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Original Article

Association of miRNA-145 expression in vascular smooth muscle cells with vascular damages in patients with lupus nephritis

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Abstract: miRNAs have been found to contribute to the regulation of multiple cellular processes, including cell apoptosis, differentiation and proliferation. The patients with lupus nephritis (LN) exhibit thickened renal vascular membrane and highly proliferative vascular smooth muscle cells (VSMCs). Of various miRNAs discovered, miR-145 is essential to mediate the proliferation of VSMCs and the formation of atherosclerotic plaques. In this study, we studied the pathological and vascular damage of renal LN, and the correlation between miR-145 expression in VSMCs and the vascular damages. Serum, urine, and renal biopsies were obtained from 41 patients with active LN. The serum and urinary VEGF levels were examined to confirm the renal damage of each patient. Biopsies were stained to observe the glomerular segmental lesions, sclerosis, and to evaluate the vascular damages. The expression of miR-145 was also examined to determine the correlation between its expression and the vascular damages. The expression of miR-145 was mainly detected in the renal VSMCs and the epithelial cells of glomerular proximal convoluted tubule. Nevertheless, the expression of miR-145 reduced as the tunicae media vasorum ratios increased, indicating the development of LN inhibits the expression of miR-145. Furthermore, our studies revealed no significant correlation among renal interstitial vascular damage, glomerular damage and severity classification of LN. Therefore, we suggest the damage of renal interstitial vascular should be considered as one of the factors to evaluate the severity of the LN.

Keywords: miR-145, lupus nephritis, renal interstitial vascular

Introduction

As the identity and function of novel microRNA (miRNAs) are revealed, it is becoming clear that they contributes to regulating multiple cellular processes, including apoptosis, differentiation, and the development of autoimmune diseases [1]. A variety of miRNAs have been reported to be significantly involved in the regulation of vascular smooth muscle cell (VSMC) proliferation, which is an essential process for the formation of atherosclerotic plaque [2]. MiR-145 acts as one of the most important communicating molecules to regulate phenotypic switch and vascular homeostasis [2, 3], and has been found to be expressed abundantly in the VSMCs composing the vascular walls [4]. The studies of Cordes, et al. revealed that miR-145 can determine the smooth muscle fate, and that miR-145 and miR-143 function to regulate the differentiation and proliferation of smooth muscle cells [5]. Also, formation of neointimal lesions were found in the VSMCs collected from miR-143/145 deficient mice [6]. Therefore, the expression of miR-143/145 may influence vascular development and repair, and attenuate the pathogenesis of arteriosclerosis. Some research outlined the effects of miRNA-145, -126, and -155 in atherosclerosis in vivo, and found that the down regulation of miR-145 controlled differentiation of smooth muscle cells and promoted lesion formation [7]. Treatment with miR-145 lentivirus in an apolipoprotein E knockout mice model resulted in reduced

Table 1. Pathological classification and immunofluorescence in 41 LN cases

classification	IgA	IgG	IgM	C1q	C3	C4	full-house	case number (%)
II	5	4	5	4	4	1	1	5 (12.2%)
III	6	7	6	6	7	5	3	7 (17.1%)
III + V	3	3	2	3	3	3	2	3 (7.3%)
IV	15	10	13	12	15	13	6	16 (39.0%)
IV + V	9	10	9	7	10	7	5	10 (24.4%)
total	38 (92.7%)	34 (82.9%)	35 (85.3%)	32 (78.0%)	39 (95.1%)	29 (70.7%)	17 (41.4%)	41 (100%)

Table 2. Clinical finding in 41 LN cases [n (%)]

class	anaemia	Hyper-Coagulation	Hypo-proteinuria	Renal dysfunction	Hypo-complementemia	hematuria	proteinuria	Hematuria+ proteinuria	Total cases [n (%)]
II	2 (40.0)	0 (0.0)	1 (20.0)	0 (0.0)	5 (100.0)	4 (80.0)	1 (20.0)	1 (20.0)	5 (12.2)
III	5 (71.4)	3 (42.9)	4 (57.1)	2 (28.6)	7 (100.0)	2 (28.6)	1 (14.3)	0 (0.0)	7 (17.1)
III+V	2 (66.7)	1 (33.3)	3 (100.0)	2 (66.7)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (7.3)
IV	13 (81.2)	11 (68.8)[1]	12 (75.0)	10 (62.5)	16 (100.0)	12 (75.0)	10 (62.5)	9 (56.3)	16 (39.0)
IV+V	8 (80.0)	8 (80.0)[1]	7 (70.0)	7 (70.0)	10 (100.0)	8 (80.0)	7 (70.0)	6 (60.0)	10 (24.4)
TOTAL	30 (73.2)	23 (56.1)	27 (65.9)	21 (51.2)	41 (100.0)	29 (70.7)	22 (53.7)	19 (46.3)	41 (100.0)

[1]compared with class II of the hypercoagulation group, $P < 0.05$.

plaque sizes [8]. The expression of miR-145 was also been found to restore the contractile phenotype of the VSMCs in the metabolic syndrome [9].

