Altered mRNA expression levels of vaspin and adiponectin in peripheral blood mononuclear cells of systemic lupus erythematosus patients

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Abstract

Objective

Increasing studies have indicated the association between adipokines and multiple autoimmune diseases. This study aimed to evaluate the mRNA expression levels of vaspin, adiponectin and adrenomedullin in peripheral blood mononuclear cells (PBMCs) of patients with systemic lupus erythematosus (SLE), as well as their clinical associations.

Methods

A total of 46 SLE patients and 51 normal controls were recruited. The three adipokines expression levels in PBMCs from SLE patients were measured by qRT-PCR, and their associations with major clinical and laboratory parameters of SLE patients were also analysed.

Results

Compared with normal controls, vaspin expression level in PBMCs was significantly decreased ($p<0.001$), whereas adiponectin expression level was significantly higher in SLE patients ($p<0.001$). There was no significant difference in adrenomedullin expression level between SLE patients and normal controls. Vaspin and adrenomedullin expression levels in more active SLE were significantly lower than those in less active SLE ($p=0.012$, $p=0.046$, respectively). No significant difference in these adipokine expression levels was observed between SLE patients with and without lupus nephritis (LN). There was also no significant association between mRNA levels of these adipokines and major clinical and laboratory parameters.

Conclusion

Altered vaspin, adiponectin expression levels, and the associations between vaspin, adrenomedullin levels and disease activity in SLE patients suggested that these adipokines might play a role in SLE.

Key words

adipokine, vaspin, adiponectin, adrenomedullin, systemic lupus erythematosus

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Introduction

Systemic lupus erythematosus (SLE) is a multi-system, inflammatory connective tissue disease characterised by several functional abnormalities of the immune system (1). Many studies have shown that increased incidence of cardiovascular events, metabolic syndrome caused by insulin resistance are observed in SLE patients, and obesity is one risk factor for SLE and other autoimmune diseases (2-4). The adipose tissue has now been recognised as a key organ with immune functions. It secretes a number of mediators known as adipokines, including adiponectin, leptin, vaspin, interleukin (IL)-6 and others; these cytokines participate in the innate immune response as anti-inflammatory or pro-inflammatory mediators (5-8). Regarding the function of adipokines during inflammatory responses, a wide variety of studies focused on the pathogenic roles and expression levels of adipokines in multiple autoimmune diseases, such as SLE (9-12). Of interest, abnormal regulations in the synthesis and expression of several adipokines genes in peripheral blood mononuclear cells (PBMCs) from patients with SLE have also been found (13). Nishitani *et al*. demonstrated that IL-6 mRNA in active LN patients was significant higher than normal controls and inactive LN patients, and it was positively correlated with disease activity (13). In addition, the IL-6 mRNA level was significantly decreased in PBMCs from active LN patients following treatment with prednisolone. These findings suggested that increased IL-6 level in PBMCs might reflected the disease activity and be associated with the progression of LN.

Vaspin has been identified as a member of the serine protease inhibitor family and a novel adipokine that was expressed principally in visceral adipose tissue (14). It have been reported that vaspin has anti-inflammatory actions as it suppressed the productions of tumour necrosis factor (TNF)-α, and proinflammatory adipokines (15). Adiponectin belonged to the collagen superfamily, sharing homologies with collagen VIII and type X and complement factor C1q (16). Shortly after the discovery of adiponectin, this adipokine has been evaluated in SLE as an immunomodulatory (17). Adrenomedullin was first discovered in human phaeochromocytoma, and known as a novel peptide consisting of 52 amino acids in 1993 (18). Adrenomedullin was also produced in mesangial cells, and inhibits mesangial cells mitogenesis, and suppressed the production of inflammatory factors in macrophage cells (13, 19). While altered plasma/serum levels of vaspin, adiponectin and adrenomedullin were reported in autoimmune disease patients (20-22), there are few studies on the mRNA levels of these adipokines in PBMCs from SLE patients. Therefore, we performed this study to explore the mRNA expression levels of vaspin, adiponectin and adrenomedullin in PBMCs of SLE patients, and analyse their associations with major clinical and laboratory parameters of SLE patients.

