organs and cause significant morbidity and mortality (1). The burden of SLE is

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substantial, with a reduced quality of life, increased risk of premature death, and high economic costs associated with the disease (2). The disease is caused by a combination of genetic and environmental factors, and is characterized by the production of autoantibodies that attack the body's own tissues and organs (3). In addition to genetic risk factors, epigenetic modifications such as DNA methylation, histone modifications, non-coding RNAs, and chromatin remodeling have been linked to SLE development and progression (4, 5).

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. They undergo several processing steps to become mature miRNAs, which can inhibit or enhance protein synthesis from target mRNAs. miRNAs have important roles in biological processes and implications in diseases (6, 7). miRNAs play important roles in regulating immune function and their dysregulation may contribute to the development of autoimmunity in SLE patients. Some of the miRNAs implicated in SLE include miR-146a, miR-155, miR-148a, miR-125a, and miR-21 (5, 8, 9). Dysregulation of these miRNAs may contribute to SLE pathogenesis by promoting autoreactivity (10). miR-21 is dysregulated in SLE and may play a complex role in SLE pathogenesis by regulating multiple pathways involved in immune function, inflammation, and apoptosis (11). It promotes the survival and proliferation of autoreactive B cells and inhibits regulatory T cell function. miR-21 downregulates PTEN expression and upregulates IL-6 expression, both of which contribute to the development of autoimmunity (12, 13).

The dysregulation of cytokine production and signaling is a critical factor in SLE development. Several cytokines, including IL-6, IFN-α, TNF-α, IL-17, and IL-10, have been implicated in SLE pathogenesis (14). IL-10 is an anti-inflammatory cytokine produced by regulatory T cells that plays a crucial role in immune response regulation. In SLE, dysregulation of IL-10 may lead to an imbalance between pro-inflammatory and anti-inflammatory cytokines (15). Elevated IL-10 levels have been found in SLE patients, particularly during disease flares, which suggests it may contribute to SLE pathogenesis. IL-10 has also been proposed as a potential SLE biomarker, as its levels correlate with disease activity and severity (16). Measuring IL-10 levels may help in monitoring disease progression and response to treatment (17). Programmed cell death protein 4 (PDCD4) is a tumor suppressor protein that regulates gene expression and inhibits cell proliferation (18). Recent studies suggest that PDCD4 may be involved in SLE pathogenesis, as it plays a role in regulating cell proliferation, survival, cytokine production, autophagy, and signaling pathways (19).

Several molecular biomarkers, including ANA, anti-dsDNA antibodies, complement levels, CRP, ESR, IFN-α, and B-cell activating factor (BAFF), are used to diagnose and monitor SLE, but have some limitations including limited specificity and sensitivity, fluctuations over time, inability to predict disease severity, cost, invasive testing, and false positives and negatives, leading researchers to seek new biomarkers for the disease (20, 21). It has been suggested that miR-21 regulates IL-10 and PDCD4 expression by targeting their mRNAs (22, 23). Dysregulation of miR-21/PDCD4, and miR-21/IL-10 axis has been implicated in various diseases, including SLE (24, 25). Although some studies have evaluated these molecules in SLE, to our knowledge, no study has evaluated the miR-21, IL-10, and PDCD4 expression in SLE patients collectively, and assessed their diagnostic and prognostic utilities. Therefore, the objective of this study was to assess miR-21, PDCD4, and IL-10 plasma expression in both SLE patients and healthy subjects, investigate their possible correlations, and determine their potentials as SLE diagnostic and prognostic biomarkers.

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Materials and Methods

Study Participants and Samplings

We recruited 200 participants, including 50 newly diagnosed (ND) and 50 under-treatment (UT) SLE patients from Rheumatology Clinic, Sayyad Shirazi Hospital, Gorgan, plus 100 healthy subjects. The SLE cases were diagnosed by consulting an expert rheumatologist based on the ACR criteria and were receiving SLE standard of care treatment but not receiving any immunomodulatory or immunosuppressive therapies that could affect the variables studied. Controls were age, sex, and ethnicity-matched healthy subjects with no histories of autoimmune diseases. All participants were 18-65 years of age and excluded if they had other autoimmune diseases, active infections, or pregnancy. Disease duration was at least six months for UT cases, and cases had registered disease activity at the time of serum collection to minimize the effect of disease activity on the variables being studied. Table 1 demonstrates the levels of miR-21, IL-10, and PDCD4 relative to various clinical characteristics of SLE patients. This study was approved by the Committee of Ethics at Golestan University of Medical Sciences (GoUMS), Gorgan, Iran, and conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants before their inclusion in the study. We collected five blood samples from all participants and transferred them to the Research Central Laboratory at GoUMS. The plasma was isolated from the whole blood by centrifugation and stored at -80 °C to avoid contamination or sample loss.

