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Case Control Study

Altered Expression of miR-125a and Dysregulated Cytokines in Systemic Lupus Erythematosus: Unveiling Diagnostic and Prognostic Markers

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Abstract

BACKGROUND

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder impacting multiple organs, influenced by genetic factors, especially those related to the immune system. However, there is a need for new biomarkers in SLE. MiR-125a levels are decreased in T cells, B cells, and dendritic cells of SLE patients. MiR-125a plays a regulatory role in controlling the levels of TNF-α and IL-12, which are crucial pro-inflammatory cytokines in SLE pathogenesis.

AIM

This study aimed to assess the levels of miR-125a, IL-12, and TNF-α in SLE patients' plasma, evaluating their diagnostic and prognostic value.

METHODS

The study included 100 healthy individuals, 50 newly diagnosed (ND), and 50 SLE patients undergoing treatment (UT). The patients were monitored for a duration of 24 wk to observe and record instances of relapses. MiR-125a expression was measured

using real-time RT-PCR, while ELISA kits were used to assess IL-12 and TNF-α production.

RESULTS

The results showed significantly reduced miR-125a expression in SLE patients compared to healthy individuals, with the lowest levels in ND patients. TNF-α and IL-12 expression levels were significantly elevated in SLE patients, especially in the early stages of the disease. ROC curve analyses, and Cox-Mantel Log-rank tests indicated miR-125a, PDCD4, and IL-10 as proper diagnostic biomarkers for SLE. A negative correlation was found between plasma miR-125a expression and IL-12/TNF-α levels in SLE patients.

CONCLUSION

Decreased miR-125a levels may be involved in the development of SLE, while elevated levels of IL-12 and TNF-α contribute to immune dysregulation. These findings offer new diagnostic and prognostic markers for SLE. Moreover, the negative correlation observed suggests an interaction between miR-125a, TNF-α, and IL-12. Further research is necessary to uncover the underlying mechanisms that govern these relationships.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a condition characterized by an overactive immune system, leading to damage in various organs and posing substantial risks to health and life [1]. The impact of SLE is significant, resulting in a decreased quality of life, heightened susceptibility to premature death, and considerable financial burdens due to the disease [2]. A combination of genetic and environmental factors is responsible for the development of the disease, wherein the immune system produces autoantibodies that target and damage the body's own tissues and organs [3, 4]. Apart from genetic risk factors, various epigenetic modifications, including DNA methylation,

histone modifications, non-coding RNAs, and chromatin remodeling, have been associated with the onset and advancement of SLE [5, 6].

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules that play a crucial role in regulating gene expression. They undergo multiple processing steps to mature into functional miRNA molecules, which can either inhibit or enhance protein synthesis from target mRNA molecules. miRNAs have significant involvement in various biological processes and hold implications in the development and progression of diseases [7, 8]. miRNAs play crucial roles in the regulation of immune function, and their dysregulation has been linked to the development of autoimmunity in individuals with SLE. Several miRNAs, such as miR-146a, miR-155, miR-148a, miR-21, and miR-125a have been specifically implicated in SLE [6, 9, 10]. The dysregulation of these miRNAs may contribute to the underlying mechanisms of SLE by promoting autoreactivity, which refers to the immune system's response against the body's own cells and tissues [11].

Research has shown that miR-125a levels are reduced in T cells, B cells, and dendritic cells of individuals with SLE, leading to increased activation, proliferation, survival, and production of inflammatory cytokines [12]. Restoring miR-125a levels in immune cells can suppress abnormal activation, indicating its potential as a treatment target. MiR-125a directly regulates cytokine levels involved in SLE, such as IFN-γ, IL-6, IL-10, and TGF-β, suggesting that controlling miR-125a expression could restore immune balance and offer therapeutic benefits [13]. MiR-125a has also been investigated as a diagnostic and prognostic marker for SLE, with lower levels observed in patients' cells and serum, correlating with disease activity, organ damage, and autoantibody presence [14, 15]. Adjusting miR-125a expression through synthetic miRNA mimics and antagomirs has shown promising results in animal models of SLE, reducing autoantibody production and improving disease outcomes [16]. However, further research is needed to ensure the safety and efficacy of miR-125a-based therapies in humans. Understanding miR-125a dysregulation and its effects on different cell types, conducting multicenter

studies, and exploring personalized treatment strategies are crucial for advancing SLE management [11].

