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Dysregulation in growth arrest-specific 5 and metastasis-associated lung adenocarcinoma transcript 1 gene expression predicts diagnosis and renal fibrosis in systemic lupus erythematosus patients

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Abstract

Background: Biomarkers that enhance overall diagnosis and prognosis of systemic lupus erythematosus (SLE) have a growing need to be recognized. The use of long non-coding ribonucleic acids (lncRNAs) as biomarkers in this regard is still largely unexplored. This study aimed to evaluate lncRNA [metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and growth arrest-specific 5 (GAS5)] expression in SLE patients with/without nephritis. Their relation to disease activity/chronicity changes has been identified. A total of 40 SLE patients and 40 healthy controls were tested using real-time quantitative polymerase chain reaction (PCR) for expression levels of MALAT1 and GAS5.

Results: MALAT1 expression was aberrantly upregulated, while GAS5 was downregulated in patients with SLE versus controls. GAS5 relative expression was significantly downregulated in lupus nephritis (LN) patients compared to non-lupus nephritis (NN) patients. GAS5 was also correlated with glomerulosclerosis, interstitial fibrosis, tubular atrophy, and hypertension.

Conclusion: The lncRNA (GAS5 and MALAT1) may serve as diagnostic biomarkers for SLE. Moreover, GAS5 may distinguish SLE LN patients from NN patients and may predict renal fibrosis in LN patients.

Keywords: Growth arrest-specific 5, Long non-coding RNA, Metastasis-associated lung adenocarcinoma transcript 1, Systemic lupus erythematosus

Background

SLE is a complex autoimmune disease defined by the existence of autoantibodies, which are reactive antibodies produced by the immune system to recognize one or more of individual proteins, deposition of immune complex, and excess proinflammatory cytokine. This causes serious damage to various organ systems [1]. Biomarkers facilitate diagnosis and prognosis [2] because of the heterogeneous presentation and unpredictable course of SLE patients. The use of lncRNAs as biomarkers in this regard is still mostly unexplored [3]. LncRNAs is a class of endogenous cellular RNAs with lengths greater than 200 nucleotides, and they are not translated into proteins. They lie within protein-coding

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gene intergenic stretches or overlap antisense transcripts [2]. They are quite numerous and involved in almost all aspects of cell biology such as cell differentiation, cell proliferation, and response to DNA damage [4].

They also play a defined role in numerous multigenetic human diseases such as cancer and neurological diseases. They currently attract many researchers to their role in autoimmune disorders including rheumatoid arthritis and SLE [5].

MALAT1, also known as nuclear-enriched abundant transcript 2 (NEAT2) or alpha, is a highly conserved lncRNA [6]. This gene was first identified in 2003. It was found in early-stage non-small cell lung cancer (NSCLC) cells with high expression levels [7]. It is highly regulated in various cancer forms: endometrial cancer, breast cancer, cervical cancer, colorectal cancer, and hepatocellular carcinoma [8]. Moreover, even normal physiological processes of cells are associated with MALAT1 [9]. GAS5 is a lncRNA, which competes with glucocorticoid response elements (GREs). Since glucocorticoids are powerful immunosuppressants, increased expression of lncRNA-GAS5 in immune cells can suppress glucocorticoid action and can make a significant contribution to autoimmune diseases [10]. Although the precise pathophysiology of SLE remains unknown, its multifactorial etiology is well-known, involving mainly genetic, epigenetic, and environmental factors [1, 11]. Recent studies emphasize the role of lncRNAs and low protein-coding potential in SLE pathogenesis [12, 13]. However, these studies are few, and further studies shall be conducted to support diagnostic and prognostic utility in SLE.

The present study aimed to evaluate the expression of long non-coding RNA gene (MALAT1 and GAS5) in SLE patients with/without nephritis and study their relation to disease activity and chronicity changes.

Methods

The study includes 40 SLE–LN/NN patients enlisted in the author's hospital outpatient clinics and 40 age-matched and sex-matched in healthy controls between October 2017 and October 2019. A written consent is obtained from all individuals concerned with this study, and it is approved by the Institutional Ethics Committee (IEC).

