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Original Article

Relationship between interstitial CD34 positive cells and active phase of lupus nephritis

Farahnaz Noroozinia[1], Leila Mahmoudzadeh[1], Farzaneh Hosseini Gharalari[1], Khadijeh Makhdoomi[2], Ata Abbasi[1]

Abstract

Objective: Lupus nephritis is one of the most serious and common complications of systemic lupus erythematosus. It has an unpredictable course, and the type, severity, and activity of renal lesions cannot be assessed only by clinical and laboratory findings. The aim of the present study was to determine the relationship between the expression of CD34 and the histopathological findings of lupus nephritis.

Methods: A total of 73 renal biopsy samples of patients with a diagnosis of lupus nephritis were examined for CD34 expression by immunohistochemistry. Samples without staining were considered as 0, mild staining as 1+, moderate as 2+, and strong staining as 3+. The relationship between CD34 expression and histopathological and clinical data (including activity index, chronicity index, lupus nephritis class, age, sex, blood pressure, complete blood count, renal function tests, and serological findings) was analyzed.

Results: The mean age of the patients was 29.3±11.3 years. CD34 was expressed in all of the cases but with different intensities. There was a significant relationship between the expression of CD34 and the activity index, as a strong expression was seen in lower activity indices ($p<0.001$). CD34 expression was correlated with patients' white blood cell (WBC) count and systolic blood pressure (SBP). Patients with strong (score 3) CD34 expression had higher SBPs and lower WBC counts ($p=0.03$ and 0.04, respectively).

Conclusion: A strong interstitial expression of CD34 was observed in lower activity indices. It seems that CD34 expression could play a protective role in lupus nephritis and could reduce renal activity.

Keywords: Lupus nephritis, CD34, histopathological findings

ORCID IDs of the authors:
F.N. 0000-0002-1302-6635;
L.M. 0000-0001-9249-2708;
F.H.G. 0000-0002-7326-2066;
K.M. 0000-0002-4503-0612;
A.A. 0000-0001-8000-8819

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[1] Department of Pathology, Urmia University of Medical Sciences School of Medicine, Urmia, Iran
[2] Nephrology and Renal Transplant Center, Urmia University of Medical Sciences, Urmia, Iran

Address for Correspondence:
Ata Abbasi, Department of Pathology, Urmia University of Medical Sciences School of Medicine, Urmia, Iran

E-mail: aabbasi@alumnus.tums.ac.ir

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology in which tissues and cells are damaged by immune complexes and pathogenic autoantibodies. Renal involvement in SLE is called lupus nephritis, which is one of the most serious and common complications of SLE (1-3). It occurs in 60% of the patients and is accompanied by infection. It is the leading cause of mortality in the first decade of the disease. Lupus nephritis is a progressive disease with exacerbations requiring treatment after many years of therapy.

Pathological changes in lupus nephritis may be found in the glomeruli, tubules, blood vessels, or renal interstitium, and renal biopsy plays an important role in the diagnosis and control of the disease (4-10). CD34, a glycosylated single-chain protein with an approximate weight of 116 kDa (11), is one of the single-pass transmembrane sialomucin proteins and intercellular adhesion factors. It is expressed in the membrane of some cell types including hematopoietic cells, endothelial cells, muscle satellite cells, hair follicle stem cells, and fibroblasts. Recent studies have shown that CD34 positive cells can cause changes in the adhesion and migration of bone marrow cells (12-14). Moreover, these cells may increase proliferation and prevent differentiation of progenitor cells, increase lymphocyte adhesion, and, in some cases, such as kidney podocytes, can prevent cell adhesion (15). Multipotency of CD34-presenting cells has made them useful agents for various therapeutic purposes, such as cancer, diabetes, cardiovascular diseases, and autoimmune disorders (16). The role of CD34-presenting cells has been studied in some recent studies, but the results show some controversies. One study showed that patients with more severe nephrosclerosis exhibit lower expression of CD34 in the tubulointerstitium (17). In contrast, another study examined 47 patients for alpha-smooth muscle antibody and CD34 and found that CD34 positivity