Table 1. The association of miR-21, PCDC4, and IL-10 with major clinical symptoms of SLE patients.

Characteristics		miR-21		PDCD4		IL-10	
Lupus Nephritis	Yes	0.366 ± 0.06	P = 0.096	0.185 ± 0.11	P = 0.079	3427 ± 751	P = 0.155
	No	0.336 ± 0.07		0.239 ± 0.12		2353 ± 317	
Malar Rash	Yes	0.366 ± 0.06	***P = 0.009***	0.154 ± 0.08	***P = 0.001***	3980 ± 559	***P = 0.000***
	No	0.330 ± 0.07		0.266 ± 0.12		1859 ± 313	
Hair Loss	Yes	0.365 ± 0.07	P = 0.011	0.169 ± 0.11	***P = 0.000***	3716 ± 570	P = 0.013
	No	0.331 ± 0.07		0.259 ± 0.12		1987 ± 320	
SLEDAI	≤ 4	0.349 ± 0.08	P = 0.935	0.265 ± 0.14	P = 0.284	2325 ± 735	P = 0.401
	5-12	0.339 ± 0.07		0.237 ± 0.11		1963 ± 377	
	≥ 12	0.342 ± 0.07		0.207 ± 0.12		3240 ± 513	

ELISA cytokine assay

IL-10 concentration was measured using commercially available ELISA kits from ZellBio (ZellBio GmbH, Germany; Cat.NO. RK00012- 96) following the manufacturer's instructions. The optical density (O.D.) values were obtained from the samples and standards on a StatFax 3300 ELISA reader (Awareness Technology, Inc., USA) (26). Non-linear

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regression was used to generate standard curves and calculate the IL-10 concentration in each sample (pg/mL).

RNA isolation, cDNA synthesis and real time RT-PCR

Total RNA was isolated from the plasma samples using TRIzol reagent (Invitrogen, USA), following a previously described protocol (27). Using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), we measured the RNA concentration and purity. Finally, we stored the RNA at -80°C or used it immediately for downstream applications, taking care to use RNase-free reagents and equipment throughout the process. cDNA was synthesized through the reverse transcription method using the SinnaClon First Strand cDNA Synthesis Kit (Cinnagen, Iran; Cat. NO. RT5201). To convert mature miRNA molecules into cDNA for further amplification and quantification by qPCR, we utilized the stem-loop method using a specific stem-loop primer and a common reverse primer (Table 1). Gene and miRNA expression were determined using Sina Green HS-qPCR Mix (Cinnagen, Iran, Cat. NO. MM2042) with specific primers, performed on a Step One Plus cycler (Thermo Fisher Scientific, Iran). The internal controls GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and U6 (small nuclear RNA U6) were used to normalize the cycle threshold (Ct) values of PDCD4 and miR-21 expression in plasma. In this study, we designed gene-specific primers that span exon junctions. These primers are listed in Table 2. The 2^-dCt method, which is a widely used approach for normalizing gene and miRNA expression levels, was employed in this study.

Table 2. List of specific primers.

Primer	Sequence (5'>3')
miR21	**F:** TCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTCAAA
	R: GTGCAGGGTCCGAGGT
U6	**F:** GCTTCGGCAGCACATATACTAAAAT
	R: CGCTTCACGAATTTGCGTGTCAT
GAPDH	**F:** GAAGGTGAAGGTCGGAGT
	R: GAAGGTGAAGGTCGGAGT
PDCD4	**F:** AGGAAGAGGAGAAGGTGGTG
	R: CTGTTTGGCTGCTGTTCTTG

Statistical analyses

SPSS 22.0 (IBM Corporation, USA), and Prism 8.0 (GraphPad Software Inc, USA) were used to compare PDCD4 and miR-21 expression, and IL-10 concentrations between all groups. Normality of results was checked using the Shapiro-Wilk test, and parametric and non-parametric tests were used accordingly. Comparisons between two groups used the Independent Samples t-Test or Mann-Whitney U test, while comparisons between more than two groups used one-way ANOVA with Tukey's post-test or Kruskal-Wallis with Dunn-Bonferroni post-test. Correlation studies used Pearson/Spearman test based on data distribution. ROC curves were analyzed to evaluate the diagnostic utility of each variable, and logistic regression was used for combined ROC curve analysis and prediction of variables' performance. The Mantel-Haenszel (also known as Mantel-Cox) log-rank test was employed to assess the prognostic value of variables in predicting flare occurrence after 24 weeks of follow-up. All experiments were performed in triplicate. The significance level of all statistical tests

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was 0.05, the degree of confidence was 95%, and the power of the test was 80%.

