Long Non-Coding RNA Negative Regulator of Interferon Response (NRIR): A Potential Marker for Disease Activity in Lupus Nephritis Patients

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Abstract

Background: Lupus nephritis (LN) is a serious complication of systemic lupus erythematosus (SLE). Existing biomarkers lack consistent sensitivity, emphasizing the need for non-invasive alternatives. The long non-coding RNA NRIR (LncRNA-NRIR), an interferon-inducible molecule that downregulates type I interferon (IFN) signaling, may serve as a biomarker for SLE activity due to its association with IFN-driven immune activation.

Objectives: To evaluate the relative expression (RE) of circulating lncRNAs-NRIR in peripheral blood mononuclear cells (PBMCs) of Egyptian patients with SLE and assess its association with LN activity.

Patients and methods: This case-control study included 45 participants: 15 healthy controls (HC), 15 SLE patients with LN, and 15 Non-lupus nephritis (NLN) SLE patients. Circulating lncRNA-NRIR was quantified using quantitative real-time polymerase chain reaction (qRT-PCR). Clinical, laboratory, and disease activity indices were recorded and statistically evaluated.

Results: The NRIR RE was significantly upregulated in LN patients compared with NLN and HC ($p<0.001$). When both SLE groups were analyzed together, NRIR RE showed significant positive correlations with various markers of disease activity. Receiver operating characteristic (ROC) curve analysis of NRIR RE demonstrated excellent diagnostic performance in discriminating LN from NLN patients (AUC=0.871,95%CI=0.73–1.00), achieving 80% sensitivity and 93.3% specificity at a cutoff > 6.45 (fold change in relative expression). Multivariate regression confirmed NRIR RE ($p=0.001$) as an independent predictor of disease activity.

Conclusion: Circulating lncRNA-NRIR is markedly upregulated in LN and correlates with disease activity. It effectively distinguishes SLE patients with nephritis and serves as an independent predictor of disease activity.

Keywords: LncRNA-NRIR; Lupus nephritis; Systemic lupus erythematosus; Interferon signaling; Biomarker.

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Introduction

Systemic lupus erythematosus is a phenotypically diverse, chronic autoimmune disorder that predominantly affects women of reproductive age. Its clinical manifestations range from mild cutaneous involvement to severe, life-threatening multi-organ disease **(Ameer et al., 2022).**

Its pathogenesis involves a complex interaction between genetic susceptibility, hormonal influences, and environmental triggers that disrupt immune tolerance. Exposure to infectious or environmental factors leads to cell damage and release of self-antigens, activating autoreactive T and B lymphocytes. Both innate and adaptive immune systems contribute to disease initiation and propagation. Toll-like receptor (TLR)–mediated pathways, type I IFN signaling, and neutrophil extracellular trap (NET) formation play central roles in amplifying inflammation. Activated T and B cells drive autoantibody production and immune complex formation, resulting in complement activation and tissue injury across multiple organs **(Takeshima et al., 2022).**

Lupus nephritis is one of the most serious complications, with presentations varying from sub-nephrotic proteinuria to progressive glomerular injury that may culminate in chronic kidney disease. LN therefore represents a major source of morbidity and mortality among SLE patients, despite therapeutic advances **(Renaudineau et al., 2023).** Currently available laboratory biomarkers for LN such as antinuclear antibodies (ANAs), anti-dsDNA, anti-C1q antibodies, and complement components C3 and C4, show inconsistent sensitivity and specificity, limiting their utility in reliably diagnosing and monitoring disease activity **(Alduraibi and Tsokos, 2024).** This has emphasized the need for novel non-invasive biomarkers that can better track LN activity and guide clinical management **(Sentis et al., 2023).**

Non-invasive molecular approaches enable repeated sampling and longitudinal monitoring of disease progression. Within this context, circulating non-coding RNAs have emerged as promising candidates for diagnosis, prognosis, and personalized care in autoimmune diseases **(Sentis et al., 2023).**

Long non-coding RNAs (lnc-RNAs), defined as transcripts longer than 200 nucleotides, regulate gene expression at multiple levels-including transcriptional, post-transcriptional, and epigenetic control-and can act as either repressors or activators. Dysregulation of lnc-RNAs has been implicated in a wide spectrum of pathological processes. Importantly, they remain stable in plasma, further supporting their potential as non-invasive disease markers **(Muntyanu et al., 2022).**

Recent systems-level transcriptomic analyses of whole blood samples from LN patients have identified differentially expressed lnc-RNAs linked to disease quiescence and flares. These studies underscored the central role of the signaling pathway in LN activity and highlighted lncRNA-NRIR as particularly relevant **(Sentis et al., 2023).**

LncRNA-NRIR (also referred to as lncRNA-CMPK2) lies immediately downstream of an -stimulated gene in a head-to-tail, non-overlapping configuration. This genomic context supports its candidacy as a circulating biomarker and potential target for precision medicine strategies in SLE and LN **(Ji et al., 2022; Ma et al., 2024)**

The present study aimed to evaluate circulating lncRNA-NRIR as a potential diagnostic and monitoring biomarker in SLE patients with or without LN, with possible utility in predicting early subclinical flares and monitoring therapeutic response.