Accelerated atherosclerosis is a major cause of mortality in SLE. Previously, we detected the thickened renal vascular membrane in children with lupus nephritis (LN), and found the fusiformal VSMCs, which represent the proliferative phenotype. Our findings confirmed the presence of vascular membrane proliferation in LN renal, but the mechanism remains unclear. miR-145 is crucial for the regulation of VSMCs phenotype. Although miR-145 is crucial for the regulation of VSMCs phenotype, little information is known about the role of miR-145 in the proliferation of VSMCs in kidney. Herein, we focused on the pathological and vascular damage of renal LN, and studied the correlation between miR-145 expression in VSMCs and vascular damages, and provided a theoretical basis for introducing miR-145 in the treatment of LN.

Patients and methods

Patients

41 patients, including 30 females and 11 males at the mean age of 11.1 ± 2.4 years, with SLE and biopsy-proven active LN between 2008 and 2014 were collected for this study. All patients fulfilled the updated revised American College of Rheumatology (ACR) classification criteria for SLE. Clinical data, blood and urinary samples were collected for laboratory tests (complete blood count, erythrocyte sedimentation rate, serum creatinine, cholesterol, urinalysis, urine protein/creatinine ratio). Written informed consent was obtained from all subjects and the regional ethics committee in Haikou, China approved the study protocol.

Assays of blood and urinary VEGF levels

The blood and urinary VEGF levels were assayed by the VEGF kit (Shenzhen Jingmei Bioengineering Co., Ltd). The renal VEGF levels were examined by SABC kit (ZhongShan Bio-Tech Co., Ltd, the primary VEGF antibody was from Santa Cruz Biotechnology, Inc.). The slides for immunohistochemistry were pretreated with polylysine. The biopsy tissues were embedded, sliced (3 μm), dewaxed, dehydrated, sealed with goat serum, microwave digested, and incubated with rabbit-anti VEGF antibodies (1:100 diluted in PBS) at 4°C overnight. After PBS washes, the tissues were incubated with biotin-conjugated goat-anti-rabbit secondary antibodies, and HRP-conjugated streptavidin at 37°C for 20 min, followed by DAB and hematoxylin staining, and sealed. Images of glomerular of the tissue slices were taken by the SECOM camera, and analyzed by the CMIAS system at

Table 3. Glomerular damage and renal interstitial damage integral of the biopsy samples from the 41 patients

classification	glomerular damage integral	renal interstitial damage integral
II	36.8 + 5.9	19.4 + 2.6
III	67.7 + 4.1[1]	34.3 + 5.9[1]
III+V	80.7 + 5.5[1,2]	42.7 + 6.0[1,2]
IV	101.0 ± 4.9[1,2,3]	54.4 + 4.7[1,2,3]
IV+V	115.8 + 5.5[1,2,3,4]	60.9 + 5.0[1,2,3,4]

1: compared with class II LN, P < 0.05; 2: compared with class III LN, P < 0.05; 3: compared with class III + V LN, P < 0.05; 4: compared with class IV LN, P < 0.05.

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$$\text{The expression level of VEGF} = \frac{\text{Average VEGF absorbance of glomerular, renal tubules, and renal interstitial}}{\text{The Absorbance of CEA}}$$

Evaluation of renal function, histopathology and renal activity

The percutaneous renal biopsy tissues were obtained from all patients, and fixed using 10% neutral buffered formalin or 4% PFA and embedded in paraffin. All the renal biopsy samples were examined by light microscopy, direct immunofluorescence and electron microscopy techniques. Lupus nephritis was re-classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification system [10].

Based on the evaluation method proposed by Katafuchi, we applied the glomerular score for the following main glomerular changes for the 41 patients: 1) glomerular hypercellularity (mesangial and endocapillary); 2) segmental lesions such as tuft adhesion, crescent and segmental sclerosis; 3) global glomerular sclerosis [11].

Glomerular hypercellularity index

Glomerular hypercellularity was defined as 3 or more nuclei in mesangial area or endocapillary hypercellularity in any extent. The severity of glomerular hypercellularity in each glomerulus was semiquantitatively graded as follows: mild indicate soccupation less than 25% of glomerular area; moderate indicates occupation with 25 to 50% of glomerular area; severe indicates occupation greater than 50% of glomerular area. Each glomerulus in biopsy specimens was given a point according to its grading of glomerular hypercellularity as follows: 1 representing no hypercellularity; 2 representing mild; 3 representing moderate; and 4 representing severe. The points of individual glomerulus were added and divided by the number of glomeruli in each biopsy.

Index of glomerular segmental lesions

The percentage of glomeruli with segmental lesions such as crescents, tuft adhesions to Bowman's capsule, and segmental sclerosis was calculated and the index of glomerular segmental lesion was determined as follows: 0 representing no segmental lesion; 1 representing segmental lesions in less than 10% of glomeruli; 2 representing segmental lesions in glomeruli more than 10% and less than 25%; 3 representing those in glomeruli over 25% and less 50% of glomeruli; 4 representing segmental lesions in over 50% of glomeruli.

Index of glomerular sclerosis

The index of glomerular sclerosis was determined according to the percentage of obliterated glomeruli due to global sclerosis as follows: 0 representing no global sclerosis; 1 representing global sclerosis in less than 10% of glomeruli; 2 representing global sclerosis in more than 10% and less than 25% of glomeruli,; 3 representing global sclerosis in over 25% and less than 50% of glomeruli; 4 representing global sclerosis in more than 50% of glomeruli. The sum of these three indices was defined to be a glomerular score.