Results

Expression Levels of miR-21, PDCD4, and IL-10 in SLE Patients

We examined the plasma expression of miR-21 in SLE patients and healthy subjects. miR21 expression was significantly greater in SLE patients than healthy subjects (P< 0.0001) miR-21 expression was significantly greater in ND than in UT patients (P< 0.01) and healthy subjects (P< 0.0001). PDCD4 expression was significantly greater in SLE patients than in healthy subjects (P< 0.0001).

PDCD4 expression was greater in in UT SLE patients than in healthy subjects (P< 0.0001) and ND patients (P< 0.01). IL-10 plasma concentrations were significantly greater in SLE patients than in healthy subjects (P< 0.0001). IL-10 plasma concentrations were significantly greater in ND than in UT patients (P< 0.01) and healthy subjects (P< 0.001) (Fig. 1).

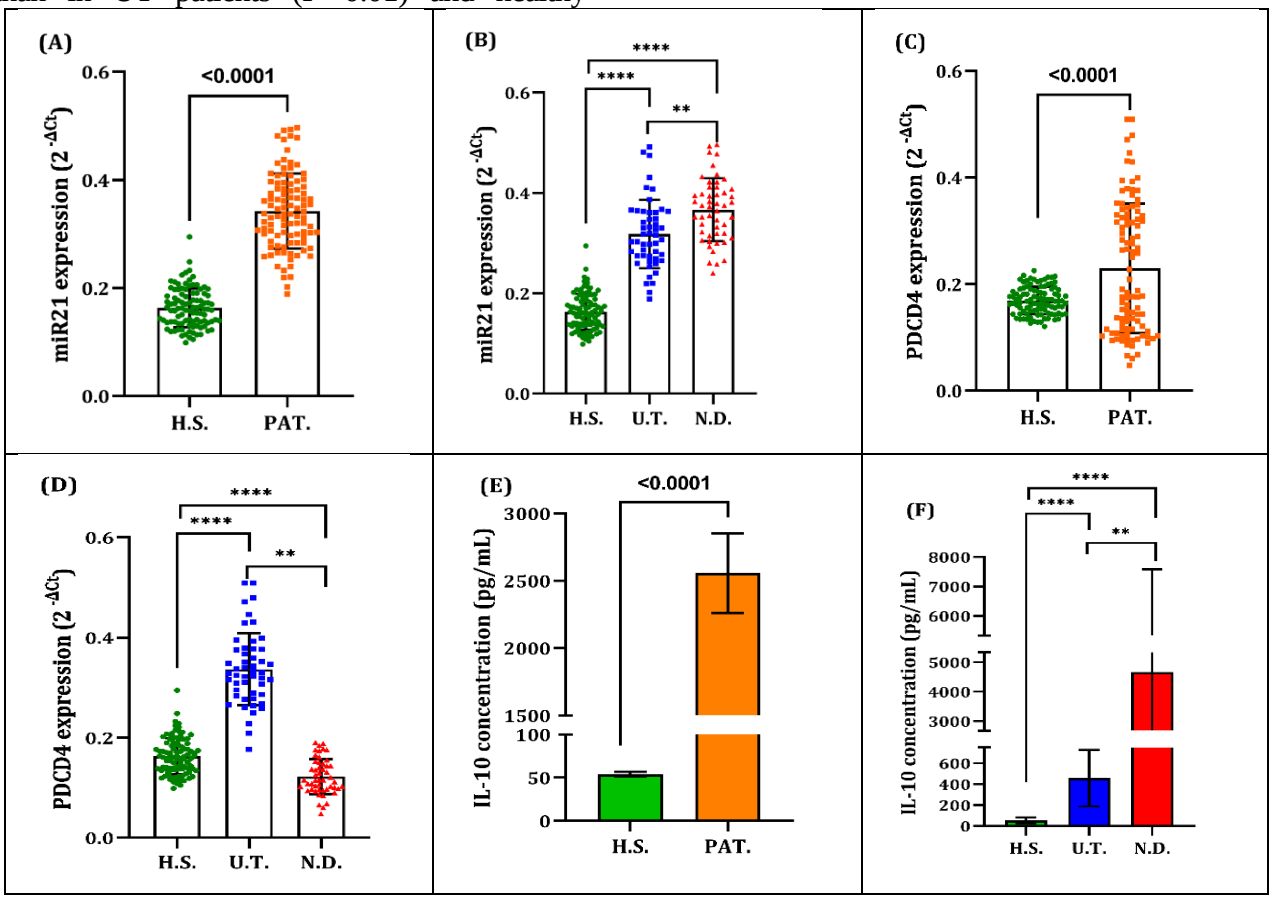

Fig. 1. miR-21, PDCD4, and IL-10 expression in SLE patients and healthy subjects. miR21 expression was significantly greater in SLE patients (PAT.) than in healthy subjects (H.S.) (A). miR21 expression was significantly greater in newly diagnosed (N.D.) SLE patients than in those under treatment (U.T.) (P< 0.01) and healthy subjects (H.S.) (P< 0.001) (B). PDCD4 expression was significantly greater in PAT than in H.S) (P< 0,001) (C). PDCD4 expression was significantly greater in U.T. patients than in H.S. (P< 0.0001) and N.D. patients (P< 0.01) (D). IL-10 was significantly greater in PAT. than in H.S. (E). IL-10 expression was significantly greater in N.D. than in U.T. patients (P< 0.01) and H.S. (P< 0.0001) (F). Comparisons between two groups used the Independent Samples t-Test or Mann-Whitney U test, while comparisons between more than two groups used one-way ANOVA with Tukey's post-test or Kruskal-Wallis with Dunn-Bonferroni post-test. Error bars demonstrate means ± SD (standard deviation). **P< 0.01, ****P< 0.0001.

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The diagnostic utilities of miR-21, PDCD4 and IL-10

ROC curves were analyzed to evaluate the diagnostic utilities of miR21, PDCD4, and IL-10 to distinguish healthy subjects from SLE patients and UT from ND SLE patients (Fig. 2). The area under the curve (AUC) for miR-21 expression (H.S. vs. PAT.) was 0.9924 (95% CI 0.9844 to 1.000; P < 0.0001). The cut-off point was set at the fold change (FC) level of 0.2305 with the sensitivity of 96.00% (95% CI 90.16% to 98.43%), the specificity of 97.00% (95% CI 91.55% to 99.18%), and likelihood ratio (LR) of 32.0. Similarly, the calculated AUC for miR21 (U.T vs. N.D) was 0.7140 (95% CI 0. 0.6127 to 0.8153; P = 0.0002). The cut-off value was set at the FC level of 0.3345 with the sensitivity of 68% (95% CI 54.19% to 79.24%), the specificity of 64% (95% CI 50.14% to 75.86%), and LR of 1.899.