Patients and methods

Study design sample size

This case–control study included 45 participants (15 per group: HC, LN, and NLN), matched for age and sex. All SLE patients were evaluated during disease relapse. The sample size was calculated to detect expected differences in mean NRIR RE (4.2, 2.2, 0.99; SD 2.7) among groups using one-way ANOVA ($\alpha = 0.05$, power = 80%) in SPSS v28, and to assess an assumed AUC of 0.8 for lncRNA-NRIR as a biomarker of LN activity using ROC curve analysis in R software **(Saleh et al., 2020; Shen et al., 2022)**.

Data Collection

Patients were enrolled from both the inpatient and outpatient settings of Alexandria Main University Hospital. The study included adults aged

18–50 years who fulfilled the 2019 EULAR/ACR classification criteria for SLE, which require a positive ANA as an entry criterion and a cumulative score ≥10 from clinical and immunologic domains **(Aringer et al., 2019).** LN was diagnosed based on clinical evidence of renal involvement—such as proteinuria ≥500 mg/24 h, active urinary sediment (hematuria or casts), or elevated serum creatinine. Exclusion criteria included hepatic or renal disease unrelated to SLE, history of HCV or HIV infection, active or severe infections, current or past malignancy, drug-induced lupus, pregnancy, and overlap with other connective tissue disorders.

Clinical parameters

Clinical and medical data were collected, including disease onset, duration, and family history. Disease activity was assessed using The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), which scores 24 clinical and laboratory variables across multiple organ systems (range 0–105; mild 1–5, moderate 6–10, high 11–19, very high ≥20) **(Gladman et al., 2002).** Assessment of renal disease activity was done by the SLE Disease Activity Index Renal Domain (SLEDAI-R) which quantifies kidney activity based on proteinuria, hematuria, pyuria, and urinary casts. **(Petri et al., 2012).**

Laboratory investigations

Routine laboratory investigations were performed for all SLE patients. Complete blood counts were analyzed using EDTA blood samples on a Sysmex XN-1000 analyzer**.** Serum albumin, urea, and creatinine were measured on a Siemens Dimension EXL system using an enzymatic colorimetric (Bromocresol green, BCG) method**.** Urinalysis was conducted on midstream samples for chemical and microscopic examination. Urine protein and creatinine were determined colorimetrically (pyrogallol red–molybdate and Jaffe methods, respectively), and the urine protein/creatinine ratio (UPCR) was calculated. C3/C4 complements were measured by nephelometry (Siemens BN ProSpec®)**.** The estimated glomerular filtration rate (eGFR) was estimated by the CKD-Epi equation **(Levey et al., 2009).** Serum anti-dsDNA antibodies were quantified using a BioFlash chemiluminescence analyzer with the QUANTA Flash assay. The erythrocyte sedimentation rate (ESR) was determined by the Westergren method.

Measurement of circulating NRIR RE was done by qRT-PCR **(Kolenda et al., 2019).** Total RNA (including lncRNA) isolation from fresh whole blood samples (collected on K3EDTA) was carried out using the miRNeasy Mini Kit (QIAGEN, Germany, cat. no. 217004) according to the manufacturer's instructions. The concentration and purity of RNA were measured at 260, 280 & 230 nm using NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA). Isolated RNA was then stored at -80°C till reverse transcribed to complementary DNA (cDNA). Reverse transcription was done using reagents from the RevertAid First Strand cDNA Synthesis Kit. (Cat. No. K1622) (Applied Biosystems, USA). qRT-PCR was performed using Thermo Scientific Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific, Cat. No. K0251), and specific primers for lncRNA-NRIR as well as Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control (Applied Biosystems, USA). Primer sequences are listed in **(Table.1).** Relative expression of lncRNA-NRIR was calculated using the comparative cycle threshold (CT) method (2–ΔΔCT) **(Livak and Schmittgen, 2001).**

Table 1.Primer sets designed for amplification of cDNA for lncRNA-NRIR and GAPDH gene sequences

Primer	Sequence
NRIR forward primer	5'-CTTGGCAACTGCTCACGATG-3`
NRIR reverse primer	5'-AGGTTAGAGGTGTCTGCTGC-3'
GAPDH forward primer	5'-GAAGGTGAAGGTCGGAGTCAAC-3'
GAPDH reverse primer	5'-CAGAGTTAAAAGCAGCCCTGGT-3'

NRIR RE: negative regulator of interferon response relative expression; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

Ethics approval and informed consent

The study was carried out in accordance with the 1975 Declaration of Helsinki and received approval from the Local Ethics Committee of the Faculty of Medicine, University of Alexandria (Serial No. 0201983). Written informed consent was obtained from all participants for the collection of blood samples and for the use of their clinical and laboratory data.