Index of interstitial cell infiltration

The severity of interstitial cell infiltration was semi quantitatively determined as follows: 0 representing none; 1 + representing occupation of less than 25% of cortical area of biopsy specimen; 2 + representing occupation between 25% and 50% of cortical area; 3 + representing occupation over 50% of cortical area.

Evaluation of vascular damage

Vascular damages from middle or near the median sagittal cross-sectional features were observed under electron microscope, and images were taken. Vascular membrane area and the total area were measured by medical image analysis system. The thickness of the intima was determined as follows: mild indicating less than 0.63; moderate indicating more than 0.63 and less than 0.75; severe indicating more than 0.75.

Table 4. Urinary, renal local and serum VEGF levels detected in the 41 LN patients and 10 healthy controls.

classification	Cases [n]	Urinary VEGF (pg/mL)	Renal VEGF (%)	Serum VEGF (pg/mL)
II*,1	5	62.3	22.1	698
		67.0	20.4	711
		59.4	23.6	708
		63.8	22.8	721
		65.5	21.6	726
III**,2	7	89.2	26.9	657
		91.3	27.2	661
		95.6	28.6	631
		92.4	29.0	648
		90.1	28.2	644
		91.6	27.3	651
		93.6	29.8	637
III+V***,3	3	329.8	36.1	572
		298.1	35.4	566
		306.5	35.7	557
IV#,4	16	289.6	31.2	501
		322.4	30.0	495
		333.0	30.5	487
		328.9	31.0	486
		329.0	31.5	480
		319.7	29.0	491
		336.2	29.5	499
		318.7	28.9+	492
		334.1	29.3	488
		346.2	29.5	467
		333.6	28.8	472
		341.0	31.5	477
		338.1	32.0	482
		306.2	31.9	480
		311.5	31.5	485
		312.9	32.0	499
IV + V€,5	10	378.0	38.9	412
		369.2	39.6	400
		397.5	39.9	403
		400.0	37.9	399
		412.5	41.3	396
		396.1	40.8	411
		399.7	39.7	408
		406.8	41.5	400
		411.0	40.0	412
		417.8	41.4	399
control	10	16.8		369
		21.0		376
		23.2		356
		17.5		367
		16.9		388
		18.2		380
		22.0		372
		23.2		368
		21.6		374
		22.8		361

miR-145 expression assay

The probe sequence was CTGGGAAAACTGGACCGTGAGG (Auragene Bioscience Corporation Inc. Changsha, China). All the incubations were at 37°C. Renal biopsy samples were dewaxed with 3% H_2O_2 for 10 min, and washed with ddH_2O twice. The samples were then incubated in pepsin containing 3% citric acid for 30 min, following three washes with 0.5 M PBS, and one wash with ddH_2O. The samples were then incubated in the hybridization buffer for 2 hr. The probe was diluted with the hybridization buffer as 1:500, and replaced the buffer without the probe, incubating with the biopsy samples overnight. After the probe incubation, the samples were washed with 2× SSC twice of 5min each, followed by a wash with 0.5× SSC for 15 min, 0.2× SSC twice, 15 min each. Sealing fluid was added, incubating for 30 min. The samples were then incubated with mouse anti digoxin for 60 min, followed by 4 washes of 0.5 M PBS, 5 min each. SABC was added with the incubation for 30 min, and the samples were washed three times with 0.5M PBS for 5 min each. Following the addition of peroxidases, and PBS washes, the samples were stained with DAB, counter stained with haematoxylin, dehydrated, and sealed with neutral balsam.

The expression level of miR-145 =

$$\frac{\text{The area of miR-145 postive region}}{\text{The average grey lever of the miR-145 postive region} \times \text{tunica media}} \times 1000$$

Statistical analysis

All the data was analyzed with SPSS19.0. Normal distributed data were indicated as mean ± standard deviation ($\bar{X} \pm S$), and underwent variance analysis. Skewed distributed data were indicated as median (25 or 75 quantile), and analyzed with Kruskal-Wallis H. $\alpha = 0.05$, and $P < 0.05$ indicates a significant difference.

Results

Pathological classification in patients with lupus nephritis and their associations with immunofluorescence and clinical features

Pathological and clinical data analysis was according to ISN/RPS 2003 classification of

*serum VEGF of class II vs III, $P < 0.05$. **serum VEGF of class III vs III + V, $P < 0.05$. ***serum VEGF of class III + V vs. IV, $P < 0.05$. #serum VEGF of class IV vs. IV+V, $P < 0.05$. ©serum VEGF of class IV + V vs. control, $P < 0.05$. [1]Urinary VEGF of VEGF of class II vs. III, $P < 0.05$. [2]Urinary VEGF of class III vs. III + V, $P < 0.05$. [3]Urinary VEGF of class III+V vs. IV, $P < 0.05$. [4]Urinary VEGF of class IV vs. IV + V, $P < 0.05$. [5]Urinary VEGF of class IV+V vs. control, $P < 0.05$.

LN, as shown in **Table 1**. All patients had an active nephritis at baseline with biopsies showing class II (n = 5), III (n = 7), III/V (n = 3), IV (n = 16), IV/V (n = 10). There was no LN class I and V in the 41 cases. Class II of cases accounted only for 12.2%, while severe cases (class III, III/V, IV, IV/V) accounted for 87.8%. In Class IV cases, there was 1 case where immunofluorescence is negative.