Materials and methods

Study subjects

In this case-control study, 46 patients with SLE, which according to the 1997 American College of Rheumatology (ACR) revised criteria for the classification of SLE (23), were consecutively enrolled from the Department of Rheumatology at the First Affiliated Hospital of Anhui Medical University and the First Affiliated Hospital of University of Science and Technology of China from February 2017 to May 2017. Then, 51 healthy volunteers, who did not have a history of SLE or other rheumatologic conditions, were selected from the same region and period to serve as controls. lupus nephritis (LN) patient was diagnosed in accordance with the ACR criteria, with one of the following clinical parameters: persistent proteinuria ≥0.5 g/day; biopsy evidence of LN; the presence of active cellular casts (24).

For all the recruited patients, the clinical features such as LN, arthritis, fever, alopecia, skin rash, myositis, oral ulcer were retrieved from hospital records. Moreover, the laboratory data including anti-Sm, anti-SSA, anti-SSB, anti-ds-DNA, thrombocytopenia, proteinuria, erythrocyte sedimentation rate (ESR), and the serum levels of C3, C4, were also accurately collected. The disease

activity of each patient was assessed at the time of blood drawing using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) score (25). Depending on the SLEDAI-2K score, SLE patients were divided into two categories: more active was defined as SLEDAI-2K score >10, while less active was defined as SLEDAI-2K score ≤10 (26). Body mass index (BMI) is defined as the patient's body weight divided by the square of his or her height, a formula universally used in clinical medicine with a unit of kg/m^2. Based on the BMI, SLE patients were classed as overweight or obese (BMI ≥24 kg/m^2), normal weight or underweight (BMI <24 kg/m^2) (27).

Informed consent of all subjects was obtained, and the study protocol was approved by the Medical Ethics Committee of Anhui Medical University.

Collection of sample, and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

PBMCs were isolated from 5 ml peripheral blood samples from SLE patients and controls by Ficoll-Hypaque density gradient centrifugation, and stored at -80°C until processed. TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from PBMCs and the RNA concentrations were quantified by a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

Total RNA were reverse-transcribed into cDNA by the PrimeScriptTM RT reagent Kit (Takara Bio Inc, Japan). In order to compare the mRNA levels of vaspin (sense primer: 5′-GACAGCACCCTGGACGAGA-3′, antisense primer: 5-CTCTGGTCAATGAACAGCG TG-3′, adiponectin (sense primer: 5′-AGGAGATGCAGGTCTTCTTGG-3′, antisense primer: 5′-CTGAGCGATACACATAAGCGG-3′) and drenomedullin (sense primer: 5′-TTGTCCTCCCCTATTTTAAGACG-3′, antisense primer: 5′-CTTCCACACAGGAGGTAATCAGTC-3′), qRT-PCR with SYBR Green (SYBR Premix Ex Taq II, Takara Bio Inc, Japan) was performed on ABI ViiA 7 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Cycle conditions were as follows: 95°C for 1 min, followed by 42 cycles at 95°C for 10 sec, 60°C for 30 sec and 72°C for 1 min. The qRT-PCR was run in duplicate, and the mRNA expression was determined by comparison with housekeeping gene β-actin (sense primer: 5′-CACGAAACTACCTTCAACTCC-3′, antisense primer: 5′-CATACTCCTGCTTGCTGATC-3′) from the same sample as internal control. The relative expression levels were calculated using $2^{-\Delta\Delta Ct}$ method normalised to endogenous control (28).

Statistical analysis

All the statistical analysis was performed by the SPSS statistical software v. 10.01 (SPSS Inc., IL, USA). Normally distributed data were described as mean ± SD, non-parametric distribution data were expressed as median value and interquartile range. The non-parametric Mann-Whitney *U*-test was used to compare the mRNA expression between two groups. The correlation analysis between adipokines mRNA expression levels and several continuous variables was conducted by Spearman's rank correlation coefficient test. A two-sided *p*-value ≤0.05 was considered as statistically significant.

Table I. The main demographic, clinical characteristics and medical therapies of study subjects.