Statistical analysis

Statistical analyses were performed using R software (version 4.4.2). Data distribution was examined with the Shapiro–Wilk test. Continuous variables were summarized as mean ± SD, median, minimum, and maximum. Comparisons between two groups were performed using the independent samples t-test or Mann–Whitney U test, as appropriate. Differences among more than two groups were analyzed using one-way ANOVA or the Kruskal–Wallis test, followed by Tukey's or Dunn's post hoc tests, respectively. Categorical variables were presented as frequencies and percentages and compared using the chi-square test. Correlations between NRIR RE, disease activity scores, and laboratory parameters were assessed using Pearson's or Spearman's correlation coefficients. Multivariate linear regression was applied to identify independent predictors of SLEDAI-2K. ROC curve analysis, including combined marker evaluation, was used to assess the diagnostic performance of lncRNA-NRIR and other clinical and laboratory indicators for LN. A p-value < 0.05 was considered statistically significant.

Results

Studied groups characteristics

As shown in **(Table.2)**, there were no significant differences among the studied groups regarding age or sex distribution (p = 0.27 and 0.76, respectively). However, the proportion of married participants was significantly lower in the NLN group compared with LN and HC (p = 0.005). The number of children also differed significantly among groups (p < 0.001), with NLN patients having the fewest.

The mean duration of SLE was significantly longer in NLN than LN patients (106 ± 110 vs 31.4 ± 38.2 months, p = 0.025). Hypertension was observed only in LN (26.7%), whereas diabetes mellitus and DVT each occurred in one LN patient. Regarding treatment, hydroxychloroquine (HCQ) use was more frequent in NLN than LN (p = 0.042), and azathioprine (AZA) therapy was exclusive to NLN (p = 0.006). The combination of corticosteroids (CS) and HCQ predominated in LN (66.7%, p = 0.028), while the triple regimen of HCQ + CS + AZA was observed only in NLN (p = 0.006).

SLE disease activity

The SLEDAI-2K scores were significantly higher in the LN group (median [range] = 19 [6–28]) compared with the NLN group (9 [2–18]; p < 0.001), reflecting markedly greater overall disease activity in patients with renal involvement. The renal SLEDAI-R domain further indicated moderate renal activity among LN patients (12 [0–16.0]), underscoring the predominance of renal components in their disease activity profile as shown in **(Table.2).**

Table 2. Studied groups characteristics

Characteristic	Group I (HC) (n = 15)	Group II (LN) (n = 15)	Group III (NLN) (n = 15)	Test of Significant	p-value
Age (years)	29.9 ± 7.76	30.0 ± 8.66	34.8 ± 8.55	F=1.34	0.27
Female no. (%)	14 (93.3%)	14 (93.3%)	13 (86.7%)	χ^2=0.549	0.76
Married no. (%)	13 (86.7%)	13 (86.7%)	6 (40.0%)	χ^2=10.6*	0.005*
Children (mean ± SD)	2.00 ± 1.36	2.33 ± 1.11	0.27 ± 0.59	H=19.876*	< 0.001*
Sig. bet. grps.	p_1=0.462,p_2=0.001*,p_3<0.001*				
Duration of SLE (months) (mean ± SD)	N/A	31.4 ± 38.2	106 ± 110	U=57.5*	0.025*
Duration of Renal Disease (months) (mean ± SD)	N/A	0.8 ± 3.1	N/A	–	-
Disease phase at sampling	N/A	15 (100.0%)	15 (100.0%)	–	-

(Relapse/Remission) no. (%)					
Associated comorbidities					
Diabetes Mellitus no. (%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	–	–
Hypertension no. (%)	0 (0.0%)	4 (26.7%)	0 (0.0%)	–	–
DVT no. (%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	–	–
Treatment received					
HCQ no. (%)	N/A	10 (66.7%)	15 (100%)	χ^2=6.000*	^{FE}p=0.042*
Corticosteroids no. (%)	N/A	11 (73.3%)	15 (100%)	χ^2=4.615	^{FE}p=0.100
Azathioprine no. (%)	N/A	0 (0.0%)	7 (15.6%)	χ^2=9.130*	^{FE}p=0.006*
Cyclophosphamide no. (%)	N/A	4 (26.7%)	0 (0.0%)	χ^2=4.615	^{FE}p=0.100
Treatment regimen combination					
HCQ no. (%)	N/A	1/15 (6.7%)	0/15 (0.0%)	χ^2=1.034	^{FE}p=1.000
CS + HCQ no. (%)	N/A	10/15(66.7%)	4/15 (26.7%)	χ^2=4.821*	0.028*
CS + CYC no. (%)	N/A	4/15 (26.7%)	0/15 (0.0%)	χ^2=4.615	^{FE}p=0.100
HCQ + CS + AZA no. (%)	N/A	0/15 (0.0%)	7/15 (46.7%)	χ^2=9.130*	^{FE}p=0.006*
HCQ + CS + MMF no. (%)	N/A	0/15 (0.0%)	4/15 (26.7%)	χ^2=4.615	^{FE}p=0.100
SLEDAI-2K median (min. – max.)	N/A	19 (6-28)	9 (2-18)	U=31.50	< 0.001*
SLEDAI-R median (min. – max.)	N/A	12 (0.0 – 16.0)	N/A	–	–

χ^2: Chi square test; FE: Fisher Exact test; U: Mann Whitney test; H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test); p: p value for comparing between the studied groups; p_1: p value for comparing between Group I (HC) and Group II (LN); p_2: p value for comparing between Group I (HC) and Group III (NLN); p_3: p value for comparing between Group II (LN) and Group III (NLN); ***bold**: Statistically significant at p ≤ 0.05; HC: Healthy Control, LN: lupus nephritis, NLN: Non-lupus nephritis; HCQ: Hydroxychloroquine; CS: Corticosteroids; CYC: Cyclophosphamide; MMF: Mycophenolate mofetil; AZA: Azathioprine; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; SLEDAI-R: Systemic Lupus Erythematosus Disease Activity Index-Renal.