The clinical variables of various pathological types are summarized in **Table 2**. The cases of III+V were not included in the analysis due to its limited sample size. As the severity of LN increases, the cases of each LN clinical manifestation increased. In the group of hypercoagulative state ($X^2 = 10.302$), renal dysfunction ($X^2 = 8.886$), proteinuria ($X^2 = 7.791$), and hematuria/proteinuria ($X^2 = 8.720$), the multisample rate showed significant difference. However, comparing the cases of each severity class, only the number of cases for class II and IV, class II and IV+V in the hypercoagulative group showed significant differences. The samples in the hypercoagulative group exhibited increased levels of D-dimer (4.8 ± 6.3 mg/L), including 15 cases of D-dimer over 2.0 mg/L, accounting for 65.2%, and a case of D-dimer of 28.71 nag/L (class III + V). Also, all 41 patients showed hypocomplementemia.

The correlation between severity grade of LN and the glomerular and renal interstitial damages. According to the ISN/RPS2003SN classification, the glomerular and renal interstitial damages of each patient were evaluated as **Table 3**. The integrals of glomerular damage, and renal interstitial damage were significantly increased, as the severity class of LN increased. Also, the glomerular damage integral and the renal interstitial damage integral were positively correlated ($r = 0.959$, $P < 0.05$).

Serum, renal, and urinary VEGF levels in patients with renal vascular damage

The vascular damage can also be evaluated by the expression levels of VEGF, which mediates angiogenesis. As summarized in **Table 4**, compared 10 healthy controls, LN patients exhibited significantly higher urinary and serum VEGF levels ($P < 0.05$). More importantly, as the severity of the disease increased, the blood VEGF levels in the patients were decreased, whereas the urinary VEGF levels increased. As biopsies were not collected from healthy controls, the renal local VEGF levels in patients and controls were not comparable, and the patient biopsies with VEGF stained were shown in **Figure 1**. The increased urinary VEGF levels and the decreased serum VEGF levels indicated more severe vascular damage.

Pathological features of renal vascular damages

Vascular damages are indicated as vascular intima exfoliation and thickened tunicae media vasorum, and the thickness of the tunicae media vasorum is positively correlated with the damage degree of the vascular. In this study, the renal artery of all patients showed thickened vascular walls, thickened/damaged intima, and thickened tunicae media vasorum, and the patients with class IV and class IV + V LN exhibited the thickest renal vascular. Specifically, the samples from the patients with class II LN had a relatively intact intima, thickened tunicae media vasorum, and obvious proliferated ECs, which accounts for the majority of the thickened vascular walls. Samples from the patients with class III and III + V LN showed partial damage of the intima, unevenly thickened tunicae media vasorum, and proliferated ECs were observed at the intact region of intima. Samples from the patients with class IV and IV + V LN showed severe damage of the vascular intima and thickened tunicae media vasorum, as well as spindle shaped proliferated SMCs, and exfoliated ECs were observed within the vessels, narrowing down the vessels.

The severity of renal artery damages was classified dependent upon the intact/damage degree of the intima. The renal artery with intact intima, from patients with class II LN, was classified as mild; the renal artery with partial damages of the intima, from patients with class III and III + IV LN, was classified as moderate; the renal artery with exfoliated intima, from patients with class IV and IV + V, was classified as severe. The measurements of the renal arteries from all patients were summarized in **Tables 5**, **6**; **Figures 2**, **3**. The ratios of the vascular thickness/outer diameter for all the

