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Is there a role of miRNA -330-3p and miRNA-362-3p in Lupus Nephritis?

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease, with lupus nephritis (LN) being one of its most serious complications. MicroRNAs, particularly miRNA-330-3p and miRNA-362-3p, were implicated in immune regulation and inflammation. This study aimed to evaluate the potential role of miRNA-330-3p and miRNA-362-3p in the progression of SLE and LN. This cross-sectional study included 150 participants: 50 controls (Group I), 50 SLE patients without nephritis (Group II), and 50 patients with lupus nephritis (Group III). Serum levels of miRNA-330-3p and miRNA-362-3p were quantified using a real-time polymerase chain reaction (PCR) test. Clinical and laboratory parameters were assessed, including disease activity and nephritis classification. miRNA-330-3p levels were significantly lower in both patients without nephritis (1.119 ± 1.289) and patients with nephritis (0.89 ± 0.518) compared to controls (1.312 ± 0.480; $p < 0.001$). miRNA-362-3p levels were significantly lower in patients with nephritis (0.623 ± 0.925) than in both controls (1.268 ± 0.419; $p < 0.001$) and patients without nephritis (1.254 ± 1.351; $p < 0.001$). The receiver operating characteristic (ROC) curve analysis revealed that miRNA-362-3p discriminated LN from non-LN SLE patients (AUC = 0.754; cut off ≤ 0.204; sensitivity 66%, specificity 72%). Both miRNA-330-3p and miRNA-362-3p are down regulated in SLE, particularly in patients with LN. These miRNAs may represent therapeutic targets, pending validation in future studies.

Keywords: Lupus Nephritis, Systemic Lupus erythematous; miRNA -330-3p; miRNA-362-3p.

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Introduction

Systemic lupus erythematosus (SLE) is a multi-factorial autoimmune disorder marked by the generation of auto-antibodies directed against nuclear and cytoplasmic elements, resulting in the formation of immune complexes that deposit in tissues and trigger inflammatory responses.[1] Multiple factors contribute to the onset of SLE, including dysfunctions in both innate and adaptive immune systems, genetic predisposition, environmental triggers, and hormonal imbalances.[2, 3]

A significant and common complication of SLE is lupus nephritis (LN), which affects the kidneys. Despite advances in treatment with immunosuppressive agents and glucocorticoids,

between 10% and 40% of LN patients still progress to end-stage renal disease within 15 years of diagnosis, underscoring the limitations of current therapies.[4] LN develops as a consequence of the immune system erroneously targeting renal tissue, which significantly contributes to the elevated morbidity and mortality experienced by patients with SLE.[5]

LN is classified as autoimmune glomerulonephritis, with varying clinical presentations ranging from asymptomatic proteinuria or hematuria to rapidly progressive glomerulonephritis. Pathological changes can range from mild hyperplasia to severe glomerulosclerosis, impacting both therapeutic responses and prognoses.[6]

MicroRNAs (MiRNAs) are endogenous, single-stranded RNA molecules, approximately 22 nucleotides long that play a regulatory role in gene expression. They achieve this by binding to complementary sequences on target mRNAs, which subsequently results in mRNA degradation or inhibition of its translation.[7, 8] MiRNAs are found abundantly in various mammalian cell types and play a crucial role in regulating thousands of protein-coding genes.[9, 10]

miRNA-330-3p, a specific miRNA, regulates gene expression by binding to particular mRNA targets.[11] Similarly, miRNA-362-3p, another small non-coding RNA, regulates gene expression by targeting mRNA for degradation or translation inhibition. Emerging evidence suggests that miRNA-362-3p may serve as a tumor-suppressive role across multiple cancer types.[12]

Consequently, the aim of this investigation was to assess the role miRNA-362-3p and miRNA-330-3p in the context of the inflammatory process of LN, which may serve as targets for treatment.