All SLE patients have met the SLE classification requirements of the American College of Rheumatology (ACR) [14, 15]. Both clinical examination and laboratory investigations were done. The Score of Systemic Lupus Erythematosus Disease Activity Index (SLED AI) for each patient is determined during blood withdrawal [2]. Depending on the SLEDAI results, patients were divided into patients with active disease (scores > 4) or patients with inactive disease (scores < 4) [16]. SLE renal involvement was defined according to ACR criteria, depending on nephritis presence/absence. All LN patients have undergone a kidney biopsy. The study excluded concurrent infection patients. Healthy controls did not have any autoimmune diseases or treatment by immunosuppressive agents.

Five millimeters of blood was collected from each subject in ethylenediaminetetraacetic acid (EDTA) collection tubes. Peripheral mononuclear blood cell (PBMC) isolation was done using Histopaque-1077 (Sigma-Aldrich) Ficoll density-gradient centrifugation according to the manufacturer instructions.

Total RNA, including lncRNA, was extracted by the TRIzol reagent from PBMCs (Zymo Research, Irvine, CA). NanoDrop 2000 (Thermo Fischer Scientific, Waltham, MA) was used to determine the concentrations of RNA. The reversed transcript on the RNA was performed using SensiFAST cDNA Synthesis Kit [Bioline, Memphis, TN] with a 20 ml final reaction volume. The expression levels of MALAT1 and GAS5 lncRNAs were evaluated according to the internal control, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), using a Hera Plus SYBR Green qPCR Kit (Willow Fort, Birmingham, UK) according to the manufacturer's protocol.

Fold change was calculated using the comparative threshold cycle [$2^{-\Delta\Delta Ct}$] for relative quantification which is normalized to an endogenous control [17]. The PCR primer sequences were as follows: MALAT1 forward primer, GAATTGCGTCATTTAAAGCCTAGTT and reverse primer, GTTTCATCCTACCACTCCCAATTAAT; GAS5 forward primer, CTTCTGGGCTCAAGTGATCCT and reverse primer, TTGTGCCATGAGACTCCATCAG; GAPDH forward primer, ACAGTCAGCCGCATCTTCTT and reverse primer, GACAAGCTTCCCGTTCTCAG. Real-time PCR was performed using 7500 Real-Time PCR Systems [Applied Biosystems] under the following conditions: 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 60 s.

Statistical analysis

Social Science Software Computer Program version 26 [SPSS, Inc., Chicago, IL, USA] was used to analyze obtained data. Non-parametric data were presented by the median and interquartile range, while means and standard deviation were used for parametric data. Student's *t* test was used to compare quantitative parametric data, while Mann-Whitney *U* test was used for quantitative non-parametric data. On the other hand, Kruskal-Wallis *H* test was followed by Dunn's post hoc test for pairwise comparison. Pearson's chi-square or Fisher exact test was used to compare qualitative data, and Spearman correlation was used to correlate different parameters. The receiver operating characteristic (ROC) curve was

Table 1 Comparison of GAS5 and MALAT1 relative expression in all studied groups

Variable	All cases		Control	*p* value
Relative expression of GAS5	0.52 (0.19–1.02)		0.98 (0.88–1.06)	0.001*
Relative expression of MALAT1	3.13 (1.69–5.15)		0.91 (0.84–1.17)	< 0.001*
	Non-nephritis	**Nephritis**	**Control**	
Relative expression of GAS5	0.91 (0.42–1.28)	0.24 (0.17–0.67) [a]	0.98 (0.88–1.06) [b]	< 0.001*
Relative expression of MALAT1	3.58 (1.50–5.63)	2.60 (1.89–4.20)	0.91 (0.84–1.17) [ab]	< 0.001*

Data are expressed as median (IQR). Tests used: Mann-Whitney *U* test and Kruskal-Wallis followed by Dunn's post hoc for data expressed as median (IQR)
p p value
*Significance < 0.05
[a]Significance vs non-nephritis
[b]Significance vs nephritis

also used to determine the diagnostic power of each test. The statistically relevant *p* value < 0.05 was regarded.

Results

The expression levels of lncRNAs (MALAT1 and GAS5) in PBMCs taken from 40 patients with SLE and 40 healthy controls were measured using RT-qPCR.