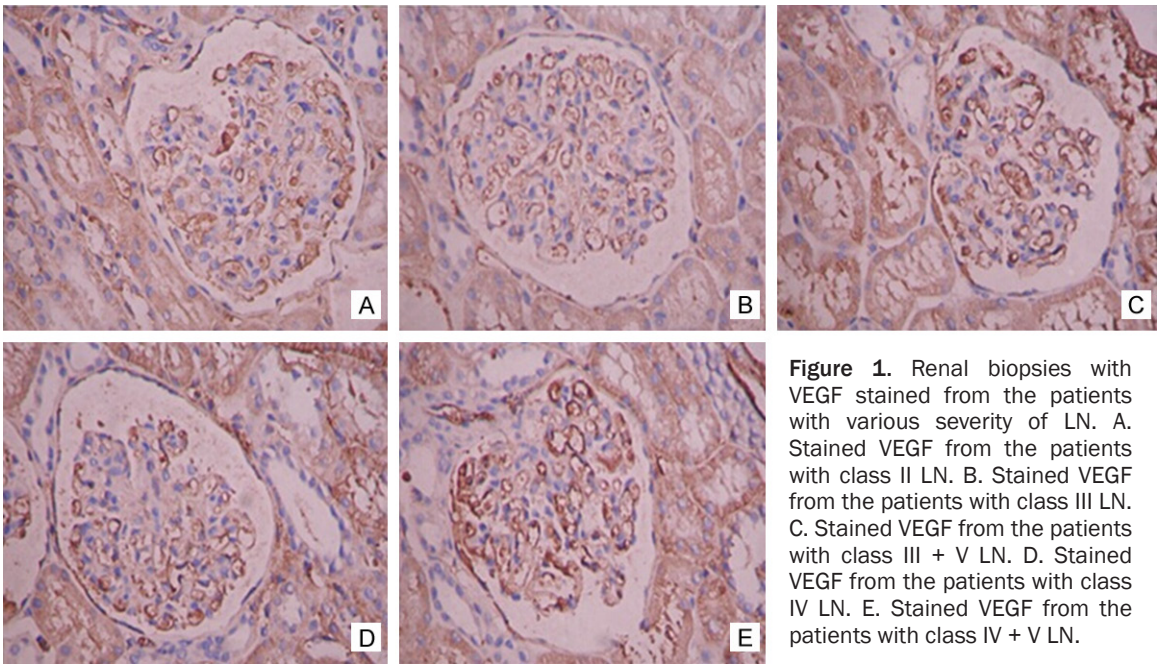

Figure 1. Renal biopsies with VEGF stained from the patients with various severity of LN. A. Stained VEGF from the patients with class II LN. B. Stained VEGF from the patients with class III LN. C. Stained VEGF from the patients with class III + V LN. D. Stained VEGF from the patients with class IV LN. E. Stained VEGF from the patients with class IV + V LN.

Table 5. Measurements of renal arteries from 41 patients with LN

Group	Vascular thickness/ outer diameter (%)	Intima thickness/vascular outer diameter (%)	tunicae media vasorum thickness/vascular outer diameter (%)
Mild	75.7 ± 27.6	25.0 ± 18.7	47.6 ± 1.8
Moderate	68.0 ± 10.2	19.9 ± 8.0	41.1 ± 16.86
Severe	78.3 ± 6.5[2]	15.5 ± 34.5	59.7 ± 5.6

2: severe group compared with moderate group, $P < 0.05$.

Table 6. Distribution of various damage degrees of tunicae media vasorum in the severity grades of LN

Damage degrees of tunicae media vasorum	II	III	III + V	IV	IV + V	Total
Mild	2	2	1	4	3	12
Moderate	1	3	1	6	4	15
Severe	2	2	1	6	3	14
Total	5	7	3	16	10	41

patients were over 0.5, indicating the thickened vascular. Variance analysis of the data suggested a significant difference between the vascular wall/outer diameter ratios for the moderate and severe group ($P < 0.05$), and the t-test suggested a significant difference between the intima thickness/vascular diameter ratios for the mild and severe group ($P < 0.05$). The correlation coefficient of the vascular thickness/ outer diameter ratio and the glomerular damage integral was r = 0.329, $P < 0.05$; and the correlation coefficient of this ratio and the renal interstitial damage integral was R = 0.360, $P < 0.05$. However, taken together, these three parameters were not significantly correlated ($P > 0.05$).

Correlation of miR-145 expression and glomerular integral and tunicae media vasorum ratios

In situ hybridization indicated miR-145 is mainly expressed in the renal VSMCs and the epithelial cells of glomerular proximal convoluted tubule (**Figure 4A**).

Increased tunicae media vasorum ratios were observed in the samples with VSMC proliferation, suggesting that the ratio of tunicae media vasorum is an indicator of the extent of VSMC proliferation. As shown in the **Figure 4C**, higher expression level of miR-145 was detected in the vascular with a larger diameter. The VSMCs were in the contractile phenotype when miR-145 showed a high expression level, proliferative phenotype when miR-145 showed a medium expression level, and the accumulation of