The imbalance and abnormal functioning of cytokine production and signaling play a vital role in the onset of SLE. The dysregulation of various cytokines, such as IL-6, IFN-α, TNF-α, IL-17, and IL-12, has been associated with the development and progression of SLE [17].

IL-12, produced by antigen-presenting cells, plays a crucial role in promoting Th1 cell differentiation and immune responses [18, 19]. In SLE, elevated IL-12 Levels are linked to disease activity and lupus nephritis. It has both pro-inflammatory and immune-regulatory effects, contributing to tissue damage and activating autoreactive cells. Targeting IL-12 as a therapy shows mixed results in SLE, likely due to disease heterogeneity and complex signaling pathways [20-24].

TNF-α, a pro-inflammatory cytokine, is produced at higher levels due to disrupted regulation. It is produced by immune cells and plays a role in inflammation and immune modulation [25]. TNF-α binds to its receptor (TNFR1) and activates signaling pathways that promote inflammation [25]. In SLE, TNF-α contributes to abnormal immune functioning by enhancing B cell activation and survival, leading to the production of harmful autoantibodies [26]. It also increases the presentation of self-antigens by antigen-presenting cells. TNF-α promotes inflammation by attracting immune cells, inducing cytokine and chemokine production, and causing endothelial cell activation and vascular permeability [27]. Prolonged elevation of TNF-α can lead to tissue damage and fibrosis in affected organs. Using inhibitors to target TNF-α has displayed potential as a therapeutic strategy in SLE, although outcomes have been inconsistent [28]. Further research is needed to identify patient subsets that may respond better to TNF-α inhibitors and optimize their use in SLE management [29].

ANA (antinuclear antibodies), anti-dsDNA antibodies, complement levels, CRP (C-reactive protein), ESR (erythrocyte sedimentation rate), IFN-α (interferon-alpha), and B-cell activating factor (BAFF) are commonly used molecular biomarkers for diagnosing and monitoring SLE. However, these biomarkers have certain limitations, including

lack of specificity, limited sensitivity, variability over time, inability to predict disease severity, high cost, invasive testing, and the potential for false positive or false negative results. Due to these limitations, researchers are actively seeking new biomarkers that can overcome these challenges and provide more accurate and reliable diagnostic and monitoring tools for SLE [30, 31]. MiR-125a plays a regulatory role in controlling the levels of pro-inflammatory cytokines TNF-α and IL-12 [32]. It directly targets their mRNA, leading to reduced expression and translation. Indirectly, miR-125a influences signaling molecules involved in TNF-α and IL-12 production, such as NF-κB, MAPKs, and STAT proteins [33]. Dysregulation of TNF-α and IL-12 is associated with inflammatory conditions, making miR-125a an attractive therapeutic target [34]. Modulating miR-125a expression or activity could restore balance to these cytokines, potentially alleviating inflammation in conditions like rheumatoid arthritis and inflammatory bowel disease [35]. However, there are still challenges and unanswered questions regarding miR-125a regulation.

To the best of our knowledge, no study has comprehensively examined the expressions of miR-125a, IL-12, and TNF-α in SLE patients collectively and evaluated their diagnostic and prognostic significance. Hence, the aim of this study was to analyze the levels of miR-125a, TNF-α, and IL-12 in the plasma of both SLE patients and healthy individuals, explore their potential correlations, and investigate their usefulness as diagnostic and prognostic biomarkers for SLE.