MALAT1 mRNA transcripts were higher in SLE patients compared to controls (p < 0.001) (Table 1, Fig. 1a). There were also significant differences between LN and NN patients compared to controls (p = <0.001) (Table 1, Fig. 1c). However, there were no statistically significant differences with respect to MALAT1 in LN patients relative to NN patients (p = 0.25) (Table 2).

The expression of GAS5 was significantly lower in SLE patients as compared to controls (p < 0.001) (Table 1, Fig. 1b). Also, the expression of GAS5 was decreased significantly in patients with LN compared with healthy donors and patients with NN (p < 0.001 and p = 0.003, respectively) (Tables 1 and 2, Fig. 1d).

SLE subgroups showed a significant difference in clinical/laboratory characteristics including blood pressure (p = 0.007), urinary output (UOP) (p < 0.001),

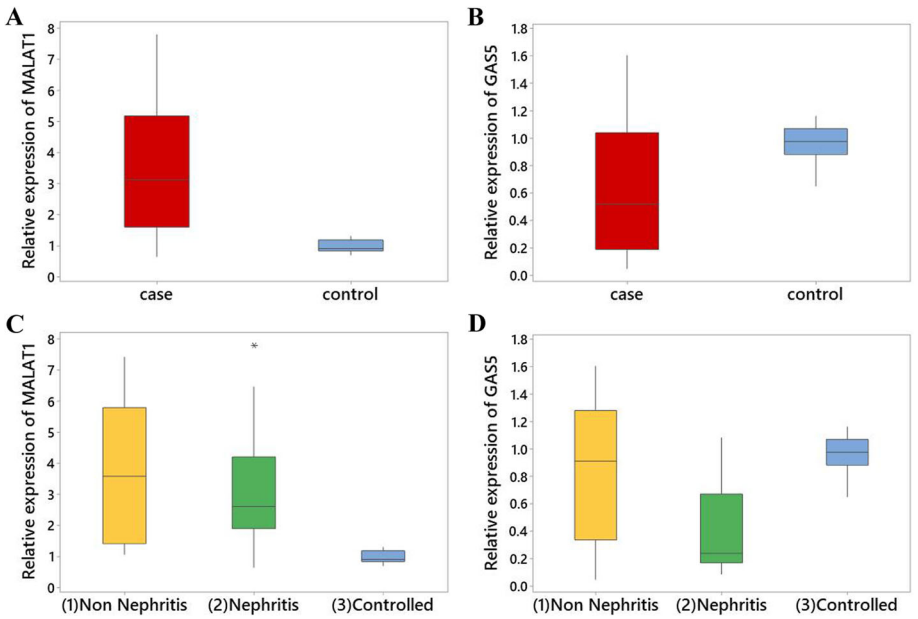

Fig. 1 The relative expression of GAS5 and MALAT1 between SLE patients and controls **a** MALAT1 relative expression between SLE groups versus controls. MALAT1 expression is significantly higher in SLE patients compared to control group (p = < 0.001). **b** GAS5 relative expression between SLE groups versus controls. GAS5 expression was significantly lower than the control group (p = < 0.001). **c** MALAT1 relative expression between two SLE subgroups, nephritis, and non-nephritis versus controls showed a significant difference in LN and NN patients compared to control cases (p = < 0.001). However, there were no statistically significant differences in LN patients compared to NN (p = 0.25). **d** GAS5 relative expression between the two SLE subgroups, nephritis, and non-nephritis versus controls showed a significant difference in LN patients compared to control groups (p = < 0.001). In addition, GAS5 showed significant downregulation in LN patients compared to NN patients (p = 0.003). Red, SLE cases; blue, control group; yellow, NN; green, LN; *p*, *p* value. The central box in the diagram represents the lower and upper quartiles (IQR). The central horizontal line in the box represents the median