Laboratory parameters in the studied groups

As shown in **(Table.3)**, LN patients had significantly lower eGFR and serum albumin and higher creatinine, urea, and urine protein/creatinine ratio compared with both controls and NLN groups (p < 0.001). Urinalysis revealed proteinuria (93.3%), pyuria (60%), and hematuria (33.3%) exclusively in the LN group. Both SLE groups showed anemia, leukopenia, and elevated ESR compared with controls, while platelet counts were lower but not significantly different between SLE subgroups. ANA positivity was universal in SLE, with anti-dsDNA titers markedly higher in LN and NLN than controls (p < 0.001). Complement C3 and C4 levels were significantly reduced in SLE groups.

NRIR relative expression

As shown in **(Table.3)**, NRIR RE differed significantly among the studied groups (p < 0.001). The LN group showed the highest NRIR RE (median = 10.56, range = 1.12–21.15), followed by the NLN group (median = 4.77, range = 0.22–8.22), while HC exhibited minimal RE (median = 0.83, range = 0.17–1.23). Post-hoc analysis demonstrated significantly higher NRIR RE in LN compared with both controls (p < 0.001) and NLN (p = 0.002), whereas the NLN–control difference was not statistically significant (p = 0.055) **(Fig.1).**

Table 3: Laboratory parameters in the studied groups

Parameter	Group I (HC) (n = 15)	Group II (LN) (n = 15)	Group III (NLN) (n = 15)	Test of Sig.	p-value	Sig. bet. grps.		
						p_1	p_2	p_3
Albumin (g/dL)	4.21 ± 0.45	2.85 ± 0.75	3.59 ± 0.25	F=25.600*	<0.001*	<0.001*	0.006*	0.001*
eGFR (ml/min/1.73 m²)	106.9 ± 13.27	65.47 ± 43.76	113.9 ± 17.06	F=12.951	<0.001*	0.001*	0.772	<0.001*

				*				
Creatinine (mg/dL)	0.75 ± 0.13	1.65 ± 0.83	0.68 ± 0.11	F=18.240*	<0.001*	<0.001*	0.932	<0.001*
Urea (mg/dL)	28.0 (19.0 – 43.0)	45.0(32.0 – 101.0)	31.0 (24.0 – 51.0)	H=20.009*	<0.001*	<0.001*	0.323	0.001*
Uric Acid (mg/dL)	4.20 (3.10 – 5.70)	7.30 (5.0 – 9.70)	5.90 (4.90 – 8.0)	H=22.865*	<0.001*	<0.001*	<0.001*	0.408
Hemoglobin (g/dL)	13.25 ± 1.32	9.36 ± 1.82	10.32 ± 1.05	F=30.096*	<0.001*	<0.001*	<0.001*	0.170
Leukocyte Count ($\times 10^3/\mu L$)	8.48 ± 1.90	4.78 ± 1.70	3.70 ± 0.86	F=39.020*	<0.001*	<0.001*	<0.001*	0.148
Platelet Count ($\times 10^3/\mu L$)	276 (155 – 384)	160 (88 – 402)	215 (15 – 350)	H=10.372*	0.006*	0.001*	0.053	0.206
ESR 1st Hour (mm/hr)	5.60 ± 2.26	59.53 ± 13.82	37.00 ± 13.99	F=84.242*	<0.001*	<0.001*	<0.001*	<0.001*
ESR 2nd Hour (mm/hr)	11.00 ± 4.00	104.60 ± 26.17	67.00 ± 26.04	F=72.417*	<0.001*	<0.001*	<0.001*	<0.001*
Urinlysis pus cells (>5)	0 (0%)	60% positive	0 (0%)	-	-	-	-	-
Urinlysis RBCs (>5)	0 (0%)	33.3% positive	0 (0%)	-	-	-	-	-
Proteinuria (dipstick)	0 (0%)	93.3% positive	0 (0%)	-	-	-	-	-
UPCR (g/g)	0.15 (0.10 – 0.20)	5.09 (0.11 – 7.60)	0.10 (0.10 – 0.20)	H=27.440*	<0.001*	<0.001*	0.240	<0.001*
ANA positive	0 (0%)	15 (100.0%)	15 (100.0%)	–	-	-	-	-
Anti-dsDNA (folds)	0.38 ± 0.20	3.77 ± 2.84	2.92 ± 1.86	F=12.091*	<0.001*	<0.001*	0.003*	0.475
C3 (mg/dL)	135.8 ± 23.76	71.20 ± 40.79	74.59 ± 41.82	F=14.955*	<0.001*	<0.001*	<0.001*	0.965
C4 (mg/dL)	27.20 ± 8.51	14.84 ± 8.99	12.49 ± 6.85	F=14.042*	<0.001*	<0.001*	<0.001*	0.712
NRIR RE	0.83 (0.17 – 1.23)	10.56(1.12– 21.15)	4.77 (0.22 – 8.22)	H=25.062*	<0.001*	<0.001*	0.055	0.002*