MATERIALS AND METHODS

Study Participants and Samplings

A total of 200 participants were enrolled in this study, including 50 newly diagnosed (ND) and 50 under-treatment (UT) SLE patients from the Rheumatology Clinic at Sayyad Shirazi Hospital in Gorgan. Additionally, 100 healthy individuals were included as controls. SLE cases were diagnosed based on the ACR criteria and UT group were receiving standard treatment for SLE without any immunomodulatory or immunosuppressive therapies that could impact the variables under investigation. The

control group consisted of age, sex, and ethnicity-matched individuals with no history of autoimmune diseases. All participants were within the age range of 18-65 years and were excluded if they had other autoimmune diseases, active infections, or were pregnant. Under-treatment cases had a minimum disease duration of 6 mo, and their disease activity was recorded at the time of serum collection to minimize the influence of disease activity on the variables of interest. The study received ethical approval from the Committee of Ethics at Golestan University of Medical Sciences (GoUMS), Gorgan, Iran, and adhered to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants prior to their inclusion. Blood samples (5 per participant) were collected and sent to the Research Central Laboratory at GoUMS. Plasma was isolated from the whole blood through centrifugation and stored at -80°C to ensure sample integrity and prevent contamination. Table 1 presents the levels of miR-125a, IL-12, and TNF-α in relation to various clinical characteristics of SLE patients.

ELISA cytokine assay

The expression of cytokines was examined using commercially available ELISA kits from ZellBio (ZellBio GmbH, Germany; IL-12 Cat.NO. RK00072-96; TNF-α Cat.NO. RK00030-96). We strictly adhered to the manufacturer's instructions provided in the kit datasheet to ensure precise and reliable results. The optical density (O.D.) values from the samples and standards were measured using a StatFax 3300 ELISA reader (Awareness Technology, Inc., USA) [36]. Non-linear regression analysis was employed to generate standard curves and calculate the concentrations of IL-12, and TNF-α in each sample, measured in picograms per milliliter (pg/mL) for IL-12 and pg/dL for TNF- α [37].

RNA isolation, cDNA synthesis and real time RT-PCR

Total RNA was extracted from the plasma samples using TRIzol reagent (Invitrogen, USA) following a previously described protocol [38]. The RNA concentration and purity were measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific,

USA). The RNA was either stored at -80°C or used immediately for subsequent applications, ensuring the use of RNase-free reagents and equipment throughout the process. For cDNA synthesis, the Sinnaclon First Strand cDNA Synthesis Kit (Cinnagen, Iran; Cat. NO. RT5201) was employed. To convert mature miRNA molecules into cDNA for amplification and quantification through qPCR, the stem-loop method was utilized, along with a specific stem-loop primer and a common reverse primer (as listed in Table 2). The expression levels of miRNA were quantitatively analyzed using Sina Green HS-qPCR Mix (Cinnagen, Iran; Cat. NO. MM2042) along with specific primers. The qPCR reactions were conducted on a Step One Plus cycler (Thermo Fisher Scientific, Iran). In this study, the cycle threshold (Ct) values of miR-125a plasma expression levels were normalized using the internal control U6 (small nuclear RNA U6). The 2^{-dCt} method, a commonly used approach for normalizing gene and miRNA expression levels, was utilized for data analysis [39].

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (IBM Corporation, USA) and Prism 8.0 (GraphPad Software Inc, USA). The Shapiro-Wilk test was used to assess the normality of the data, and parametric or non-parametric tests were chosen accordingly. Independent Samples t-Test or Mann-Whitney U test was employed for comparisons between two groups, while one-way ANOVA with Tukey's post-test or Kruskal-Wallis with Dunn-Bonferroni post-test was used for comparisons involving more than two groups. Pearson/Spearman tests were utilized for correlation analyses based on the distribution of the data. ROC curve analyses were conducted to evaluate the diagnostic utility of each variable, and logistic regression was employed for combined ROC curve analysis and prediction of variable performance. The Mantel-Haenszel (Mantel-Cox) log-rank test was utilized to assess the prognostic value of variables in predicting flare occurrence after a 24-week follow-up. All experiments were performed in triplicates. The significance level for all statistical tests was set at 0.05, with a 95% confidence interval and a test power of 80%.