Table 1. Clinical data of enrolled patients

	Age	SBP	Up24	GFR	Cr	Urea	ESR	Plt	HGB	WBC	DBP
Mean	29.6±11.3	124.1±22.2	2427.3±2915.4	63.1±29.6	1.2±0.7	52.1±44.1	48.1±31.5	207.5±86.9	10.7±1.8	8.2±5.2	79.7±15.1

SBP: systolic blood pressure; DBP: diastolic blood pressure; Cr: creatinine; Plt: platelet; Up24: 24-hour urine protein; GFR: glomerular filtration rate; HGB: hemoglobin; ESR: erythrocyte sedimentation rate; WBC: white blood cell

Table 2. Serological data of enrolled patients

	Anti-cardiolipin	CH50	C4	C3	dsDNA	ANA	β2-Glycoprotein
Mean	4.5	80	0.14	0.55	4.2	7.64	3.7
Min	0.12	3	0.01	0	0.1	0.8	0.5
Max	43	135	100	180	600	1180	25.7

ANA: antinuclear antibod

Table 3. The relationship between CD34 expression and WBC and SBP

		SBP (mm Hg)	p	WBC (count/μL)	p
CD34	1+	124.7±23.2	0.03	9.21±4.42	0.04
	2+	111.5±20.7		9.81±8.24	
	3+	131.7±19.2*		6.12±2.59*	
	Total	124.17±22.2		8.21±5.27	

SBP: systolic blood pressure; WBC: white blood cell

*p<0.05 is significant

Table 4. The relationship between the expression of CD34 and the activity index in patients with lupus nephritis

		Activity index		
		<9	≤10	Total
CD34 expression	0	0	0	0
	1	22	7	29
	2	14	5	19
	3	25*	0*	25
Total		34	12	73

*p<0.05 is significant

in severely sclerotic lesions is more than the other lesions (18). In addition, the CD34 positivity rate in proliferative glomerulonephritis (GN) was significantly higher in the control group and non-proliferative lesions.

However, most of the studies have explored CD34-presenting cells in cases other than lupus nephritis. To the best of our knowledge, there are few studies that explored the role of CD34 in lupus nephritis. Considering the lack of data in lupus nephritis and also the present controversies, we tried to examine the immunohistochemical expression of CD34 antigen in renal biopsies of patients with lupus nephritis and explore its relationship with various histopathological and clinical findings of the disease in the present study.

Methods

In the present study, 73 paraffin-embedded blocks of kidney needle biopsy samples of patients with lupus nephritis collected by the pathology department of Urmia University of Medical Sciences, Urmia, Iran from 2009 to 2014 were enrolled. The slides were evaluated and classified for lupus nephritis according to the International Society of Nephrology/Renal Pathology Society classification (19). Suboptimal samples (<8 glomeruli or <0.5 cm sample length) were excluded from the study. Then, suitable blocks were selected for immunohistochemical (IHC) staining. A 4 μm section was prepared from the selected blocks, and IHC staining for CD34 antigen was performed according to the manufacturer's instructions (Dako Corporation, Glostrup, Denmark).

Briefly, after deparaffinization and re-hydration, endogenous peroxidase activity was blocked using hydrogen peroxide. Primary antibody for CD34 (Dako Corporation, Glostrup, Denmark) was added after antigen retrieval and incubated for 45 min. Then, the antibody was washed, and the slides were incubated with envision (Dako Corporation, Glostrup, Denmark). Diaminobenzidine tetrahydrochloride was used for visualization. The membranous staining pattern in interstitial cells was considered as positive. The semi-quantitative method was used for examination, and scoring was as follows: 0=no reaction, 1=mild reaction, 2=moderate reaction, and 3=strong reaction (20). A negative control was applied on one of the samples by the secondary antibodies removal. Control samples were stained with the same method.

Patients' clinical data including age, gender, systolic and diastolic blood pressure (SBP and DBP), blood count indices (white blood cell (WBC), red blood cell, hemoglobin, and platelet count), erythrocyte sedimentation rate, serum urea and creatinine levels, and serological markers, such as antinuclear antibody, dsDNA, anti-cardiolipin, β2-glycoprotein, and complement levels, were also obtained from their medical records for further evaluation.