Patients and Methods

Design and population

The present cross-sectional study included 100 patients diagnosed with SLE and 50 age-matched controls, recruited from the Internal Medicine Clinic at the university hospital during the period from January 2024 to September 2024.

The study included patients above 18 years of age and diagnosed with systemic lupus according to the 2015 ACR/SLICC Revised Criteria for the Diagnosis of SLE.[13] Lupus nephritis was diagnosed with laboratory investigations (urine analysis, albumin creatinine ratio) and was confirmed by renal biopsy for diagnosis and classification of nephritis. Patients were categorized into three groups: Group I, included 50 adults as a control group, Group II, included 50 SLE patients without nephritis, and Group III, included 50 SLE patients with lupus nephritis.

We excluded patients younger than 18 years, those with renal impairment not confirmed to be resulting from lupus nephritis. Individuals diagnosed with other autoimmune disorders, and patients with active disease (all patients with active disease whether SLE patients without nephritis or SLE patients with nephritis), were also excluded from the study.

Methods

All studied cases were subjected to a thorough medical history, general clinical examination, and laboratory investigations. Serum miRNA-330-3p and miRNA-362-3p levels were quantified using a real-time polymerase chain reaction (PCR) test. Routine investigations for lupus diagnosis and activity, including complete blood count, renal functions (urea and creatinine), urine analysis, liver functions, electrolytes, protein-creatinine ratio in urine, antinuclear antibody (ANA), anti-double stranded DNA (anti-dsDNA), C3, C4, and renal pathology examination to diagnose lupus nephritis and for disease classification.

A blood sample (4 ml) was drawn from each participant and promptly placed in a heparinized collection tube, followed by centrifugation at 1,789 × g (assuming a rotor radius of 10 cm) for 10 minutes. After centrifugation, 100 µl of freshly isolated serum was collected and allocated for extraction of total RNA, encompassing microRNA. The rest of the serum was preserved at -80°C, designated for future assays to determine levels of serum

urea, ANA, creatinine, and anti-dsDNA titers. Serum urea and creatinine were measured using standard enzymatic colorimetric methods on an automated chemistry analyzer. ANA were detected by indirect immunofluorescence on HEp-2 cell substrates, and anti-dsDNA antibodies were quantified using a commercial ELISA kits, following the manufacturers' protocol.

Quantification of plasma miR-330-3p and miR-362-3-p gene expression: Purification of miRNA from plasma was conducted using miRNeasy® commercial Kits (QIAGEN, USA), strictly following the manufacturer's guidelines. RNA quality, purity, and concentration were then assessed using Nanophotometer N-60 (Implen, Germany). The purity of RNA was determined by calculating A260/A280 ratio, representing absorbance values at 260 nm and 280 nm, respectively. In this study, A260/A280 ratios of RNA extracts consistently fell within acceptable range of 1.8 to 2. Post-purification, isolated miRNA was stored at -80°C.

Subsequently, miScript II RT Kit (QIAGEN, USA, starter protocol) was utilized to synthesize single-stranded cDNA through reverse transcription of purified miRNA. A reaction mixture totaling 20 µl was prepared by combining 4 µl of miScript HI Spec RT buffer, 2 µl of miScript Nucleic Mix, 2 µl of miScript™ reverse transcriptase, 2 µl of nuclease-free water, and 10 µl of extracted miRNA. The entire reaction was performed on ice. The reaction was conducted using a thermal cycler (2720 Applied Bio Systems thermal cycler, Singapore). The reaction started with an initial cycle at 37°C for 6 minutes, followed by a second cycle at 95°C for 5 minutes to inactivate reverse transcriptase enzyme. The synthesized cDNA was subsequently stored at -20°C until its use in real-time PCR phase. The real-time PCR was performed using commercial kits (miScript SYBR Green PCR kit, USA, initial protocol). Reaction mixture, with a final volume of 25 µl, consisted of 12.5 µl SYBR Green Master Mix, 2.5 µl MiScript universal primer, 4 µl of diluted cDNA, 3.5 µl nuclease-free water, and 2.5 µl miScript primer assay. A 1:5 ratio was used to dilute cDNA with nuclease-free water for assay preparation. RNU6 was used as reference miRNA. Mature miR-330-3p, 362-3p, and RNU6 primers were supplied (QIAGEN, USA, starter protocol) and are detailed in Table 1.