RESULTS

Expression Levels of miR-125a, TNF-α, and IL-12 in SLE Patients

This study analyzed the expression levels of miR125a, TNF-α, and IL-12 in SLE patients and normal subjects. The results indicated that the expression level of miR-125a was significantly decreased in SLE patients compared to normal subjects (p-value < 0.0001) (Fig 1A). However, it was shown that miR-125a expression was lowest in newly diagnosed (ND) SLE patients compared to those under treatment (UT) (p-value < 0.01) (Fig 1B). TNF-α was significantly elevated in SLE patients compared to normal subjects (p-value < 0.0001) (Fig 1C). The expression level of TNF-α was compared among SLE patients with different disease states (under treatment and newly diagnosed). The results showed that TNF-α expression was highest in ND SLE patients compared to UT patients (p-value < 0.01) (Fig 1D). Independent samples t-test indicated that the expression level of IL-12 was significantly elevated in SLE patients compared to normal subjects (p-value < 0.0001) (Fig 1E). The expression level of IL-12 was highest in ND SLE patients compared to those with longer disease durations (p-value < 0.01) (Fig 1F).

The diagnostic utilities of miR-125a, TNF-α and IL-12

ROC curve analysis was conducted to evaluate the diagnostic utility of miR-125a, TNF-α and IL-12 to distinguish SLE patients from normal subjects, and newly diagnosed (ND) SLE patients from under treatment (UT). The area under the curve (AUC) for the expression of miR-125a (H.S. vs PAT.) (Fig 2A) was 0.8370 (95%CI 0.7803 to 0.8936; P < 0.0001). The cut-off point was set at the fold change level of 0.2365 with the sensitivity of 72.00% (95%CI 62.51% to 79.86%), the specificity of 88.00% (95%CI 80.19% to 93.00%), and likelihood ratio (LR) of 6.0. The calculated AUC for miR-125a (N.D. vs U.T.) (Fig 2B) was 0.8102 (95%CI 0.7279 to 0.8925; P < 0.0001). The cut-off value was set at the FC level of 0.1849 with the sensitivity of 70.0% (95%CI 56.25% to 80.90%), the specificity of 78.0% (95%CI 64.76% to 87.25%), and likelihood ratio (LR) of 3.182.

The AUC for the expression of TNF-α (H.S. *vs* PAT.) (Fig 2C) was 0.9668 (95%CI 0.9476 to 0.9860; P < 0.0001). The cut-off point was set at the level of 34.50 with the sensitivity of 84.00% (95%CI 75.58% to 89.90%), the specificity of 92.00% (95%CI 85.00% to 95.89%), and likelihood ratio (LR) of 10.50. Similarly, the calculated AUC for TNF-α (N.D. *vs* U.T.) (Fig 2D) was 0.9748 (95%CI 0.9513 to 0.9983; P < 0.0001). The cut-off value was set at the level of 44.50 with the sensitivity of 88.00% (95%CI 76.20% to 94.38%), the specificity of 92.00% (95%CI 81.16% to 96.85%), and likelihood ratio (LR) of 11.00. The AUC for the expression of IL-12 (H.S. *vs* PAT.) (Fig 2E) was 0.9778 (95%CI 0.9599 to 0.9957; P < 0.0001). The cut-off point was set at the level of 69.50 pg/mL with the sensitivity of 91.00% (95%CI 83.77% to 95.19%), the specificity of 98.00% (95%CI 93.00% to 99.64%), and likelihood ratio (LR) of 45.5. Similarly, the calculated AUC for IL-12 (N.D. *vs* U.T.) (Fig 2F) was 0.9600 (95%CI 0.9289 to 0.9911; P < 0.0001). The cut-off value was set at the level of 119.50 pg/mL with the sensitivity of 72% (95%CI 58.33% to 82.53%), the specificity of 98% (95%CI 89.50% to 99.90%), and likelihood ratio (LR) of 36.0.