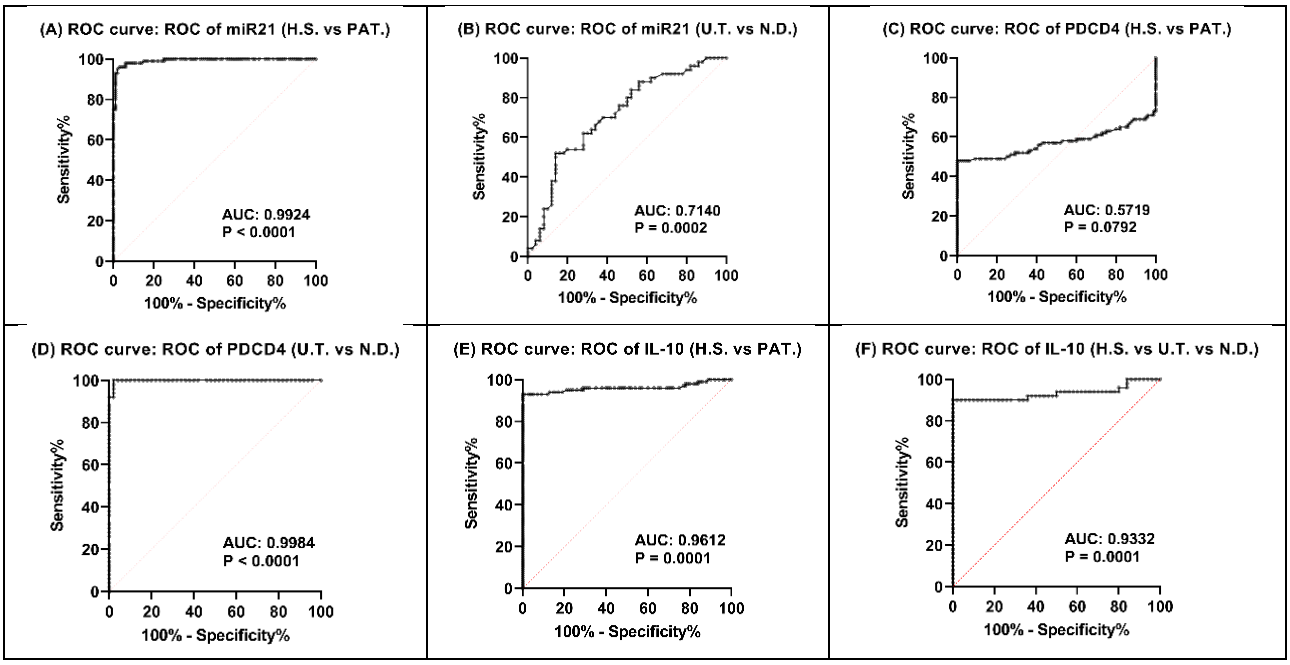

Fig. 2. Diagnostic utilities of miR-21, PDCD4, and IL-10 in SLE patients. We conducted ROC curve analyses to evaluate the diagnostic abilities of miR21, PDCD4, and IL-10 to distinguish between SLE patients (PAT.) and healthy subjects (H.S.), as well as newly diagnosed (N.D.) SLE patients and those under treatment (U.T.). miR-21 (A) had high accuracy with an AUC of 0.9924, while PDCD4 had low accuracy with an AUC of 0.5719 (C) in distinguishing between SLE patients and healthy subjects. In terms of distinguishing between N.D. and U.T. patients, miR-21 (B) had an AUC of 0.7140, while PDCD4 had an AUC of 0.9984 (D). IL-10 had good diagnostic accuracy in both tests, with AUCs of 0.9612 (E) and 0.9332 (F), respectively.

The AUC for PDCD4 expression (H.S. vs PAT.) was 0.5719 (95% CI 0.4820 to 0.6617; P = 0.0792). The cut-off point was set at the FC of 0.1749 with the sensitivity of 56.00% (95% CI 46.23% to 65.33%), the specificity of 59.00% (95% CI 49.20% to 68.13%), and LR of 1.366. Similarly, the calculated AUC for PDCD4 (U.T. vs. N.D.) was 0.9984 (95% CI 0.9946 to 1.000; P < 0.0001). The cut-off value was set at the FC level of 0.1994 with the sensitivity of 100% (95% CI 92.87% to 100.0%), the specificity of 98% (95% CI 89.50% to 99.90%), and LR of 50.0.

The AUC for IL-10 expression (H.S. vs PAT.) was 0.9612 (95% CI 0.9289 to 0.9935; P< 0.0001). The cut-off point was set at the level of 99 pg/mL with the sensitivity of 93.00% (95% CI 86.25% to 96.57%), the specificity of 99.00% (95% CI 94.55% to 99.95%), and LR of 93.0. Similarly, the calculated AUC for IL-10 (U.T. vs. N.D) was 0.9332 (95% CI 0.8740 to 0.9924; P < 0.0001). The cut-off value was set at the level of 963 pg/mL with the sensitivity of 90% (95% CI 78.64% to 95.65%), the specificity of 98% (95% CI 89.50% to 99.90%), and LR of 45.0.

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The correlations of miR21 with PDCD4 and IL-10

We evaluated the correlation between the plasma expression of miR-21 with IL-10 and PDCD4 plasma levels. Pearson correlation study showed that IL-10 and miR21 were positively correlated (r = 0.735, P< 0.0001). Our findings also showed that PDCD4 and miR21 were positively, but not significantly, correlated (r = 0.128, P = 0.071) (Fig. 3).