Table 1. Real-time polymerase chain reaction (PCR) primer sequences for MicroRNAs (miRNA).

Gene name	Specific no for each primer of gene
1) miRNA -330-3p	YP00204017
2) miRNA-362-3p	YP00205612
3) RNU6-6P	ENSG00000227055

To analyze the study samples, a real-time PCR system (ABI 7500 real-time PCR system) was operated using the software version 2.0.1. After an initial phase of 15 minutes at 95°C, thermal cycling protocol proceeded with 40 cycles, each of which consisted of three stages: an initial phase of 15-second duration at 94°C, followed by a 30-second interval at 55°C, and culminating in a 30-second period at 70°C. Data were analyzed through software (version 2.0.1) integrated into Applied Biosystems 7500 real-time PCR system. Gene expression levels were quantified using comparative ΔΔCt method to determine relative quantification (RQ).[14]

Statistical Analysis

The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Corp., Armonk, NY). Chi-square test was applied to assess qualitative variables, with categorical data presented as frequency distributions and percentages. Normality of continuous data was assessed using the Kolmogorov-Smirnov test, and the results are presented as range, mean, standard deviation, median, and IQR. Normally distributed data were analysed using the student t-test, while the Mann-Whitney test was applied for skewed data. Spearman's coefficient was used for correlation between quantitative variables, and the receiver operating characteristic (ROC) curve analysis assessed the anticipated role of the molecules, with an AUC of 50% or higher considered satisfactory.[15] A *p*-value less than 0.05 was considered statistically significant.

Results

There was no significant difference in sex distribution across the three groups (p = 0.125), with females predominating in all groups. The mean age did not differ significantly (p = 0.240), with averages of 26.14 ± 7.12, 29.52 ± 8.85, and 28.04 ± 9.69 years in Groups I, II, and III respectively (Table 2).

Table 2. Demographic Data Comparison among the three studied Groups.

		Group I (n = 50)		Group II (n = 50)		Group III (n = 50)		p value
Sex (No. %)	Female	37	74.0	30	60.0	41	82.0	χ^2NS
	Male	13	26.0	20	40.0	9	18.0	
Age (years) Mean ± SD		26.14 ± 7.12		29.52 ± 8.85		28.04 ± 9.69		^{H}NS

χ^2: Chi square test, H: H for Kruskal Wallis test, p: p value for comparing between studied groups, SD: Standard deviation, Group (I): Control group, Group (II) Patients of systemic lupus without nephritis, Group (III) Patients of lupus nephritis. $p > 0.05$ is not significant (NS).

miRNA-330-3p levels were significantly different among the study groups ($p < 0.001$). Group I had higher levels than Groups II and III (p = 0.001 and $p < 0.001$, respectively), while the difference between Groups II and III was not significant (p = 0.145). miRNA-362-3p levels were significantly lower in Group III compared to both Group I ($p < 0.001$) and Group II ($p < 0.001$), Table 3.

Table 3. Comparative analysis of miRNA-330-3p and miRNA-362-3p levels across three studied groups.

	Group I (n = 50)	Group II (n = 50)	Group III (n = 50)	$^H p$ value	Sig. bet. grps. I vs II	I vs III	II vs III
miRNA330-3p Mean ± SD.	1.312 ± 0.480	1.119 ± 0.289	0.89 ± 0.518	<0.001	0.001	<0.001	NS
miRNA362-3p Mean ± SD.	1.268 ±0.419	1.254 ± 0.351	0.623 ± 0.925	<0.001	0.022	<0.001	<0.001

SD: Standard deviation, p: p value for comparing between studied groups, $p > 0.05$ is not significant (NS)., H: Kruskal-Wallis test was applied, and pairwise comparisons between the three groups were performed using Dunn's Post Hoc Test for multiple comparisons, Group (I): Control group, Group (II) Patients of systemic lupus without nephritis, Group (III) Patients of lupus nephritis.