H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test); F: F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey); p: p value for comparing between the studied groups; p_1: p value for comparing between Group I (HC) and Group II (LN); p_2: p value for comparing between Group I (HC) and Group III (NLN); p_3: p value for comparing between Group II (LN) and Group III (NLN); ***bold**: Statistically significant at $p \leq 0.05$; HC: Healthy Control, LN: lupus nephritis, NLN: Non-lupus nephritis; eGFR: estimated glomerular filtration rate; ESR: erythrocyte sedimentation rate; UPCR: urine protein/creatinine ratio; ANA: antinuclear antibody; Anti-dsDNA: anti–double-stranded DNA antibody; C3: complement component 3; C4: complement component 4; NRIR RE: negative regulator of interferon response relative expression.

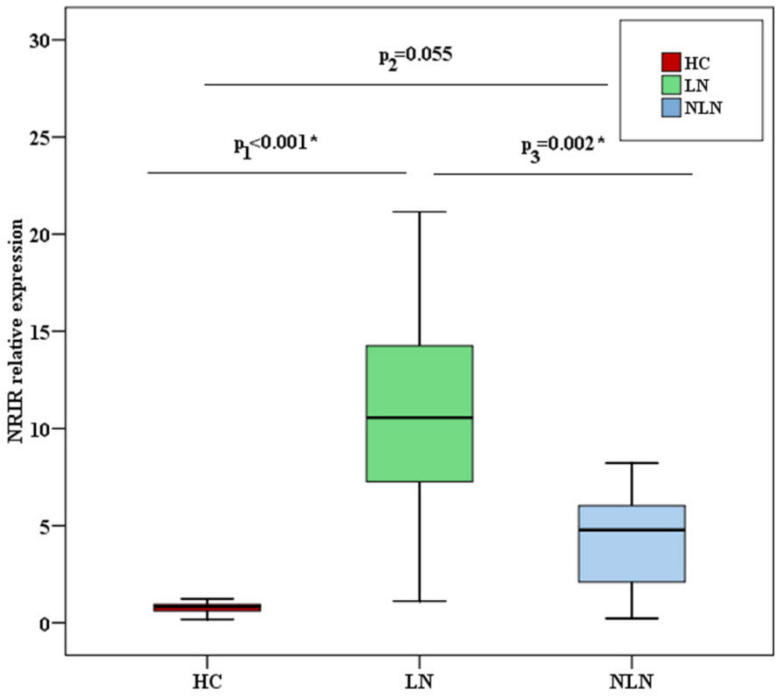

Fig.1. Negative Regulator of Interferon Response Relative Expression (RE) in the studied groups (HC: Healthy Control; LN: Lupus Nephritis; NLN: Non-Lupus Nephritis).

Correlation between NRIR RE with different parameters in each studied group

As shown in **(Table.4)**, NRIR RE in LN patients exhibited a strong negative correlation with eGFR (r = –0.809, p < 0.001), indicating higher NRIR RE levels with declining renal function. A significant positive correlation was also observed with platelet count (r = 0.640, p = 0.010). In the NLN group, NRIR RE correlated positively and significantly with anti-dsDNA titers (r = 0.678, p = 0.005) and showed a moderate, non-significant association with SLEDAI-2K (r = 0.467, p = 0.079). No significant correlations were found with complement levels, proteinuria, or ESR in either group.

Table 4.Correlation between NRIR RE with different parameters in each studied group

NRIR RE vs.	Group II (LN) (n = 15)		Group III (NLN) (n = 15)	
	r	p	r	p
SLEDAI-2K #	-0.236	0.398	0.467	0.079
SLEDAI-R #	-0.305	0.268	–	–
Anti-dsDNA (folds)	-0.476	0.073	0.678	0.005*
C3 (mg/dL)	0.168	0.550	-0.103	0.716
C4 (mg/dL)	0.177	0.528	0.175	0.534
Platelet Count (×10³/µL) #	0.640	0.010*	0.347	0.205
eGFR (ml/min/1.73 m²)	-0.809	<0.001*	0.093	0.742
UPCR (g/g)	-0.224	0.421	0.214	0.444
ESR 1st Hour (mm/hr)	0.214	0.443	0.382	0.160
ESR 2nd Hour (mm/hr)	0.237	0.395	0.315	0.253

r: Pearson coefficient; #: Spearman coefficient; ***bold** : Statistically significant at p ≤ 0.05; NRIR RE: negative regulator of interferon response relative expression; LN: lupus nephritis, NLN: Non-lupus nephritis; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; SLEDAI-R: Systemic Lupus Erythematosus Disease Activity Index-Renal; Anti-dsDNA: anti–double-

stranded DNA antibody; C3: complement component 3; C4: complement component 4; eGFR: estimated glomerular filtration rate; UPCR: urine protein/creatinine ratio; ESR: erythrocyte sedimentation rate.