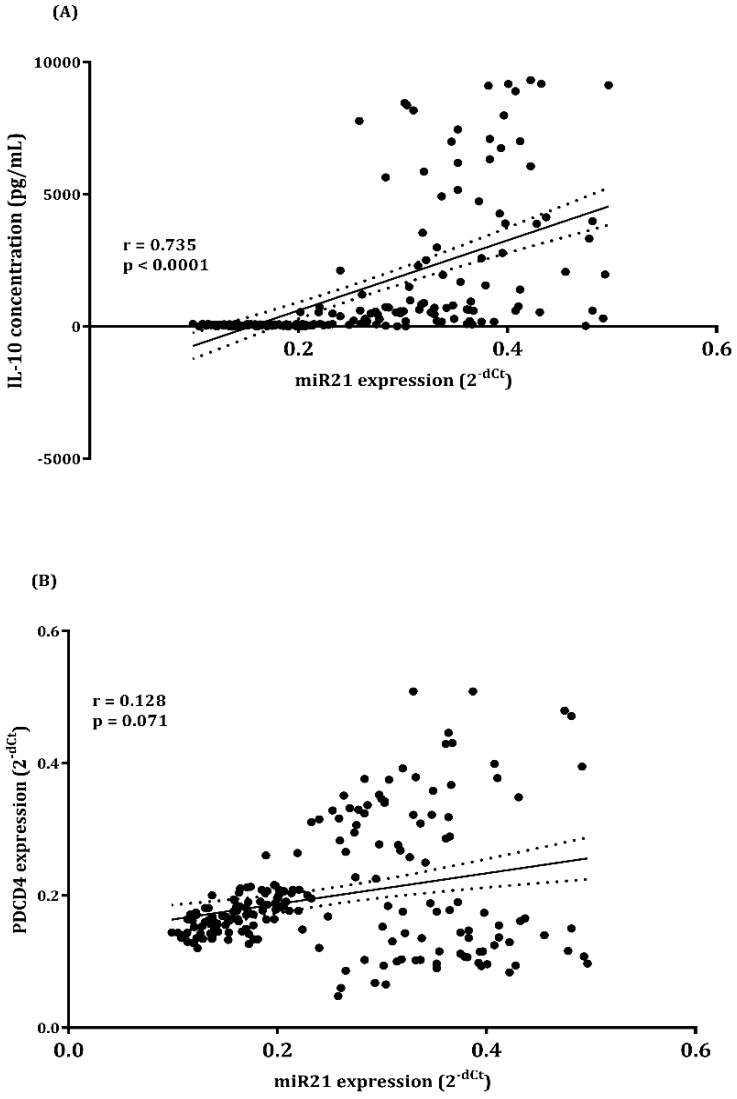

Fig. 3. The correlations of miR21 with PDCD4 and IL-10. We evaluated the correlation between plasma miR-21 expression and IL-10 (A) and PDCD4 (B)s. Pearson correlation showed that IL-10 and miR21 were positively correlated (r = 0.735, P< 0.0001). Our findings also showed that miR21 and PDCD4 were positively, but not significantly, correlated (r = 0.128, P = 0.071).

The prognostic utilities of miR21, PDCD4 and IL-10