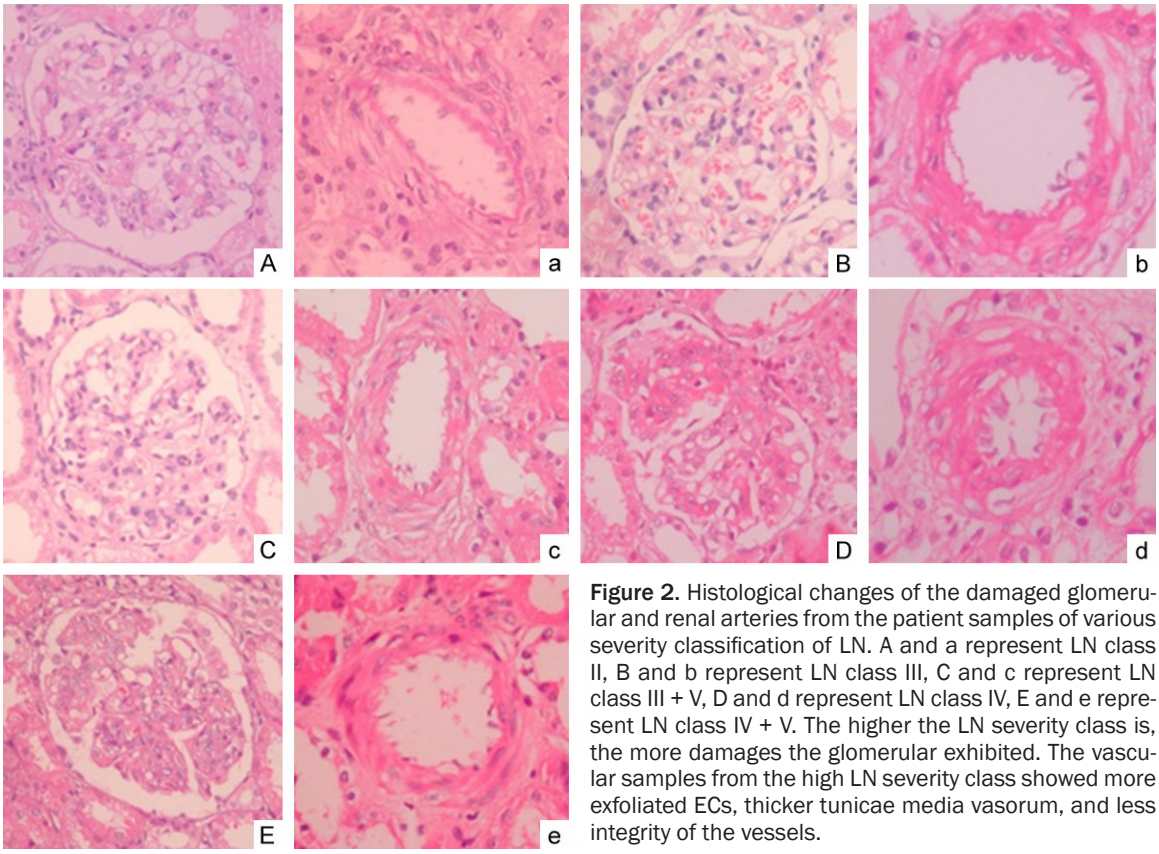

Figure 2. Histological changes of the damaged glomerular and renal arteries from the patient samples of various severity classification of LN. A and a represent LN class II, B and b represent LN class III, C and c represent LN class III + V, D and d represent LN class IV, E and e represent LN class IV + V. The higher the LN severity class is, the more damages the glomerular exhibited. The vascular samples from the high LN severity class showed more exfoliated ECs, thicker tunicae media vasorum, and less integrity of the vessels.

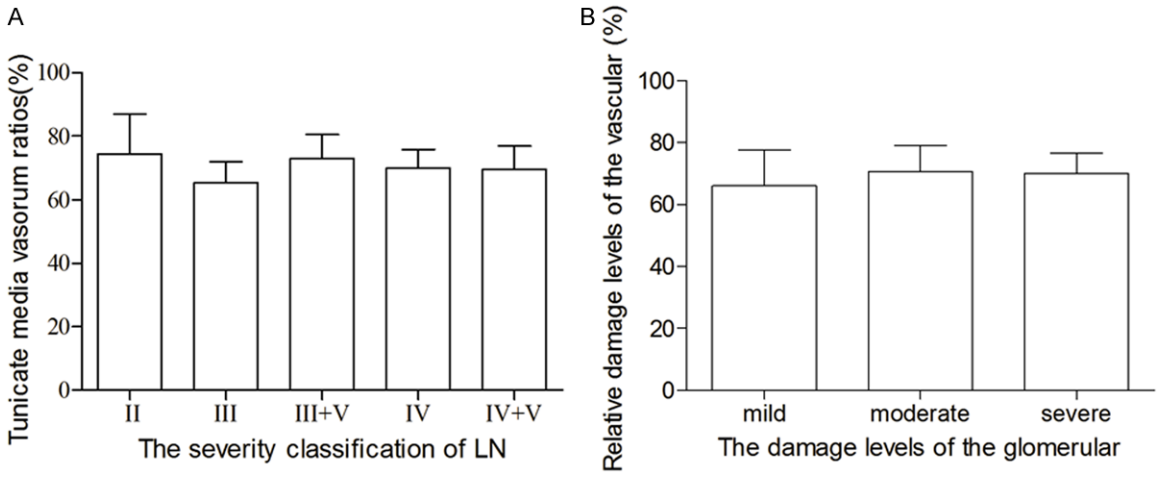

Figure 3. A. The tunicae media vasorum ratios in various LN severity classes did not show significant differences, F = 0.228, *P* = 0.921. B. The damage degrees of glomerular in the samples with mild, moderate, or severe vascular damages, did not show significant differences, F = 0.244, *P* = 0.785.

proliferated VSMCs when miR-145 showed a low expression level. The patient samples were grouped based on the tunicae media vasorum ratios to analyze the expression levels of miR-145 for each group (**Figure 4B**). The expression of miR-145 reduced as the tunicae media vasorum ratios increased, indicating the development of LN inhibits the expression of miR-145.

Furthermore, in order to investigate the correlation between the damage degrees of the vascular and clinical manifestation, all the patient samples were grouped depending on the damage degrees of the vascular, and the data from clinical tests were analyzed as shown in **Table 7**. However, no significant differences were discovered, indicating the damage degrees of the