Correlation between different parameters in SLE patients

As shown in **(Table.5)**, NRIR RE in the combined SLE cohort (LN + NLN) showed a significant positive correlation with SLEDAI-2K (r = 0.435, p = 0.016), UPCR (r = 0.435, p = 0.016) and ESR (1st hr: r = 0.527, p = 0.003; 2nd hr: r = 0.506, p = 0.004), and a strong negative correlation with eGFR (r = –0.792, p < 0.001), indicating higher NRIR RE levels with increasing disease activity and declining renal function. SLEDAI-2K itself correlated positively with anti-dsDNA (r = 0.516, p = 0.004), UPCR (r = 0.0.772, p = <0.001) and ESR (r = 0.799, p < 0.001), but negatively with complement components C3 (r = –0.563, p = 0.001) and C4 (r = –0.449, p = 0.013). Complement levels were positively associated with each other (r = 0.788, p < 0.001) and inversely related to proteinuria and inflammatory markers. In addition, the UPCR correlated positively with ESR (1st hr: r = 0.644, p = <0.001; 2nd hr: r = 0.570, p = <0.001) and inversely with eGFR (r = –0.395, p = 0.031).

Table 5. Correlation between different parameters in SLE patients (n = 30)

Variables		NRIR RE	SLEDAI-2K	Anti-dsDNA (folds)	C3 (mg/dL)	C4 (mg/dL)	eGFR (ml/min/1.73 m²)	UPCR (g/g)	ESR 1st Hour (mm/hr)	ESR 2nd Hour (mm/hr)
NRIR RE	**r**	1.000	0.435#	-0.071	0.039	0.225	-0.792	0.435#	0.527	0.506
	p		0.016*	0.708	0.837	0.231	<0.001*	0.016*	0.003*	0.004*
SLEDAI-2K	**r**		1.000	0.516	-0.563	-0.449	-0.070	0.772#	0.799	0.680
	p			0.004*	0.001*	0.013*	0.712	<0.001*	<0.001*	<0.001*
Anti-dsDNA (folds)	**r**			1.000	-0.488	-0.423	0.213	0.449	0.297	0.189
	p				0.006*	0.020*	0.259	0.013*	0.111	0.317
C3 (mg/dL)	**r**				1.000	0.788	-0.218	-0.374	-0.399	-0.332
	p					<0.001*	0.248	0.042*	0.029*	0.073
C4 (mg/dL)	**r**					1.000	-0.462	-0.149	-0.273	-0.235
	p						0.010*	0.433	0.144	0.211
eGFR (ml/min/1.73 m²)	**r**						1.000	-0.395	-0.240	-0.254
	p							0.031*	0.201	0.176
UPCR (g/g)	**r**							1.000	0.644	0.570
	p								<0.001*	0.001*
ESR 1st Hour (mm/hr)	**r**								1.000	0.932
	p									<0.001*
ESR 2nd Hour (mm/hr)	**r**									1.000
	p									

r: Pearson coefficient; #: Spearman coefficient; ***bold**: Statistically significant at p ≤ 0.05; NRIR RE: negative regulator of interferon response relative expression; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; SLEDAI-R: Systemic Lupus Erythematosus Disease Activity Index-Renal; Anti-dsDNA: anti–double-stranded DNA antibody; C3: complement component 3; C4: complement component 4; eGFR: estimated glomerular filtration rate; UPCR: urine protein/creatinine ratio; ESR: erythrocyte sedimentation rate.

Diagnostic performance for different parameters to discriminate SLE cases with LN

As shown in **(Table.6)**, NRIR RE demonstrated excellent diagnostic accuracy in differentiating LN from NLN cases, with an AUC of 0.871 (p = 0.001, 95% CI = 0.733–1.000). At a cutoff > 6.45 (fold change in relative expression), NRIR RE achieved 80% sensitivity and 93.3% specificity, with a PPV of 92.3% and NPV of 82.4%. The diagnostic performance of NRIR RE was superior to conventional markers such as anti-dsDNA, C3, and C4, which showed nonsignificant AUC values. SLEDAI-2K also showed good discriminative ability (AUC = 0.860, p = 0.001, 95% CI = 0.724–0.996), and combining NRIR RE with SLEDAI-2K further improved the diagnostic power (AUC = 0.929, p < 0.001), highlighting the additive value of lncRNA-NRIR in identifying LN among SLE patients **(Fig.2).**

Table 6.Diagnostic performance for different parameters to discriminate SLE cases with LN (n = 15) from NLN (n = 15)

Variables	AUC	p	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
NRIR RE	0.871	0.001*	0.733 – 1.000	>6.45#	80.0	93.33	92.3	82.4
SLEDAI-2K	0.860	0.001*	0.724 – 0.996	>14	73.33	80.0	78.6	75.0
Anti-dsDNA (folds)	0.580	0.455	0.369 – 0.791					
C3 (mg/dL)	0.522	0.836	0.296 – 0.748					
C4 (mg/dL)	0.558	0.590	0.345 – 0.770					
Combination NRIR RE + SLEDAI-2K	0.929	<0.001*	0.838 – 1.000		80.0	93.33	92.31	82.35

AUC: Area Under a Curve; p value: Probability value; CI: Confidence Intervals; NPV: Negative predictive value; PPV: Positive predictive value; ***bold**: Statistically significant at p ≤ 0.05; #Cut off was choose according to Youden index; NRIR RE: negative regulator of interferon response relative expression; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; Anti-dsDNA: anti–double-stranded DNA antibody; C3: complement component 3; C4: complement component 4.