The Mantel-Cox log-rank test was conducted to assess the prognostic value of miR21, PDCD4, and IL-10 in predicting flare occurrence after 24 weeks of follow-up. The miR21, PDCD4 and IL-10 levels as putative biomarkers were subdivided into two categories of low and high levels based on their optimum cut-off points derived from ROC curve analyses. Log-rank test results revealed that miR-21, PDCD4, and IL-10 could all predict the outcomes (flare) of SLE patients at 24 weeks (P< 0.0001 for all) (Fig. 4).

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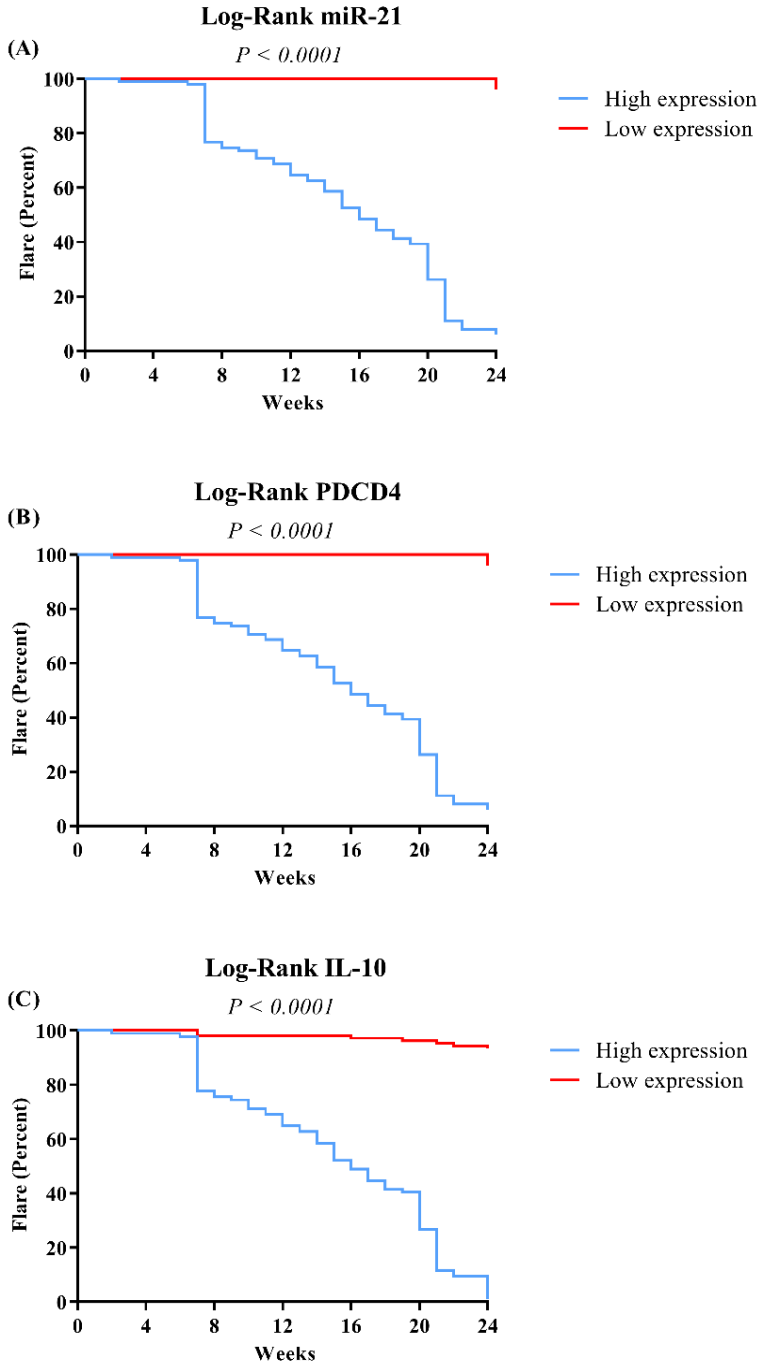