In Group III, miRNA-362-3p negatively correlated with platelets (r = -0.280, p = 0.049), anti-dsDNA (r = -0.364, p = 0.009), creatinine (r=-0.400, p = 0.004), and C4 (r = -0.360, p = 0.010); and positively correlated with C3 (r = 0.319, p = 0.024), albumin (r = 0.434, p = 0.002), and LDH (r = 0.499, $p < 0.001$), Table 4.

Table 4. Correlation between miRNA362-3p and different parameters in each group.

	miRNA362-3p			
	Group II		Group III	
	r_s	p value	r_s	p value
Age	-0.103	NS	-0.011	NS
Age of onset	0.024	NS	0.000	NS
Duration of disease	0.177	NS	0.281	NS
Hb	-0.083	NS	-0.202	NS
Platelets	-0.034	NS	-0.280*	0.049
WBCs	-0.074	NS	0.007	NS

Table 4. Continued.

	miRNA362-3p			
	Group II		Group III	
	r_s	*p* value	r_s	*p* value
ANA	0.207	NS	-0.302	0.033
Anti dsDNA	-0.015	NS	-0.364	0.009
S Creatinine	0.004	NS	-0.400*	0.004
C3	0.114	NS	0.319	0.024
C4	-0.102	NS	-0.360*	0.010
CRP	-0.073	NS	-0.196	NS
ESR	-0.043	NS	-0.113	NS
Albumin	0.044	NS	0.434*	0.002
LDH	0.111	NS	0.499*	<0.001
Renal class	–	–	-0.454	0.001
PCR	-0.268	NS	-0.299	0.035

p > 0.05 is not significant (NS)., rs: Spearman coefficient.

In Group II, miRNA-330-3p was positively correlated with anti-dsDNA (r = 0.432, *p* = 0.002) and C4 (r = 0.299, *p* = 0.035); but negatively correlated with creatinine (r = -0.286, *p* = 0.044). In Group III, miRNA-330-3p was negatively correlated with hemoglobin, WBCs, anti-dsDNA, C3, and PCR; but positively correlated with C4 however, this was not significant, Table 5.

Table 5. Correlation between miRNA330.3p and different parameters in each group.

	miRNA330-3p			
	Group II		Group III	
	r_s	*p* value	r_s	*p* value
Age	0.082	NS	0.128	NS
Age of onset	0.173	NS	0.054	NS
Duration of disease	-0.148	NS	0.008	NS
Hb	0.218	NS	-0.337*	0.017
Platelets	-0.162	NS	-0.171	NS
WBCs	-0.147	NS	-0.388*	0.005
ANA	0.170	NS	-0.353	0.012
Anti dsDNA	0.432*	0.002	-0.320	0.024
S Creatinine	-0.286*	0.044	-0.248	NS
C3	0.062	NS	-0.674	<0.001
C4	0.299*	0.035	0.227	NS
CRP	0.236	NS	0.260	NS
ESR	-0.018	NS	-0.036	NS
Albumin	0.180	NS	0.087	NS
LDH	-0.125	NS	-0.155	NS
Renal class	–	–	-0.312	0.027
PCR	-0.048	NS	-0.323	0.022

rs: Spearman coefficient, *p* > 0.05 is not significant (NS).