Table 2 Clinical and laboratory characteristics of the study patients

Variables		Non nephritis		Nephritis		Total		p
Blood pressure	**Average**	18	85.7%	8	42.1%	26	65.0%	**0.007***
	Hypotensive	0	0.0%	0	0.0%	0	0.0%	
	Hypertensive	3	14.3%	11	57.9%	14	35.0%	
UOP	**Average**	14	66.7%	5	26.3%	19	47.5%	**< 0.001***
	Anuria	1	4.8%	2	10.5%	3	7.5%	
	Oliguria	6	28.6%	11	57.9%	17	42.5%	
	Polyuria	0	0.0%	1	5.3%	1	2.5%	
Proteinuria	**No**	20	95.2%	0	0.0%	20	50.0%	**< 0.001***
	Yes	1	4.8%	19	100.0%	20	50.0%	
Casts in urine	**None**	19	90.5%	8	42.1%	27	67.5%	**< 0.001***
	Granular	1	4.8%	4	21.1%	5	12.5%	
	Hyaline	0	0.0%	1	5.3%	1	2.5%	
	Mixed "Granular&Hyaline"	1	4.8%	5	26.3%	6	15.0%	
	Crystal	0	0.0%	1	5.3%	1	2.5%	
Relative expression of GAS5 (Median [IQR])		0.91 [0.42–1.28]		0.24 [0.17–0.67]		0.52 [0.19–1.02]		**0.003***
Relative expression of MALAT1 (median [IQR])		3.58 [1.50–5.63]		2.60 [1.89–4.20]		3.13 [1.69–5.15]		**0.25**
Hb (mean ± SD)		9.3 ± 1.9		8.8 ± 2.0		9.1 ± 1.9		**0.38**
RBC (mean ± SD)		3,761,905 ± 763,869		3,584,211 ± 977,993		3,677,500 ± 865,305		**0.52**
WBC (median [IQR])		5600 [4400–9800]		6790 [5600–15,000]		6500 [4400–13,850]		**0.27**
Lymphocytes (median [IQR])		1300 [900–1700]		1400 [1000–1800]		1400 [950–1800]		**0.66**
PLT (median [IQR])		210,000 [135,000–295,000]		162,000 [12,7000–218,000]		203,500 [128,000–267,500]		**0.15**
S. creatinine (median [IQR])		0.9 [0.8–0.9]		1.5 [0.9–3.2]		0.9 [0.8–2.0]		**0.004***
cNa (mean ± SD)		138.0 ± 4.5		138.6 ± 6.7		138.3 ± 5.6		**0.73**
ck (mean ± SD)		3.74 ± .56		3.81 ± .67		3.77 ± .61		**0.71**
ANA (median [IQR])		52.0 [40.0–93.5]		81.8 [45.0–133.0]		70.8 [41.1–99.0]		**0.29**
Anti.ds.DNA (median [IQR])		31.6 [9.9–68.0]		79.0 [20.0–210.0]		38.0 [15.0–161.8]		**0.1**
C3 (median [IQR])		76.0 [50.0–93.0]		77.0 [28.0–98.0]		76.5 [40.6–95.0]		**0.76**
C4 (median [IQR])		11.0 [8.0–22.0]		12.0 [7.0–29.4]		12.0 [7.5–27.1]		**0.48**
SLEDAI_2K.Score (median [IQR])		20.0 [14.0–29.0]		43.0 [28.0–55.0]		28.5 [19.5–48.0]		**0.001***
Biopsy class	**Non**	**0**	**0.0%**	**0**	**0.0%**	**0**	**0.0%**	
	Class 1	**0**	**0.0%**	**0**	**0.0%**	**0**	**0.0%**	
	Class 2	**0**	**0.0%**	**1**	**5.3%**	**1**	**5.3%**	
	Class 3	**0**	**0.0%**	**2**	**10.5%**	**2**	**10.5%**	
	Class 4	**0**	**0.0%**	**12**	**63.2%**	**12**	**63.2%**	
	Class 5	**0**	**0.0%**	**3**	**15.8%**	**3**	**15.8%**	
	Class 4-5	**0**	**0.0%**	**0**	**0.0%**	**0**	**0.0%**	
	Class 6	**0**	**0.0%**	**1**	**5.3%**	**1**	**5.3%**	
Biopsy.AI (median [IQR])		**.**		**5.5 [5.0–8.0]**		-		
Biopsy.CI (median [IQR])		**.**		**3.5 [1.5–6.0]**		-		