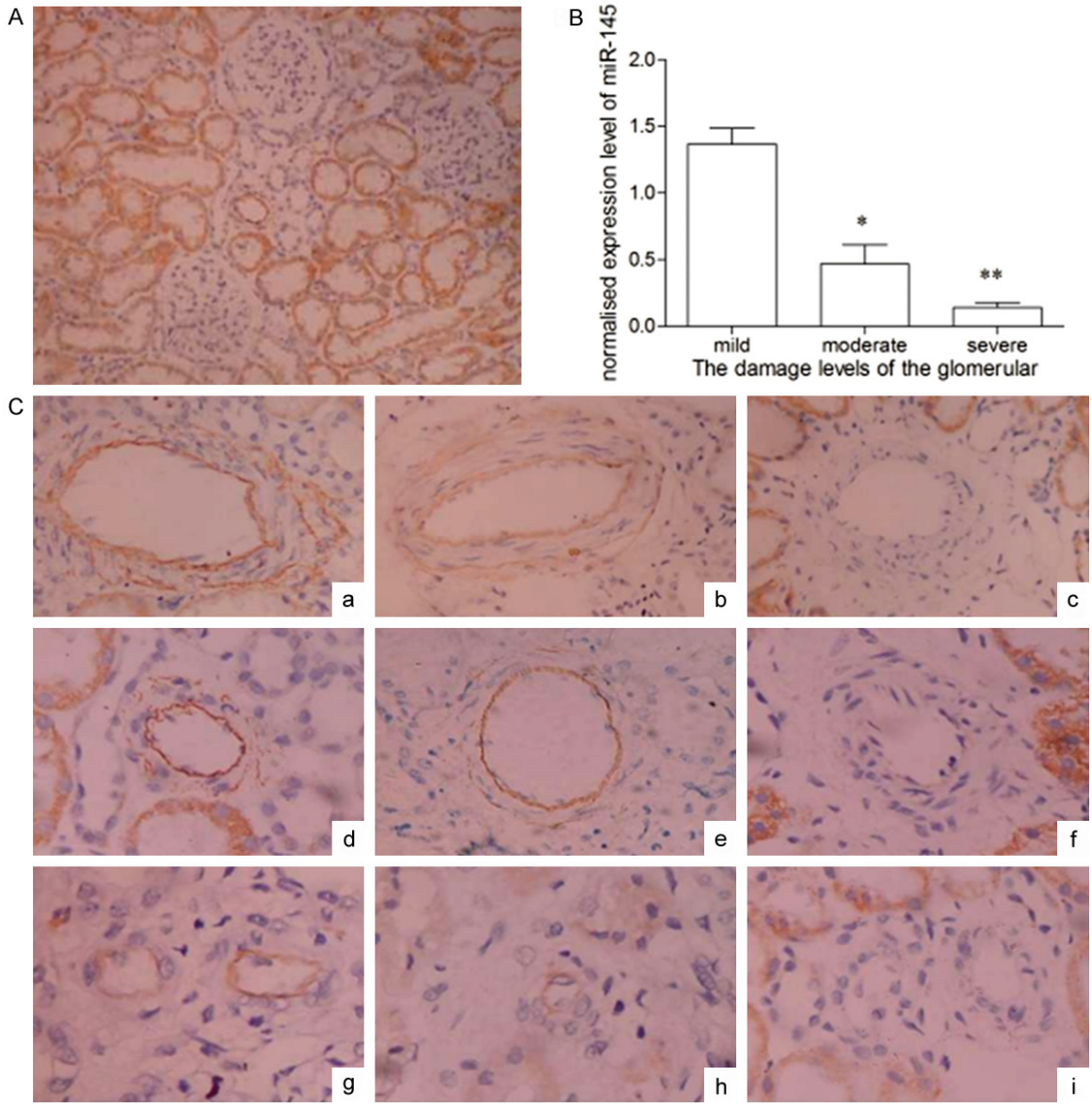

Figure 4. Expression of miR-145 in different tissues. A. The expression of miR-145 in the renal cortex. miR-145 is mainly expressed in the VSMCs of the vessel, as well as the epithelial cells of the glomerular proximal convoluted tubule. B. The expression levels of miR-145 in the samples with various tunicae media vasorum ratios showed significant differences, F = 146.002, $P < 0.001$. *Moderate samples vs. mild samples, $P < 0.05$. ** Severe samples vs. moderate samples, $P < 0.05$. C. The expression of miR-145 in the vascular with various diameters. a, b, and c showed the highest expression of miR-145 in the vessel with longer diameters. d, e, and f showed medium expression levels of miR-145 in the vascular with medium diameters. g, h, and i showed the lowest expression of miR-145 in the vessel with shorter diameters.

renal vascular cannot represent the clinical manifestation of the patients.

Discussion

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by a heterogeneous set of clinical presentations including renal manifestations and the presence of autoantibodies. Lupus nephritis (LN) of varying severity affects up to 60% of SLE patients [12]. It does not only cause glomerular and interstitial damage, but also associate with renal vascular disease, which has great significance for judging the clinical course and treatment [13]. It has been confirmed that the existence of renal vascular changes affect directly the prognosis of LN [14].