The correlations of miR-125a with TNF-a and IL-12

We examined the relationship between plasma levels of miR-125a and IL-12 as well as miR-125a and TNF-α. The results of a Pearson correlation analysis revealed a negative correlation between IL-12 and miR-125a (r = -0.569, p < 0.0001) (Fig 3A). Similarly, a negative correlation was observed between TNF-α and miR-125a (r = -0.570, p < 0.0001) (Fig 3B).

The prognostic utilities of miR-125a, TNF-a and IL-12

The levels of miR-125a, TNF-α, and IL-12 were categorized as low or high based on the optimal cut-off points determined from ROC curve analyses. The predictive ability of these biomarkers for the outcome (Flare) of SLE patients was assessed using the log-rank test. The results showed that miR-125a did not significantly predict the outcome of SLE patients (P < 0.7151) (Fig 4A). TNF-α, on the other hand, had a predictive potential

for the outcome of SLE patients, but the association was not statistically significant (P = 0.4828) (Fig 4B). In contrast, IL-12 demonstrated a significant predictive ability for the outcome of SLE patients (P = 0.0508) (Fig 4C) according to the log-rank test.

DISCUSSION

Recent research has focused on exploring the diagnostic and prognostic potential of various biomarkers in SLE. Our study investigated the levels of miR-125a, IL-12, and TNF-α in the plasma of individuals with SLE. The main objective was to evaluate the usefulness of these biomarkers in predicting SLE flares after 24 wk. By analyzing their diagnostic utilities and prognostic power, this study aimed to improve the detection and management of SLE.

Our study found a significant decrease in miR125a expression in SLE patients compared to normal individuals. Furthermore, miR125a expression was observed to be lowest in newly diagnosed SLE patients as compared to those who were already under treatment. These results are consistent with the findings of Zhao et al [40], who showed reduced expression of miR-125a and increased expression of its predicted target gene KLF13 in SLE patients. The study by Zhao et al had limited samples, while our study had a larger sample size. Furthermore, while Zhao et al did not categorize their SLE patients based on disease duration, our study did so and revealed that miR-125a expression is more reduced in newly-diagnosed SLE patients. Consequently, our findings suggest that treatment may have an impact on miR-125a expression levels in SLE patients. A study conducted by Zhang et al also aimed to investigate the roles of circRNAs in SLE and their findings were consistent with ours. They utilized microarray analysis, which was verified by qPCR, to demonstrate that miR-125a was downregulated in SLE patients as compared to healthy controls, and this reduction was linked to SLE characteristics. However, their sample size was limited (n = 3), which may have affected the generalizability of their results [41]. Our results were consistent with those reported by Nascimento et al, who observed downregulation of miR-125 in peripheral blood mononuclear cells (PBMCs) of childhood-onset systemic lupus

erythematosus (cSLE) patients. However, their study was limited to cSLE patients and PBMC samples, while our investigation focused on adult females and plasma samples [42]. While our findings regarding reduced miR-125a expression in SLE patients are similar to those reported by Eissa *et al*, there is a difference in the focus of our studies. Eissa *et al* evaluated the plasma expression of miR-125a specifically in juvenile SLE (jSLE) patients, whereas our study did not differentiate between adult and juvenile SLE patients [43]. In summary, our study provides additional evidence to support the idea that levels of miR-125a are lower in patients with SLE and decrease further in more severe cases of the disease.