The ethics committee of Urmia University of Medical Sciences approved the study. Written informed consent was obtained from the patients.

Statistical analysis

The results are expressed as mean±standard deviation. Statistical analysis was performed using the SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). The difference among proportional variables was analyzed by the chi-square tests. Numerical data were evaluated by analysis of variances followed by post hoc test. A p value <0.05 was considered as significant.

Results

The mean age of the patients was 29.63±11.3 years. Of the patients, 83.6% were females. Tables 1 and 2 show the clinical data and also the serological findings. Our results showed that CD34 expression was correlated with patients' WBC count and SBP. Patients with a strong (score 3) CD34 expression had higher SBPs and lower WBC counts (p=0.03 and 0.04, re-

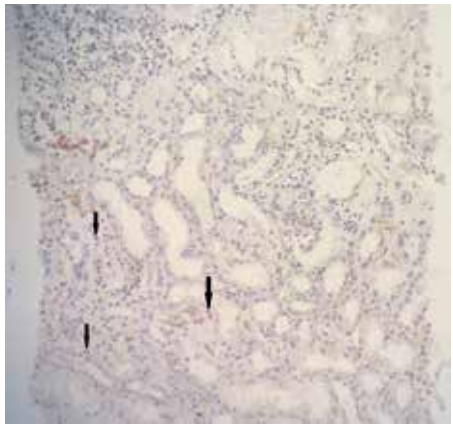

Figure 1. Immunostaining of the kidney tissue with CD34 marker, showing mild staining density in interstitial cells (arrows). Vascular endothelial cells that also stain by CD34 are indicated by arrowheads (IHC, 20× magnification)

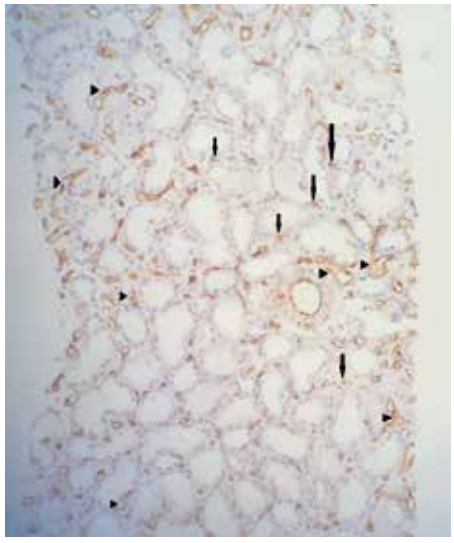

Figure 2. Immunostaining of the kidney tissue with CD34 marker, showing moderate staining density in interstitial cells (arrows). Vascular endothelial cells that also stain by CD34 are indicated by arrowheads (IHC, 20× magnification)

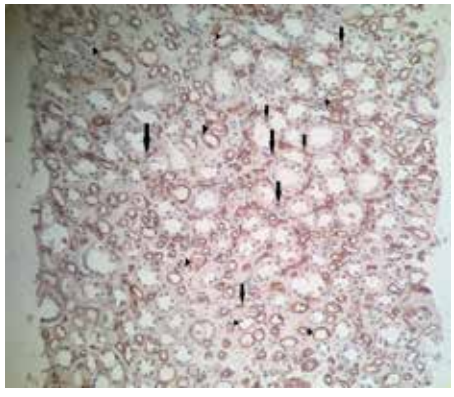

Figure 3. Immunostaining of the kidney tissue with CD34 marker, showing marked (severe) staining density in interstitial cells (arrows). Vascular endothelial cells that also stain by CD34 are indicated by arrowheads (IHC, 20× magnification)

spectively) (Table 3). There was no relationship between CD34 expression and other clinical or laboratory findings.

Histological evaluation

The most frequent type of lupus nephritis was class IV (47.9%), and no case with class I lupus nephritis was observed. Of the cases, 23.3% were class II, 6.8% were class III, and 17.8% were class V. Activity index analysis showed that the most frequent score was 2 (12.3%). Score 1 was seen in 9.6%, score 3 in 8.2%, score 4 in 9.6%, score 5 in 6.8%, score 6 in 8.2%, score 7 in 6.8%, score 8 in 5.5%, score 9 in 9.6%, score 10 in 6.8%, score 11 in 1.4%, score 12 in 5.5%, score 13 in 4.1%, score 14 in 2.7%, and score 15 in 2.7% of the patients. None of the patients had a score ≥16. There was no difference between male and female patients in activity index.