Data are expressed as frequency (*N*, %), mean ± SD, and median (IQR). Test used: Fisher exact or Monte-Carlo for data expressed as frequency, Mann-Whitney *U* test for data expressed as median (IQR), and Student's *t* test for data expressed as mean ± SD

p p value

*Significance < 0.05

Table 3 Correlation between GAS5, MALAT1, and clinical and laboratory variables

Variable	Non-nephritis				Nephritis			
	Relative expression of GAS5		Relative expression of MALA T1		Relative expression of GAS5		Relative expression of MALA T1	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age	− 0.052	0.824	− 0.327	0.148	− 0.277	0.252	− 0.002	0.994
Marital status	0.412	0.064	− 0.023	0.921	− 0.138	0.572	− 0.23	0.344
Blood pressure	− .562	**0.008***	− 0.214	0.352	− 0.078	0.751	0	1
Hb	0.064	0.782	0.3	0.186	− 0.213	0.382	− 0.079	0.748
RBC	0.174	0.45	0.278	0.222	− 0.02	0.935	− 0.056	0.819
WBC	0.351	0.119	0.169	0.463	0.26	0.282	− 0.067	0.786
Lymphocytes	0.418	0.059	0.051	0.827	0.172	0.482	− 0.074	0.764
PLT	− 0.146	0.529	0.041	0.859	− 0.04	0.871	0.056	0.819
S. creatinine	− 0.152	0.511	0.026	0.912	0.244	0.314	0.353	0.138
Can	0.039	0.868	0.151	0.514	0.12	0.625	0.105	0.67
Ck	0.12	0.605	0.225	0.326	0.196	0.421	0.052	0.833
ANA	0.014	0.953	− 0.017	0.941	− 0.021	0.932	0.009	0.972
Anti.ds.DNA	0.023	0.92	0.116	0.616	0.127	0.603	0.128	0.601
C3	0.126	0.586	0.261	0.253	− 0.069	0.778	0.258	0.286
C4	0.268	0.24	0.405	0.068	− 0.001	0.997	0.256	0.289
UOP	− 0.288	0.205	− 0.188	0.414	0.321	0.18	0.045	0.853
SLEDAI_2K.Score	0.456	**0.038***	0.093	0.687	-0.216	0.375	− 0.137	0.576

r Spearman correlation coefficient, *p p* value
**r* is significant at $p < 0.05$

proteinuria ($p < 0.001$) and serum creatinine ($p = 0.004$), and casts present in the urine ($p < 0.001$), and SLEDAI score ($p = 0.001$) (Table 2).

There was no correlation among lncRNA (GAS5 and MALAT1) expression, activity markers, including anti-dsDNA titers, complement level c3 and c4, and other hematological parameters. In addition, SLEDAI score was not correlated to MALAT1 relative expression, but it was positively correlated to GAS5 relative expression in the NN subgroup (Table 3).

GAS5 expression was significantly correlated to glomerulosclerosis ($p = 0.044$), interstitial fibrosis (0.028), and tubular atrophy degree ($p = 0.031$). This might be attributed to GAS5 association with chronicity pathological markers in LN patients. On the other hand, it showed no significant correlation with

Table 4 Correlation between GAS5, MALAT1, and histopathological markers of activity and chronicity

Variable	Relative expression of GAS5		Relative expression of MALAT1	
	r	*p* value	*r*	*p* value
Endocapillary hypercellularity percentage	.218	.604	− .289	.487
Hyaline lesions	.184	.636	− .244	.527
Cellular crescents	− .105	.773	− .276	.440
Fibrinoid necrosis	− .501	.116	.300	.370
Karyorrhexis	.256	.475	− .294	.409
Interstitial infiltration	.071	.809	.322	.261
Sclerotic glomeruli	.615*	**.044**	− .080	.816
Fibrous crescents	.413	.270	− .411	.272
Atrophic tubules	.647*	**.031**	.077	.821
Interstitial fibrosis	.565*	**.028**	− .047	.869

r Spearman correlation coefficient, *p p* value
**r* is significant at $p < 0.05$