Table 7. Clinical examination of the 41 patients

Clinical data	Mean of all cases N = 41	Mild N = 12	Moderate N = 15	Severe N = 14	F	P
Hb (g/l)	98.9 + 18.0	92.7 + 16.6	100.5 + 22.2	102.4 + 13.5	1.040	0.363
D-dimer (μg/ml)	2.8 (1.2, 4.4)	4.1 (2.1, 10.7)	3.5 (0.8, 6.7)	2.5 (1.1, 3.5)	1.093	0.579
Alb (g/l)	26.3 + 7.3	26.6 + 6.0	25.8 + 9.3	26.5 + 6.5	0.049	0.952
TC (ml/dl)	4.8 + 1.8	4.6 + 2.2	4.9 + 2.1	4.9 + 1.1	0.126	0.882
BUN (mmol/l)	10.1 + 8.6	9.8 + 9.0	10.8 + 10.1	9. 8+ 7.0	0.063	0.939
Cr (μmmol/l)	56.8 (34.8, 114.0)	49.3 (34.2, 118.7)	46.8 (34.7, 143.0)	66.8 (33.9, 107.6)	0.121	0.942
UA (μmmol/l)	317.8 (255.6, 503.5)	309.2 (272.4, 391.5)	301.0 (231.0, 571.5)	420.5 (230.9, 520.2)	0.341	0.843
Hematuria (10^4/ml)	1.6 (0.8, 12.8)	1.3 (0.8, 11.9)	1.3 (0.8, 15.5)	2.4 (0.8, 5.9)	0.052	0.974
Proteinuria (g/d)	1.0 (0.3, 3.8)	1.9 (0.5, 5.5)	0.9 (0.2, 3.2)	1.0 (0.1, 5.4)	1.896	0.387

Renal vascular lesions, including vascular endothelial injury, cellulose degeneration, non-inflammatory necrosis, micro thrombosis, are the most common causes of renal dysfunction, poor treatment, and poor long-term prognosis. Active SLE in the patients can activate the immune system, resulting in the disruption of epithelial cells, which directly influences clinical development, treatment, and prognosis of LN [14]. Although SLE has various etiologies and clinical manifestations, with unknown development mechanisms, renal involvement is a common complication of SLE and the cause of death. In particular, the renal involvement in pediatric SLE accounts for 60-80%, with a death rate of twice of adult patients [15-18]. Notably, adolescents with LN exhibit more severe clinical manifestations, and poorer prognosis [19, 20]. Therefore, more attention needs to be paid to the diagnosis and treatment of adolescent LN, as well as related studies.

In a study of adolescent LN of 365 patients (WHO classified), patients with class I-II accounted for 25.0%, whereas patients with class III-IV accounted for 65.0% of all cases, indicating the distribution of adolescent LN patients skewed into high severity [21]. In our study, all 41 patients showed renal injury after taking the renal biopsy, including 10 cases with class IV, 16 cases with class IV + V, accounting 63.4% of the total.

The glomerular damage integral and the severity classification of LN is positively correlated, this is because the current classification of LN is based on the damage degrees of the glomerular. The observed renal vascular damage of LN patients can be caused by intima proliferation, or unevenly thickened tunicae media vasorum resulted from intima damages [22-24]. Also, the vascular damage has been found to be significantly correlated with the prognosis of LN [25, 26]. Our study also revealed that the vascular damage was indicated as intima exfoliation, thickened tunicae media vasorum, which led to more intima exfoliation, resulting in the loss of integrity of the vascular. The reduced integrity of the vascular is unable to maintain normal physiological functions. It has been discovered that the presence of ECs can inhibit the over proliferation of SMCs, and the amount of exfoliated endothelial cells can evaluate the vascular damage [27].

The vascular damage was not significantly correlated with the severity of LN, this is because the severity classification system has not included renal interstitial damage as one of the parameters. In our study, no significant correlation was discovered among renal interstitial vascular damage, glomerular damage and severity classification of LN. This might be due to the immune complex of the SLE patients pass through renal interstitial vascular, depositing onto the glomerular basement membrane, resulting in persistent injuries of the glomerular, which would become more severe as the development of LN. Therefore, we suggest the damage of renal interstitial vascular should be considered as one of the factors to evaluate the severity of the disease.

Studies have detected the expression of miR-145 in the VSMCs, mesangial cells, and undifferentiated proximal medullary cells [28]. Our study of the renal cortex detected the expression of miR-145 in the epithelial cells of the glomerular, and VSMCs. miR-145 can mediate cell differentiation, the role of miR-145 in the glomerular epithelial cells requires further studies. Many studies have found that VSMCs can switch between contractile and proliferative phenotypes. The majority of VSMCs from healthy people exhibit the contractile phenotype, whereas the VSMCs from LN patients

exhibit the proliferative phenotype, resulting in thickened tunicae media vasorum and more severe vascular damages. Also, the switch of VSMC phenotypes was found to be accompanied with accelerated migration and accumulation of extracellular matrix, leading to damages of fresh intima, which is the cause of various proliferative angiopathy [29]. The study also suggested miR-145 mediated the phenotype switch of the VSMCs in the renal interstitium, indicating transfection of miR-145 to recover renal vascular injuries could be a possible therapy. SLE also resulted in nodular vasculitis in various organs, including the heart, lungs, kidneys, intestines, and brains, suggesting the onset of the cardiovascular disease in adolescents with SLE might be one of the causes of death [29]. As the reverse of cardiovascular injuries by the transfection of miR-145 has been validated in several studies [8, 9], we will next investigate the effect of transfecting miR-145 into adolescent patients with LN.

Renal vascular injuries is highly correlated with prognosis of LN [25, 26], however, clinical statistical data suggested the severity of clinical manifestation is not significantly correlated with the vascular injuries. This might be due to an alternative between acute onset of LN and chronic relief of the disease. Therefore, the severity of the clinical manifestation is insufficient to evaluate the vascular injuries, which need to bring more attention.

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Disclosure of conflict of interest

None.

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