The expression of miR-125a was found to be significantly different between individuals with SLE and those without the disease. To determine whether miR-125a could be a useful diagnostic tool for detecting SLE and distinguishing between newly diagnosed patients and those already undergoing treatment, we analyzed ROC curves. The results showed that miR-125a has an AUC of 0.8370 and 0.8102 for distinguishing SLE patients from normal individuals and newly diagnosed patients from those already under treatment, respectively. This suggests that miR-125a is effective in identifying SLE patients and differentiating them from healthy individuals or those at different stages of treatment. Biomarkers used for diagnosis can be classified based on their AUC values, which determine how accurately they can differentiate between two groups, such as healthy and diseased individuals. An AUC value of 0.9-1.0 indicates an excellent biomarker, meaning that it is highly dependable in identifying the presence or absence of a target condition. A biomarker with an AUC value between 0.8-0.9 is considered good, indicating reasonably accurate diagnostic ability. An AUC value of 0.7-0.8 is classified as fair, meaning that it has moderate diagnostic accuracy but may not be as reliable. Biomarkers with an AUC value below 0.7 should be considered poor and should not be relied upon for diagnoses [44]. Therefore, miR-125a is classified as an effective diagnostic biomarker for SLE. While Zhang *et al* have suggested that miR-125a could serve as a diagnostic biomarker for SLE based on its significant reduction in SLE patients [41], and several studies have introduced miR-125a as diagnostic biomarkers for

malignancies such as Pancreatic Cancer [45], and Cervical Cancer [46], our study is, to the best of our knowledge, the first to propose miR-125a as an effective diagnostic biomarker for both discriminating between SLE patients and healthy subjects, as well as distinguishing under-treatment patients from newly diagnosed ones. In this study, patients were also divided into high and low expression groups based on the suggested miR-125a cut-off point. These individuals were then monitored for 24 wk to determine if they experienced a flare or not. Based on the results of the log-rank test, it was concluded that miR-125a is not a predictor of SLE patient outcomes after 24 wk. It should be noted that, to date, no other research has explored the utility of miR-125a as a prognostic marker for SLE flare outcome. Further research is required to confirm these findings.

Regarding the function of miR-125a in SLE pathogenesis, Zhang and his colleagues suggested that miR-125a may play a role in the development of SLE. They speculated that miR-125a could help maintain self-tolerance by limiting the activity of T cells that promote inflammation, but they found that its expression is lower in T cells from individuals with SLE. Based on these findings, they proposed that increasing levels of miR-125a might be a promising strategy for treating SLE [47]. In their study, Zhao and colleagues discovered that the expression of miR-125a was lower in individuals with SLE. They also observed an increase in KLF13, a gene that miR-125a is predicted to target. When miR-125a was overexpressed, it led to a significant decrease in the expression of RANTES and KLF13. The researchers found that miR-125a inhibited KLF13 expression in a dose-dependent manner using gain- and loss-of-function methods. Additionally, introducing miR-125a into T cells from SLE patients reduced the high levels of RANTES expression. Notably, the expression of miR-125a in T cells increased in a dose- and time-dependent manner [40]. However, further research is needed.

Our study analyzed the expression level of TNF-α in SLE patients and normal subjects. The results showed that the expression level of TNF-α was significantly elevated in SLE patients compared to normal subjects. Additionally, TNF-α expression

was highest in newly diagnosed SLE patients compared to those with longer disease durations. ROC curve analysis was used to assess the diagnostic accuracy of TNF-α in distinguishing SLE patients from normal subjects and newly diagnosed SLE patients from those under treatment. The AUC for TNF-α expression was high in both cases, indicating excellent diagnostic utility. However, Log-rank test results revealed that TNF-α was not capable of predicting the outcome (Flare) of SLE patients, after 24 wk. The role of TNF-α in the pathogenesis of SLE is well established, and our findings are supported by several confirmatory studies, further strengthening their significance. According to the results of their research, Idborg and colleagues carried out a study aimed at determining potential biomarkers for identifying disease activity in patients with SLE by evaluating a large group of cytokines and basic laboratory tests. They discovered that TNF-α had the most significant ability to differentiate between SLE patients and healthy individuals, with higher levels detected in SLE patients. The researchers also observed a strong association between TNF-α and measures of disease activity, particularly in the subgroup of patients with nephritis [48]. In research conducted by Ma and colleagues, it was observed that SLE patients had higher levels of plasma TNF-α than healthy individuals, which is consistent with our own results. Additionally, the study found that TNF-α levels were elevated in SLE patients who were experiencing active symptoms compared to those who were not, as well as compared to healthy controls, which agrees with previous findings of increased TNF-α expression in patients who have recently been diagnosed with SLE [49]. In addition to their other findings, Rana and colleagues discovered that the TNF-α gene was highly expressed in most patients with SLE. Furthermore, they observed a strong association between these expression levels and both renal involvement and disease activity as measured by SLEDAI scores [50]. According to Sabry and colleagues, SLE patients with active hematological disease exhibited elevated levels of TNF-α as compared to those with inactive disease or healthy individuals. Additionally, they found a strong positive correlation between the TNF-α levels and the SLE Disease Activity Index (SLEDAI) score. The results of their study indicated that increased levels of TNF-α may contribute