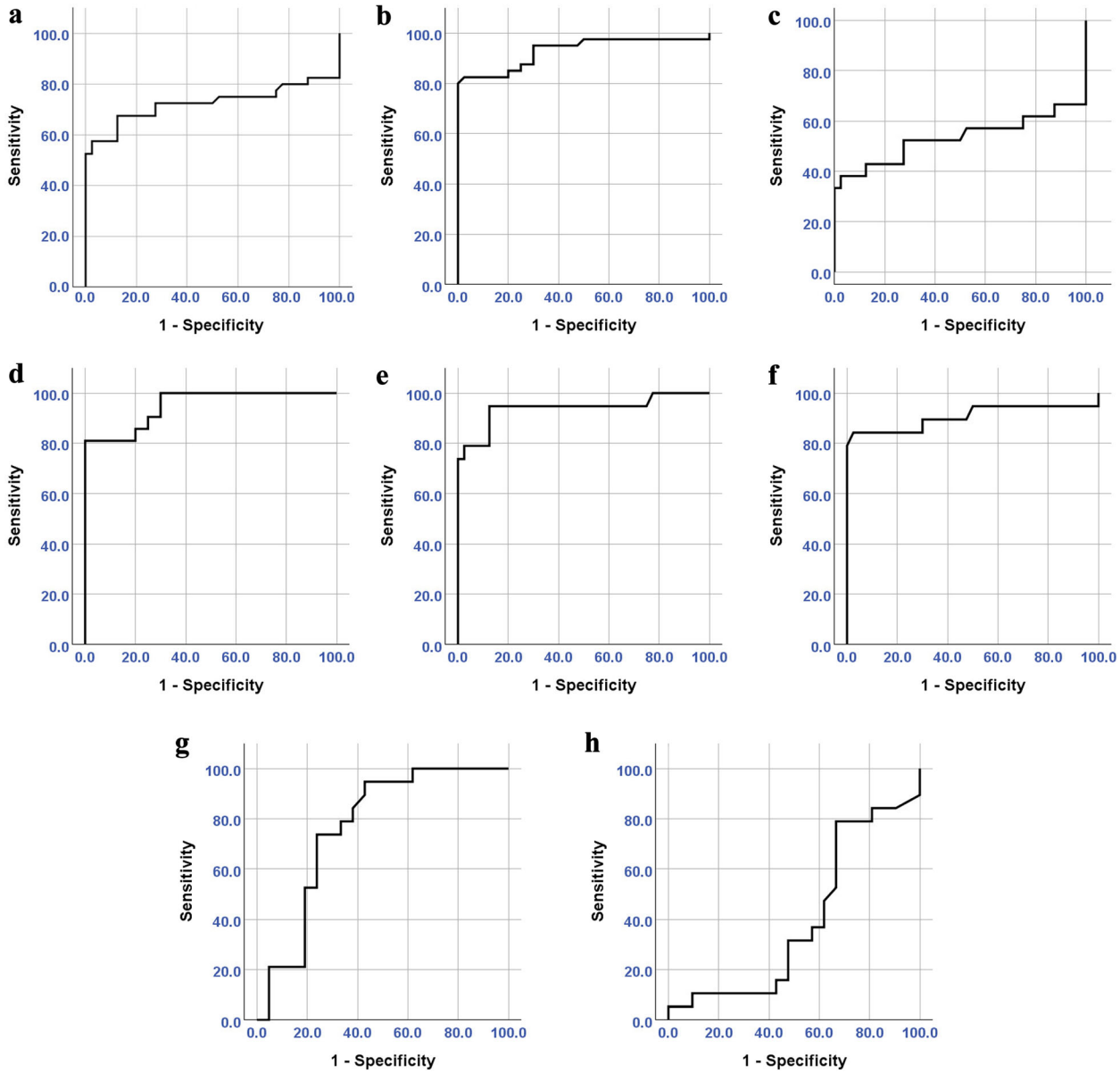

Fig. 2 ROC curve studying the validity as diagnostic biomarker **a** relative expression of GAS-5 in all cases vs. control groups area under curve [AUC] 0.725, *p* value 0.001*. **b** Relative expression of MALAT1 in all cases vs. control groups AUC 0.929 *p* value < 0.001*. **c** Relative expression of GAS5 control vs. con-nephritis. **d** Relative expression of MALAT1 control vs. non-nephritis AUC 0.95, *p* value < 0.001*. **e** Relative expression of GAS5 control vs. nephritis AUC 0.94, *p* value < 0.001*. **f** Relative expression of MALAT1 control vs. nephritis AUC 0.91, *p* value < 0.001*. **g** Relative expression of GAS5 nephritis vs non-nephritis AUC 0.77 *p* value (0.004*). **h** Relative expression of MALAT1 nephritis vs non-nephritis. AUC, area under curve; *p*, probability. *Significance < 0.05

any of the renal histopathological activity parameters, including endocapillary hypercellularity, cellular crescents, fibrinoid necrosis, karyorrhexis, and active interstitial infiltration (Table 4).