The ROC analysis revealed a significant discriminatory ability of miRNA-330-3p (AUC = 0.637, *p* = 0.018) and miRNA-362-3p (AUC = 0.675, *p* = 0.003) between Group II and Group I. For miRNA-330-3p, the optimal cut-off was ≤0.823, yielding a sensitivity of 60% and a specificity of 88%. For miRNA-362-3p, the best cut-off was ≤0.677, with 56% sensitivity and 92% specificity. Figure 1

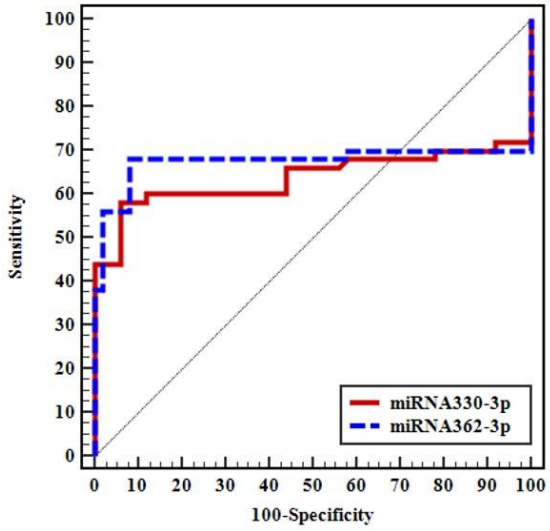

Figure 1. Receiver Operating Characteristic (ROC) curve for miRNA330-3p and miRNA362-3p to discriminate Group II (n = 50) from Group I (n = 50).

Both miRNA-330-3p and miRNA-362-3p exhibited strong discriminatory performance between Group III and Group I, with AUCs of 0.831 (p < 0.001) and 0.802 (p < 0.001), respectively. The optimal cut-off for miRNA-330-3p was ≤1, yielding a sensitivity of 74% and a specificity of 56%, while for miRNA-362-3p, the best cut-off was ≤0.881, with 80% sensitivity and 92% specificity. Figure 2

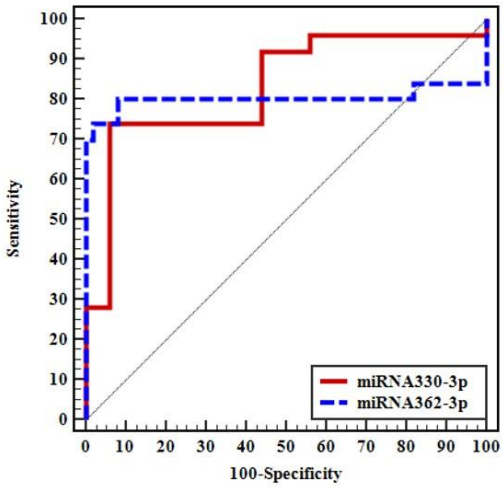

Figure 2. Receiver Operating Characteristic (ROC) curve for miRNA330-3p and miRNA362-3p to discriminate Group III (n = 50) from Group I (n = 50).

The ROC analysis showed that miRNA-330-3p had poor discriminatory ability between Group III and Group II (AUC = 0.530, p = 0.605). In contrast, miRNA-362-3p demonstrated better performance, with an AUC of 0.754 (*p* < 0.001). The optimal cut-off for miRNA-330-3p was ≤0.34, providing 34% sensitivity and 52% specificity, while miRNA-362-3p had a best cut-off of ≤0.204, yielding 66% sensitivity and 72% specificity. Figure 3

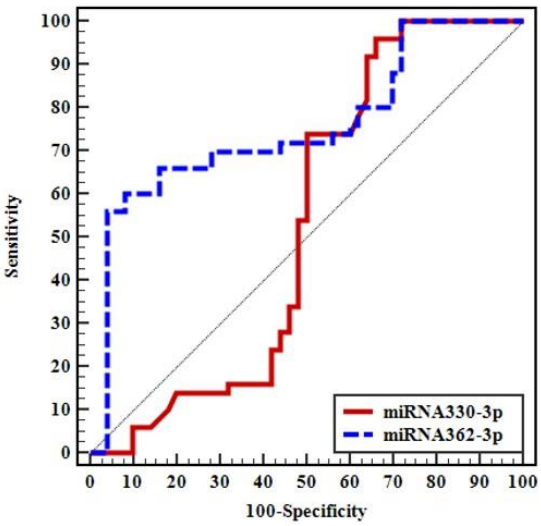

Figure 3. Receiver Operating Characteristic (ROC) curve for miRNA330-3p and miRNA362-3p to discriminate Group III (n = 50) from Group II (n = 50).