to the onset of anemia among Egyptian patients with Lupus Nephritis [51]. According to our research, a study conducted by Sabry *et al* came up with similar results. They found that patients who had active SLE had significantly higher levels of TNF-α compared to those who had inactive SLE or were healthy. They concluded that measuring the levels of TNF-α in the blood could be a useful way to predict disease activity and distinguish between individuals with SLE and those without [27]. The elevated levels of TNF-α and its possible role as a diagnostic marker was also confirmed in other studies by Aringer *et al* [52], Umare *et al* [53], and Zhu *et al* [54].

Regarding the association between TNF-α and diverse clinical manifestations in SLE, notably, patients with CNS involvement exhibit markedly elevated TNF-α levels in contrast to those without CNS involvement. Furthermore, TNF-α levels are significantly heightened in lupus patients overall, particularly in those with NPLE. Additionally, a substantial TNF-α level increase is observed in patients presenting with multiple focal pattern hypoperfusion, the predominant SPECT pattern in individuals with NPLE [55]. Diffusion tensor imaging (DTI) relies on assessing water diffusion within cellular compartments. In this context, DTI holds the potential to serve as an imaging biomarker for neuropsychiatric systemic lupus erythematosus (NPSLE) and a valuable tool for correlation with TNF-α levels [56].

We also analyzed the expression level of IL-12 in SLE patients and normal subjects. The results revealed that the expression level of IL-12 was significantly higher in SLE patients than in normal subjects. Additionally, among SLE patients, those who were newly diagnosed had the highest levels of IL-12 expression compared to those who had been under treatment for longer periods. The diagnostic ability of IL-12 to distinguish SLE patients from normal subjects and newly diagnosed SLE patients from those under treatment was evaluated through ROC curve analysis, suggesting that IL-12 may be a useful diagnostic marker for SLE, particularly in distinguishing between newly diagnosed and under-treated patients. Following the patients for 24 wk, Log-rank test results revealed that IL-12 was capable of predicting the outcome (Flare) of SLE patients. Similarly, the role of IL-12 in SLE pathogenesis is established in previous

studies, and there are several studies highlighting the elevation of IL-12 in SLE patients. According to our discoveries, Capper and colleagues demonstrated that the levels of IL-12 were noticeably elevated in individuals with SLE when compared to those who did not have the condition. This difference was observed irrespective of whether the SLE patients were having an active episode of the disease or not [57]. Lauwerys and colleagues also noted similar results, where they observed an increase in IL-12 p40 Levels in the blood of SLE patients. They further demonstrated that the administration of immunosuppressive therapy resulted in a significant decrease in serum IL-12 Levels, which verified the lower levels of IL-12 found in individuals without SLE in our study [20], supported in the studies conducted by Qiu *et al* [58], and, Uzrail *et al* [59].