Receiver operating characteristic (ROC) curve analysis was performed to discriminate SLE patients from healthy controls using MALAT1 and GAS5 gene expression as discrimination marker (Fig. 2).

Discussion

In this study, we investigated lncRNA expression (MALAT1 and GAS5) in SLE patients with/without nephritis. We found that the lncRNA (MALAT1) was aberrantly expressed in comparison with healthy controls, while GAS5 expression levels in SLE patients was significantly reduced in comparison with controls. Results suggested that MALAT1 and GAS5 plasma levels could serve as potential biomarkers for SLE.

Previously, it has been shown that lncRNA (GAS5)—which is important for normal growth, apoptosis, and cell cycle function—was consistent with an increased risk of developing SLE in a mouse model [18]. GAS5 was also involved in human SLE development [19]. Previous studies have indicated that GAS5 is significantly lower in SLE patients compared to healthy controls [20, 21].

It has been found that MALAT1 expression increases abnormally in SLE patients. Previous studies have proven that MALAT1 was involved in Sirtuin 1 (SIRT1) signaling regulation, which contributes to lupus disease initiation and maintenance [22]. SIRT1 expression was reduced significantly after MALAT1 knockdown, which suggested its main regulatory function in SLE pathogenesis [23, 24]. The role of other lncRNAs (NEAT2, CTC-471 J1.2, and lnc-DC) was investigated in LN patients in previous reports [25]. To investigate whether GAS5 and MALAT1 expression is related to renal affection in SLE, we contrasted their relative expression levels in SLE patients with/without nephritis. Our findings showed that GAS5 relative expression in LN patients is significantly downregulated compared to patients with NN. This indicates that GAS5 could be used as a biomarker to distinguish the SLE with/without LN.

GAS5 relative expression was significantly associated with the degree of glomerulosclerosis, interstitial fibrosis, and tubular atrophy. This may indicate an association between the dysregulated lncRNA [GAS5] and poor renal outcomes.

By further investigations on the correlation of lncRNA (GAS5, MALAT1) with SLEDAI-2 K score between LN and NN, a positive correlation was found between GAS5 and SLEDAI-2 K score in the NN subgroup, not in the LN subgroup. Currently, there is no study exploring GAS5 association and lupus activity in differentiating between patients with/without nephritis. This observation needs to be further studied to clarify this point.

lncRNA (GAS5) was negatively correlated to blood pressure in NN group, which denotes the higher risk of hypertension in SLE patients with downregulated GAS5. This can be explained by the previous observation of GAS5 inhibitory effect on the proliferation and migration of vascular smooth muscle cells, for which it can serve as a potential hypertension therapeutic target [26]. Considering GAS5 as a potential biomarker for hypertension has been previously elucidated in animal models [27].

This research may have many limitations. First is examining patients from only one hospital, which may limit finding generalization. Second, potential confounding factors such as different treatment strategies among patients should be considered in data interpretation. To reveal the exact further function of these lncRNAs in SLE and further evaluate their diagnostic and prognostic efficacy in lupus nephritis, future studies must be conducted.

Conclusion

The lncRNA (GAS5 and MALAT1) may serve as diagnostic biomarkers for SLE. Moreover, GAS5 may distinguish SLE LN patients from NN patients and may predict renal fibrosis in LN patients.

Abbreviations

GAS5: Growth arrest-specific 5; LncRNA: Long non-coding RNA; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; PBMCs: Peripheral blood mononuclear cells; SLE: Systemic lupus erythematosus; LN: Lupus nephritis; NN: Lupus non-nephritis; SLEDAI: Systemic lupus erythematosus disease activity index; Anti-dsDNA: Anti-double stranded DNA; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

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Authors' contributions

ME, NM amd ME designed the research and performed the experiments. RS and AE recruited the patients and processed the samples. All authors analyzed the data, shared in writing the manuscript, and read and approved the final version of this manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Mansoura Faculty of Medicine with approval number [R. 20.01.731]. Informed written consent was obtained from all individuals included in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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