Discussion

LN is a frequent and severe complication of SLE, affecting up to 60% of patients as the disease progresses.[16] miRNAs are noncoding RNA sequences, typically 21 to 24 nucleotides in length that regulate gene expression at the post-transcriptional level. Emerging research indicated that miRNAs play a pivotal role in the onset of various human diseases and are also involved in critical biological processes such as cell differentiation and embryonic development.[17] Expression patterns of miRNAs in renal tissue have garnered significant interest, particularly following the work of Dai et al., 2008, who conducted an in-depth investigation of differently expressed miRNAs in kidney biopsy specimens from LN patients, involving 11 affected individuals and three control subjects in an in vivo setting.[17] Their findings revealed marked down-regulation of miR-423 and miR-663 in LN patients,[18] suggesting that miRNA expression profiles may be specific to the cell type and organ involved.

To the best of our knowledge, this investigation is the first to specifically evaluate the role of miRNA-362-3p and miRNA-330-3p in the context of LN. Our study showed that sex distribution was similar across all groups, with a higher proportion of females in each group. Additionally, the mean age was consistent among the groups, with no significant differences observed.

Hanly et al., 2016, investigated nephritis outcomes in a prospective cohort study involving 1,827 SLE patients from diverse ethnic and racial backgrounds. Participants were categorized into those with LN and those without LN. The study reported that 89% of the subjects were female, with a mean age of 35.1 ± 13.3 years.[19] The differences in demographic characteristics may be attributed to variations in population and study methodology.

In our study, miRNA-330-3p levels were significantly higher in Group I compared to Groups II and III, with no significant difference observed between Groups II and III. Similarly, miRNA-362-3p levels were elevated in Group I compared to both Groups II and III, and Group II also exhibited higher levels than Group III.

Wu and Liang, 2021, conducted an animal model study on cartilage injury using 30 male New Zealand rabbits. The rabbits were randomly assigned to three distinct groups: a sham surgery control group, a model group, and an intervention group. The study aimed to explore the roles and interactions between S100 calcium-binding protein B (S100B) and microRNA (miR)-330-3p. The findings demonstrated a significantly decreased expression of miR-330-3p in the model group compared to the control group.[20]

Previous studies by Lee et al., 2016, and Zhu et al., 2019, demonstrated that circulating exosomes play a crucial role in the development of SLE by promoting the expression of certain inflammatory genes.[21, 22] However, most prior studies investigated circulating miRNAs and proteins in SLE, have primarily focused on monocytes or analyzed whole serum, plasma, or urine samples.[23] The current understanding of the roles played by miRNAs and proteins within plasma exosomes in SLE patients remained limited, highlighting the need for further investigative studies to expand this knowledge.

In the present study, miRNA-362-3p in Group III showed negative correlations with platelets, anti-dsDNA, serum creatinine, and C4, and positive correlations with C3, albumin, and LDH. miRNA-330-3p in Group II correlated positively with anti-dsDNA and C4, and correlated negatively with serum creatinine. In Group III, it showed negative correlations with hemoglobin, white blood cells (WBCs), anti-dsDNA, C3, and PCR, along with a positive correlation with C4.

In LN, platelet count decreases due to immune-mediated destruction or consumption during inflammation.[24] Elevated levels of miRNA-362-3p may reflect heightened immune activity, potentially contributing to suppressed platelet production or increased platelet destruction, thereby resulting in a reduced platelet count.