Maximum chronicity index was 9 (1.4%), the minimum was 0 (5.5%), the most frequent index was index 2 (30.1%), and the least one was index 6. None of the patients had score 7 or higher.

CD34 expression was seen in all 73 (100%) cases. Of the cases, 29 (39.7%) were grade 1, 19 (26%) were grade 2, and 25 (34.2%) were grade 3 (Figure 1-3). Data analysis showed a significant relationship between CD34 expression and activity index, as higher staining score (grade 3, strong staining) for CD34 was seen in activity indices <9, and none of the cases with activity indices >10 showed a strong staining pattern for CD34 (p=0.023) (Table 4).

However, no significant relationship was seen between CD34 antigen expression and chronicity index (p=0.139). There was no significant relationship between CD34 expression and pathological class of lupus nephritis (p=0.552).

Discussion

Lupus nephritis is one of the most serious complications of SLE. There is no distinct method for the early diagnosis and prediction of patients' prognosis. Several studies have attempted to find new markers in addition to routine histopathological evaluation in which CD34 is one of the markers recently used for this purpose. Although some studies had encouraging results, there are some others with equivocal or controversial findings. In one study, angiogenesis factors in GN with different etiologies consisting of lupus nephritis were examined and showed that patients with more severe nephrosclerosis exhibit lower expression of CD34 in the tubulointerstitium (17). There are few studies focusing purely on lupus nephritis. Most of the recent studies on CD34 have evaluated the relationship between CD34 and GN with etiologies other than SLE. Most of them have shown different results. For example, in one study, only 27% of the patients were CD34 positive (20), but another study showed 77% positivity for CD34 (21). In addition, in one study, 96.66% of the patients with interstitial fibrosis were CD34 positive (22).

The relationship between CD34 expression and activity indices was also explored previously, but the results were not similar (21, 23). Some studies have found no relationship, whereas others have found a significant relationship between CD34 expression, disease activity, and also prognosis in patients with various types of GN, claiming that proliferative GN had higher expression of CD34 than non-proliferative GN and control groups. As previously mentioned, most of these studies have evaluated various GNs other than SLE, and data on lupus nephritis are inconclusive.

We found a significant relationship between CD34 expression and activity index, as patients with lower activity index had higher CD34 expression, suggesting that CD34 positive mesenchymal cells may have a protective role in lupus nephritis.

The relationship between CD34 expression and clinical and serological data was also studied in various types of GNs other than lupus nephritis, but no significant relationship was found (24).

We found a significant relationship between CD34 expression, WBC count, and SBP, as patients with higher SBP and lower levels of WBC count showed higher expressions of CD34, which was a novel finding in this topic.

Data on the relationship between CD34 expression and disease chronicity are controversial. Some studies have found significant relationships (either positive or negative) (17, 18), whereas others have shown no correlation between them (21, 23). In the present study, we did not find any significant relationship between chronicity index and CD34 expression (p=0.139).

Overall, we found that CD34 was expressed in all of our lupus nephritis samples. We also found a reverse correlation between activity index and CD34, as patients with higher and stronger CD34 expression had lower activity index. We concluded that CD34 expression could play a protective role, and a weak expression of CD34 should raise the possibility of higher activity index in lupus nephritis. We also found

that patients with higher SBP and lower WBC count showed a strong expression of CD34. This was a novel finding in these patients that has not been expressed before. Some studies have shown that autologous transplantation of CD34 positive cells can induce remission and reduce severity of symptoms in lupus nephritis (25-27). Considering these findings, we suggest that a thorough investigation of CD34 functions in the kidney may introduce new methods in the treatment of patients presenting with active symptoms of renal involvement during SLE.

Ethics Committee Approval: Ethics committee approval was received for this study from the Urmia University of Medical Sciences Ethical Committee.

Informed Consent: Written informed consent was obtained from all the patients who participated in this study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflict of interest to declare.

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