Fig. 4. The prognostic utilities of miR-21, PDCD4, and IL-10 to predict flare in SLE patients. The Mantel-Cox log-rank test was conducted to assess the prognostic value of miR21, PDCD4, and IL-10 in predicting flare occurrence over 24 weeks of follow-up. miR-21, PDCD4, and IL-10 all predicted flare occurrences after 24 weeks (A, B, and C, respectively).

Discussion

The purpose of this research was to determine miR-21, PDCD4, and IL-10 levels in the plasma of SLE patients and healthy subjects.

Additionally, the study aimed to explore any potential connections between these molecules and determine whether they could serve as useful SLE diagnostic or prognostic biomarkers. We found that miR-21 expression in SLE patient plasma was significantly greater than in healthy subjects, especially ND patients. Consistent with our findings,

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Nakhjavani et al. reported significantly elevated miR-21expession in SLE patient plasma, particularly in those with lupus nephritis (LN) (28). However, our study had a larger sample size, which more accurately reflects the group differences. Stagakis et al. found significant upregulation of miR-21 expression in SLE patient PBMCs through microarray analysis, which was confirmed by real-time PCR and western blots, consistent with our findings (29). However, it should be noted that their sample source was PBMCs while ours was plasma. The study also revealed that miR-21 demonstrated the most noteworthy association with disease activity, as it was found to be upregulated in both T and B lymphocytes of individuals diagnosed with active SLE. Furthermore, miR-21 was observed to be upregulated in activated effector and memory T cells in mice, and considerably impacted cellular proliferation (29). Tang et al, evaluated miR-21 expression in the plasma of 44 new-onset SLE patients using relative qRT-PCR. Increased miR-21 expression was seen in SLE patients, consistent with our findings. However, the study's sample size was limited and did not include SLE patients who were receiving treatments (30). Kourti et al. measured miR-21expression in PBMCs of both inactive and active SLE patients. Their findings revealed significantly greater miR-21 expression in active than in in inactive SLE patients, which is consistent with our research demonstrating elevated miR-21 levels in ND patients (31). Khoshmirsafa et al. showed that miR-21 expression was significantly greater in SLE patients, particularly in those with active lupus nephritis, than in healthy subjects. Despite different sample types and smaller sample size, these findings support our results (32).

To date, studies investigating the role of PDCD4 in SLE are limited, and none of these studies have specifically examined PDCD4 expression in SLE patient plasma. However, some reports have evaluated PDCD4 expression in cancer (33, 34). Our findings indicated that SLE patients had greater PDCD4 expression than healthy subjects, particularly in those who were receiving treatment, suggesting a potential correlation between PDCD4 and disease management. Numerous studies have identified PDCD4 as playing a significant role in SLE pathogenesis, with particular emphasis on its association with miR-21. Stagakis and colleagues reported less PDCD4 mRNA and protein expression in individuals with active SLE than in healthy subjects, which aligns with our findings indicating lower PDCD4 levels in ND patients than in those under treatment. Additionally, they conducted a prospective study of two active SLE patients until they achieved remission, demonstrating an inverse relationship between miR-21 and PDCD4. Specifically, upon attaining remission, miR-21 mRNA expression was reduced while PDCD4 protein expression significantly increased. While Stagakis et al. evaluated these expressions in CD4+ T cells, we analyzed plasma (29, 35). Garchow et al. reported that miR-21 was consistently overexpressed in mouse SLE lymphocytes and demonstrated that silencing miR-21 in vivo reversed key autoimmune manifestations, increased PDCD4 expression, and altered lymphocyte populations (36). Given the limited research in this area, further investigation is warranted to better understand the potential role of PDCD4 in SLE.

Diagnostic biomarkers can be categorized based on their AUC values, which determine the accuracy of a biomarker in distinguishing between two groups, such as healthy versus diseased. Biomarkers with an AUC value of 0.9-1.0 are considered excellent, indicating high reliability in identifying the presence or absence of a target condition. AUC values between 0.8-0.9 indicate good diagnostic accuracy, while values of 0.7-0.8 are considered fair, meaning that they have moderate diagnostic accuracy but may not be as reliable as those with higher values. Poor biomarkers have AUC values less than 0.7 and should not be relied upon for diagnoses (37). Evaluating the diagnostic utilities of miR-21, PDCD4, and IL-10, miR-21 was identified as an excellent biomarker (AUC = 0.9924) to