When miRNA-362-3p levels are reduced, the immune system may produce more autoantibodies, such as ANA and anti-dsDNA, contributing to heightened disease activity. Thus, the inverse relationship suggests that miRNA-362-3p may play a role in suppressing autoantibody production, and its down-

regulation is associated with increased antibody levels, indicating more active disease.[25]

Higher miRNA-362-3p levels are associated with lower creatinine, possibly due to a protective role of miRNA-362-3p in kidney function, with lower creatinine reflecting better renal health. Decreased C3 and C4 levels indicate complement consumption during active immune responses in LN. Elevated miRNA-362-3p levels may be linked to increased immune activity, leading to complement activation and subsequent consumption, resulting in lower C3 and C4 levels. A higher renal class reflects more severe kidney involvement, and increased miRNA-362-3p levels could indicate its role in modulating immune responses in advanced stages of LN, potentially signifying greater disease severity.[26]

In LN, inflammation and immune-mediated processes are more pronounced, leading to increased consumption or destruction of WBCs. Kidney involvement in nephritis can trigger a more robust immune response, resulting in greater immune cell consumption. Additionally, certain treatments for LN, such as immunosuppressive therapies, may further reduce WBC counts compared to those in non-nephritis SLE patients.[27]

In SLE, chronic inflammation and immune-mediated damage can lead to protein loss, including albumin. LN further exacerbates this, as the kidneys are responsible for filtering blood and retaining essential proteins. In nephritis, damage to the glomeruli results in increased proteinuria (loss of protein in the urine), leading to significantly lower albumin levels compared to non-nephritis SLE patients. This protein loss is more pronounced in nephritis, contributing to even greater reductions in albumin levels.[28]

ROC analysis demonstrated that both miRNA-330-3p and miRNA-362-3p effectively discriminated between Group II and Group I, as well as between Group III and Group I, with miRNA-362-3p showing stronger performance. miRNA-362-3p also outperformed miRNA-330-3p in distinguishing Group III from Group II. The optimal cutoffs varied across comparisons, with miRNA-362-3p generally exhibiting higher sensitivity and specificity than miRNA-330-3p in all groups.

These findings indicated that both miRNA-330-3p and miRNA-362-3p may play a potential role in lupus, particularly in LN, as they are down-regulated in lupus patients overall and more so in those with LN. miRNA-362-3p may be specifically involved in the pathogenesis of LN, while miRNA-330-3p could be associated with general SLE-related inflammation and autoimmune activity. Both may serve as potential therapeutic targets, pending confirmation by further studies.

Despite the promising findings, this study is limited by its small sample size and cross-sectional design, which restrict the generalization of its findings and limit insights into disease progression. Additionally, confounding factors such as medication use were not accounted for, and the absence of functional studies prevents a deeper understanding of the biological roles of miRNA-330-3p and miRNA-362-3p in LN. Further rigorous research is essential to validate and strengthen these findings. A deeper understanding of lupus nephritis is needed to improve its management.

In conclusion, both miRNA-330-3p and miRNA-362-3p showed a potential role in lupus, particularly in nephritis, as they are down-regulated in lupus patients in general and in LN patients in particular. miRNA-362-3p may be particularly involved in LN, while miRNA-330-3p may play a broader role in SLE-related inflammation and autoimmunity. These findings suggest that they may be potential therapeutic targets pending confirmation in further studies.

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Author Contributions

All the authors contributed to the study as MA, conceptualized the study and developed the main idea of the paper. ST, was responsible for data collection, drafting, and preparing the manuscript for

publication. EB, provided guidance in the biochemical and molecular biology analysis. AE, contributed to the biochemical analysis and laboratory work. BE, offered critical insights and assisted in molecular biology methodologies. All authors reviewed and approved the final version of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Menoufia University (approval 1/2024 BI O15).

Informed consent

An informed consent was obtained from each study participant. Adequate provisions were made to maintain the privacy of participants and the confidentiality of the data.

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