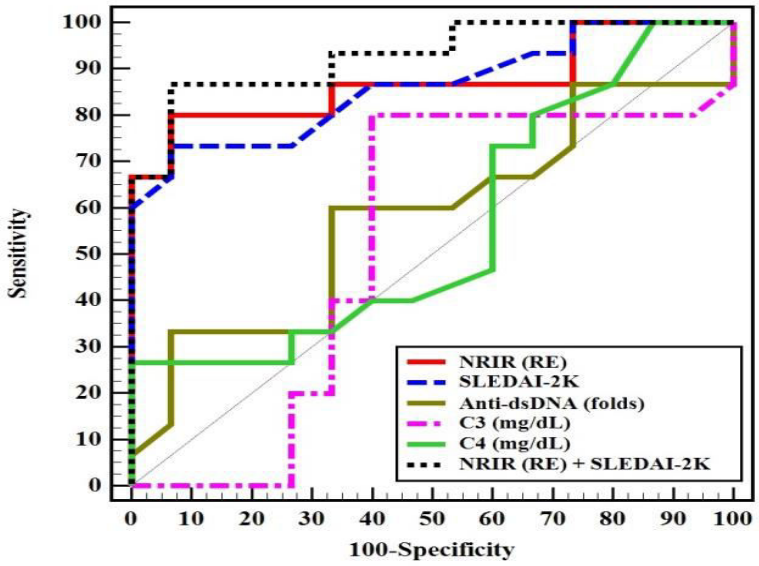

Fig.2. ROC Curve for Different Parameters to Discriminate SLE Cases with LN (n = 15) from NLN (n = 15).
NRIR RE: Negative Regulator of Interferon Response Relative Expression; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; Anti-dsDNA: Anti–Double-Stranded DNA Antibody; C3: Complement Component 3; C4: Complement Component 4.

Multivariate linear regression analysis for the parameters affecting SLEDAI-2K in SLE patients (n = 30)

Multivariate linear regression analysis, as represented in **(Table.7),** identified NRIR RE (B = 0.588, p = 0.001), anti-dsDNA (B = 1.071, p = 0.021), and C3 (B = –0.074, p = 0.008) as independent predictors of SLEDAI-2K scores (F = 13.177, p < 0.001, R^2 = 0.603). Together, these findings indicate that elevated NRIR RE levels not only distinguish LN from NLN but also independently reflect higher disease activity in SLE patients

Table 7. Multivariate linear regression analysis for the parameters affecting SLEDAI-2K in SLE patients (n = 30)

Variables	p	B (LL – UL 95%C.I)
NRIR RE	0.001*	0.588 (0.262 – 0.914)
Anti-dsDNA (folds)	0.021*	1.071 (0.175 – 1.967)
C3 (mg/dL)	0.008*	-0.074 (-0.127 – -0.022)
Calculations	F=13.177*,p<0.001*	
	R^2=0.603, Adjusted R^2=0.557	

B: Unstandardized Coefficients; C.I: Confidence interval; LL: Lower limit; UL: Upper Limit; All variables with p<0.05 was included in Multivariate; ***bold**: Statistically significant at p ≤ 0.05; NRIR RE: negative regulator of interferon response relative expression; Anti-dsDNA: anti–double-stranded DNA antibody; C3: complement component 3.

Discussion

Systemic lupus erythematosus is a heterogeneous autoimmune disease with variable clinical presentation and unpredictable course. LN remains one of its most serious and frequent manifestations, affecting approximately 50% of SLE patients worldwide and representing a major cause of morbidity and long-term kidney failure **(Almaani et al., 2017).**

Early recognition and accurate assessment of LN activity are essential to prevent irreversible renal damage. Consequently, identifying novel, non-invasive biomarkers that reflect LN activity and overall disease burden has become a central objective in lupus research **(Liu et al., 2024).**

The studied cohort was comparable in age and sex distribution across groups, ensuring reliable intergroup comparisons. Disease duration was significantly longer in NLN than LN patients, consistent with prior observations that nephritis often develops early in the disease course **(Musa et al., 2025).** All SLE patients were evaluated during disease relapse. Hypertension occurred exclusively in LN patients, while diabetes and DVT were rare comorbidities. Treatment regimens reflected standard clinical practice **(Fanouriakis et al., 2024).** These comparable baseline characteristics suggest that differences in NRIR RE were primarily attributable to disease mechanisms rather than demographic or therapeutic variations.

Clinically and biochemically, LN patients exhibited higher SLEDAI-2K scores, elevated ESR, lower eGFR, and increased proteinuria, consistent with active LN. Complement consumption (low C3 and C4) and elevated anti-dsDNA titers further supported immune-complex–mediated injury. The positive correlations observed between NRIR RE and both disease activity (SLEDAI-2K, ESR) and renal indices like UPCR indicate that NRIR RE upregulation parallels LN severity and systemic inflammation, while its strong negative correlation with eGFR reflects worsening renal involvement.