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distinguish SLE patients (PAT) from healthy subjects. However, it was identified as a fair diagnostic biomarker in distinguishing ND from UT patients (AUC = 0.7140). Nakhjavani et al. found that miR-21 was a reliable diagnostic biomarker for LN, with an AUC of 0.912, which was consistent with our findings (28). Our findings are at odds with Tang et al.'s conclusions regarding the diagnostic potential of miR-21 in differentiating new-onset SLE patients from healthy subjects. While they reported miR-21 to be a poor biomarker with an AUC of 0.64, we found it to have excellent diagnostic accuracy. The discrepancies between our results and those of Tang et al. may be attributed to differences in sample size or patient selection criteria, including the possible exclusion of patients receiving treatment in their study (30). Khoshmirsafa et al. identified miR-21 as a reliable biomarker for differentiating between SLE patients and healthy subjects (AUC = 0.84), as well as distinguishing SLE patients with active LN from those who were currently asymptomatic (AUC = 0.89) (32). These results corroborate our findings. According to Zheng et al.'s meta-analysis, miR-21 demonstrated an AUC of 0.8281, suggesting its potential as a valuable biomarker for diagnosing SLE. These results are consistent with our own findings (38). While Tangtanatakul et al. found that miR-21 was significantly less in LN patients with active disease than in those with inactive disease, and long-term follow-up of patients showed down-regulation of miR-21 during disease flare. Our study goes further by demonstrating that miR-21 predicted the flare in SLE patients at 24 weeks (39). However, to our knowledge, no study has yet introduced plasma miR-21 expression as a predictor of SLE flare. Further research is required to fully understand the potential of miR-21 as a biomarker for predicting SLE flare.

While PDCD4 has limited ability to differentiate between healthy subjects and patients with a low AUC value of 0.5719, it could be considered an exceptional biomarker for distinguishing between ND and UT SLE patients (AUC = 0.9984). Furthermore, the findings of this study suggest that PDCD4 has the potential to predict flare occurrence in SLE patients after 24 weeks. While there have been previous reports on the association between PDCD4 and miR-21 in SLE, this is the first study to establish PDCD4 as a reliable biomarker for SLE flare prognosis. Nevertheless, additional research is necessary to validate these results.

Our findings showed that IL-10 expression was significantly greater in SLE patients than in healthy subjects, with the highest levels observed in the ND group. IL-10 was identified as a promising diagnostic biomarker for SLE, with an AUC of 0.9612 for distinguishing SLE patients from healthy subjects and an AUC of 0.9332 for discriminating between ND and UT patients. Additionally, IL-10 has demonstrated potential in predicting flare outcomes in SLE patients. The reports regarding the overexpression of IL-10 and its diagnostic utilities are consistent with previous reports by Chun et al. (40), Godsell et al. (41), and Hu et al. (42). Although previous reports, such as the one by Jin et al. (43), have evaluated the prognostic utilities of IL-10, they were limited by factors such as small sample size and outcome selection. Our study addresses these limitations and provides stronger evidence for the prognostic role of IL-10 in predicting flare-ups.

While our study yielded promising results regarding the potential diagnostic and prognostic utilities of miR-21, PDCD4, and IL-10 in SLE patients, we acknowledge that there were limitations to our research. Despite having a larger sample size than previous studies, the generalizability of our findings could be further improved by further increasing the sample size. Furthermore, as our study assessed these molecules only in plasma, it may be worthwhile to explore their expression in different cell types or tissues to gain a more comprehensive understanding of their role in SLE pathogenesis. Moreover, it is important to note our study had a cross-sectional design, which limits our ability to establish a cause-and-effect relationship between the levels of these molecules and

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disease progression. Therefore, we suggest conducting a longitudinal cohort study to further investigate the potential causal relationships between miR-21, PDCD4, and IL-10 expression and SLE progression.

Based on the findings of this study, we conclude that miR-21 and IL-10 have great potential as biomarkers for diagnosing SLE. miR-21 demonstrated excellent accuracy, while IL-10 showed good accuracy. Both these biomarkers also proved to be effective prognostic indicators for predicting flare-ups in SLE patients. Furthermore, PDCD4 may be a valuable biomarker for distinguishing between ND and UT SLE patients. It could also potentially help predict the occurrence of flares after 24 weeks of treatment. However, it is important to note that further research is needed to validate these findings and establish causal relationships.

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Ethical considerations

Ethical considerations were taken seriously during this research study, which involved human subjects. The study received approval from the ethics committee at Golestan University of Medical Sciences (Code of Ethics: IR.GOUMS.REC.1401.280), and all participants provided informed consent before participating. Confidentiality and privacy were maintained throughout the study, and measures were taken to prevent any undue influence or coercion.

Conflict of interest

We disclose that we have no conflicts of interest.

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