Despite the significant role of IL-12 in SLE pathogenesis, few studies assessed its diagnostic and prognostic accuracy for SLE. In a study by Ye *et al* SLE patients had significantly higher plasma levels of IL-12, and the area under the curve (AUC) for IL-12 was 0.756, indicating its potential as biomarkers for SLE diagnosis [22]. Through our study on a larger sample size, we have discovered that IL-12 can serve as an excellent biomarker for SLE. While Ye *et al* identified it as a fair biomarker, our findings indicate its potential as a stronger indicator of the disease. Additionally, our study is the first to evaluate the occurrence of flares in SLE patients after 24 wk of follow-up. Thus, our results provide valuable insights into the long-term management and prognosis of SLE.

While our study yielded promising results regarding the potential diagnostic and prognostic utilities of miR-125a, TNF-α, and IL-12 in SLE patients, we acknowledge that there were limitations to our research. Despite having a larger sample size compared to previous studies, the generalizability of our findings could be further improved by increasing the sample size even more. Furthermore, as our study only assessed the plasma levels of these molecules, it may be worthwhile to explore their expression in different types of cells or tissues to gain a more comprehensive understanding of their role in SLE pathogenesis. Moreover, it is important to note that 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 disease progression. Therefore, we suggest

conducting a longitudinal cohort study to further investigate the potential causal relationships between miR-125a, TNF-α, and IL-12 Levels and SLE progression.

CONCLUSION

Our study has shed light on the potential of miR-125a, TNF-α, and IL-12 as biomarkers for SLE diagnosis and management. We found that the downregulation of miR-125a is an effective diagnostic tool for distinguishing between SLE patients and healthy individuals, as well as newly diagnosed patients from those under treatment. Furthermore, TNF-α and IL-12 Levels were elevated in SLE patients, with TNF-α serving as a useful diagnostic marker and IL-12 being both a diagnostic and prognostic marker for SLE flare outcome. These findings could contribute to improved patient outcomes and better management of SLE. However, further research is needed to fully understand the mechanisms by which these biomarkers are involved in SLE pathogenesis and their potential as therapeutic targets.

ARTICLE HIGHLIGHTS

Research background

SLE is a long-lasting autoimmune disorder that impacts multiple organs and significantly increases the risk of morbidity and mortality. MiR-125a levels are decreased in T cells, B cells, and dendritic cells of SLE patients. MiR-125a plays a regulatory role in controlling the levels of TNF-α and IL-12, which are crucial pro-inflammatory cytokines in SLE pathogenesis.

Research motivation

Recent research has focused on exploring the diagnostic and prognostic potential of various biomarkers in SLE. Since the levels of miR-125a, TNF-α, and IL-12 are altered in SLE, these molecules could be introduced as novel biomarkers.

Research objectives

The aim of this study was to analyze the levels of miR-125a, TNF-α, and IL-12 in the plasma of both SLE patients and healthy individuals, explore their potential correlations, and investigate their usefulness as diagnostic and prognostic biomarkers for SLE.

Research methods

The study included 100 healthy individuals, 50 newly diagnosed (ND), and 50 SLE patients undergoing treatment (UT). The patients were monitored for a duration of 24 wk to observe and record instances of relapses. MiR-125a expression was measured using real-time RT-PCR, while ELISA kits were used to assess IL-12 and TNF-α production.

Research results

The results showed significantly reduced miR-125a expression in SLE patients compared to healthy individuals, with the lowest levels in ND patients. TNF-α and IL-12 expression levels were significantly elevated in SLE patients, especially in the early stages of the disease. ROC curve analyses, and Cox-Mantel Log-rank tests indicated miR-125a, PDCD4, and IL-10 as proper diagnostic biomarkers for SLE. A negative correlation was found between plasma miR-125a expression and IL-12/TNF-α levels in SLE patients.

Research conclusions

Decreased miR-125a levels may be involved in the development of SLE, while elevated levels of IL-12 and TNF-α contribute to immune dysregulation. These findings offer new diagnostic and prognostic markers for SLE. Moreover, the negative correlation observed suggests an interaction between miR-125a, TNF-α, and IL-12.

Research perspectives

Further research is necessary to uncover the underlying mechanisms that govern the relationships between miR-125a, TNF-α, and IL-12 in SLE pathogenesis.

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