The present study evaluated the relative expression of the lncRNA-NRIR as a potential biomarker of disease activity and LN involvement in SLE patients. Our results demonstrated that NRIR RE was markedly upregulated in LN patients compared with both NLN SLE patients and HC. Our findings are similar to those of **Ma et al., (2024)**, who demonstrated elevated NRIR RE in patients with SLE, without distinguishing between LN and NLN cases.

In SLE patients, NRIR RE correlated positively with SLEDAI-2K, UPCR, and ESR, and negatively with eGFR, indicating an association with LN activity and systemic inflammation. This correlation pattern aligns with that reported by **Ma et al., (2024)**, who found NRIR RE associated with SLEDAI-2K and ESR in SLE patients.

Importantly, NRIR RE effectively discriminated LN from NLN, with ROC curve analysis showing an AUC of 0.871 (95% CI = 0.733–1.000), achieving 80% sensitivity and 93.3% specificity at a cutoff > 6.45 (fold change in relative expression). Combining NRIR RE with SLEDAI-2K further enhanced this discriminatory power (AUC = 0.929). In contrast, significantly higher NRIR RE was reported in NLN compared with LN patients, as well as higher NRIR RE in NLN patients than in HC **(Ma et al., 2024).**

Moreover, multivariate regression identified NRIR RE, anti-dsDNA, and C3 as independent predictors of SLEDAI-2K, confirming the strong association between NRIR RE and lupus activity. To the best of our knowledge, no previous studies have evaluated lncRNA-NRIR in conjunction with classical biomarkers such as anti-dsDNA and C3 as independent predictors of SLEDAI-2K. This suggests a potential role for lncRNA-NRIR as an additional indicator of lupus activity beyond conventional serological markers.

The biological plausibility of these findings stems from lncRNA-NRIR's role in IFN-mediated immune regulation. LncRNA-NRIR is an IFN-stimulated lncRNA that modulates type I IFN signaling by repressing transcription of downstream antiviral genes. Dysregulated type I IFN pathways are central to SLE and LN pathogenesis, promoting dendritic cell activation, autoantibody production, and immune-complex deposition. Prior studies have reported upregulation of several IFN-related lncRNAs, such as NRIR, NEAT1, and GAS5, in autoimmune disorders, including SLE. LncRNA-NRIR has also been identified as a key IFN-inducible transcript elevated in chronic immune activation **(Baechler et al., 2003; Suarez et al., 2020).**

Our findings extend this observation by demonstrating that lncRNA-NRIR overexpression is specifically linked to LN activity, suggesting its involvement in IFN-driven renal inflammation and its potential utility in distinguishing LN from NLN SLE patients. These results also raise the possibility that targeting lncRNA-NRIR or its downstream IFN-related pathways could represent a novel therapeutic approach in LN.

The diagnostic and discriminatory ability of lncRNA-NRIR further underscores its clinical value. The AUC of 0.871 for NRIR RE in differentiating LN from NLN surpasses that of traditional biomarkers such as anti-dsDNA, C3, and C4, which often fluctuate independently of nephritis activity **(Renaudineau et al., 2023).** The combination of NRIR RE with SLEDAI-2K (AUC = 0.929) yielded superior diagnostic precision, emphasizing its additive role in composite indices. These findings align with growing evidence that circulating lncRNAs serve as stable, non-invasive molecular indicators of LN activity. Thus, lncRNA-NRIR may complement conventional serological tests, improving diagnostic accuracy and facilitating early identification of LN **(Mihaylova et al., 2020).**

From an epidemiological perspective, LN remains a major health concern in Egypt, where it affects a substantial proportion of patients with SLE and contributes significantly to morbidity and renal failure rates. Limited access to specialized nephrology services and advanced diagnostic tools in many healthcare settings often results in delayed diagnosis and suboptimal therapeutic outcomes **(Abdulrahman and Sallam, 2020).** The incorporation of sensitive molecular biomarkers such as lncRNA-NRIR could facilitate earlier detection of LN, provide a reliable means of assessing disease activity, enable more individualized immunosuppressive therapy, and ultimately improve renal prognosis.

Limitations: This study has several limitations. Being conducted at a single center may limit the generalizability of the results, and the relatively small sample size could reduce statistical power and mask subtle associations. The cross-sectional design also precludes causal inference, and residual confounding cannot be fully excluded. Therefore, larger multi-center longitudinal studies are needed to validate these findings and better define their clinical relevance.

Conclusion

In conclusion, lncRNA-NRIR is significantly overexpressed in LN patients and correlates with disease activity and key clinical indicators in SLE, including SLEDAI-2K, ESR, proteinuria, and eGFR. NRIR RE demonstrated

excellent diagnostic performance in distinguishing SLE cases with and without renal involvement. Multivariate regression analysis further identified NRIR RE, alongside anti-dsDNA and C3, as an independent predictor of disease activity, underscoring its potential clinical relevance. lncRNA-NRIR therefore, represents a promising non-invasive biomarker for assessing disease activity, identifying renal involvement, and improving the clinical evaluation and early detection of kidney disease in SLE.

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