Characteristics	SLE patients (n=46)	Healthy controls (n=51)	*p*-value
Demographic characteristics			
Age (years)	34.96±13.05	38.92±14.31	0.159
Sex (male/female)	4/42	4/47	0.879
Clinical characteristics			
Active (SLEDAI-2K>10)	2 6	NA	NA
Disease duration(years)	5.04(1.00, 8.19)	NA	NA
Lupus nephritis (yes/no)	25/21	NA	NA
Medical therapies			
Prednisone ≥30 mg/day[a] (yes/no)	15/31	NA	NA
Immunosuppressants[b] (yes/no)	17/29	NA	NA

SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; Median (interquartile range); [a]≥30 mg/day means a medium to high doses of prednisone; [b]Immunosuppressants included methotrexate, cyclophosphamide, leflunomide, cyclosporine, tacrolimus, azathioprine, and mycophenolate mofetil; NA: not applicable.

Table II. Comparison of vaspin, adiponectin, adrenomedullin mRNA levels in PBMC between different subgroups.

Group	Number	Vaspin mRNA level	Adiponectin mRNA level	Adrenomedullin mRNA level
Healthy controls	51	0.036(0.016,0.247)	0.011(0.004,0.061)	0.019(0.011,0.045)
SLE patients	46	0.010(0.004,0.049)[a]	0.101(0.022,0.398)[b]	0.023(0.010,0.105)
SLE with LN	25	0.007(0.003,0.096)	0.108(0.025,0.856)	0.020(0.010,0.202)
SLE without LN	21	0.011(0.004,0.050)	0.069(0.017,0.188)	0.025(0.011,0.082)
More active SLE	26	0.006(0.002,0.027)	0.055(0.022,0.221)	0.015(0.009,0.071)
Less active SLE	20	0.015(0.008,0.070)[c]	0.113(0.019,0.799)	0.039(0.017,0.196)[d]
BMI ≥24 in SLE	12	0.012(0.003,0.046)	0.109(0.019,0.490)	0.025(0.010,0.148)
BM I<24 in SLE	34	0.010(0.004,0.050)	0.095(0.022,0.398)	0.020(0.010,0.105)

Median (interquartile range); more active SLE was defined as a SLEDAI score >10, those patients with SLEDAI ≤10 were classed as less active. BMI ≥24 kg/m^2 was defined overweight or obese, BMI <24 kg/m^2 was defined normal or underweight. [a]vs. Healthy controls, $p<0.001$; [b]vs. Healthy controls, $p<0.001$; [c]vs. More active SLE, $p=0.012$; [d]vs. More active SLE, $p=0.046$.

Results

The main demographic, clinical characteristics and medical therapies of SLE patients and normal controls in this study were shown in Table I. The mean age of SLE patients was 34.96±13.05 years, and 91.30% (42/46) of them were women. The mean age of normal controls was 38.92±14.31 years, and 92.16% (47/51) of them were women.

No significant differences in age and gender distribution among SLE patients and normal controls were observed (both $p>0.05$). The main medical therapies of all the patients were prednisone and immunosuppressants (methotrexate, cyclophosphamide, leflunomide, cyclosporine, tacrolimus, azathioprine, and mycophenolate mofetil).

mRNA expression of vaspin, adiponectin and adrenomedullin in PBMCs from SLE patients and normal controls

The vaspin, adiponectin and adrenomedullin mRNA expression levels in PBMCs from SLE patients and normal controls were normalised to housekeeping gene (β-actin). While the vaspin mRNA expression level was significantly lower in PBMCs of SLE patients when compared with normal controls ($p<0.001$), the adiponectin mRNA level in SLE patients was significantly higher than that in normal controls ($p<0.001$) (Table II, Fig. 1). However, there was no significant difference with regard to adrenomedullin mRNA levels between SLE patients and normal controls ($p=0.814$) (Table II, Fig. 1).

No significant differences in the three adipokines mRNA expression levels were observed between the SLE patients with LN and those without LN. The mRNA expression levels of vaspin and adrenomedullin mRNA in more active SLE were significantly lower than those in less active SLE groups ($p=0.012$, $p=0.046$, respectively) (Fig. 2). However, no significant differences in vaspin, adiponectin and adrenomedullin mRNA levels were found between different BMI groups in SLE patients.

Associations of vaspin, adiponectin and adrenomedullin mRNA expression levels with clinical and laboratory data in SLE patients

The associations of vaspin, adiponectin, and adrenomedullin mRNA expression levels with major clinical and laboratory parameters of SLE patients were further analysed, and the results were shown in Tables III, IV, V. However, we found that there were no significant associations of vaspin, adiponectin, and

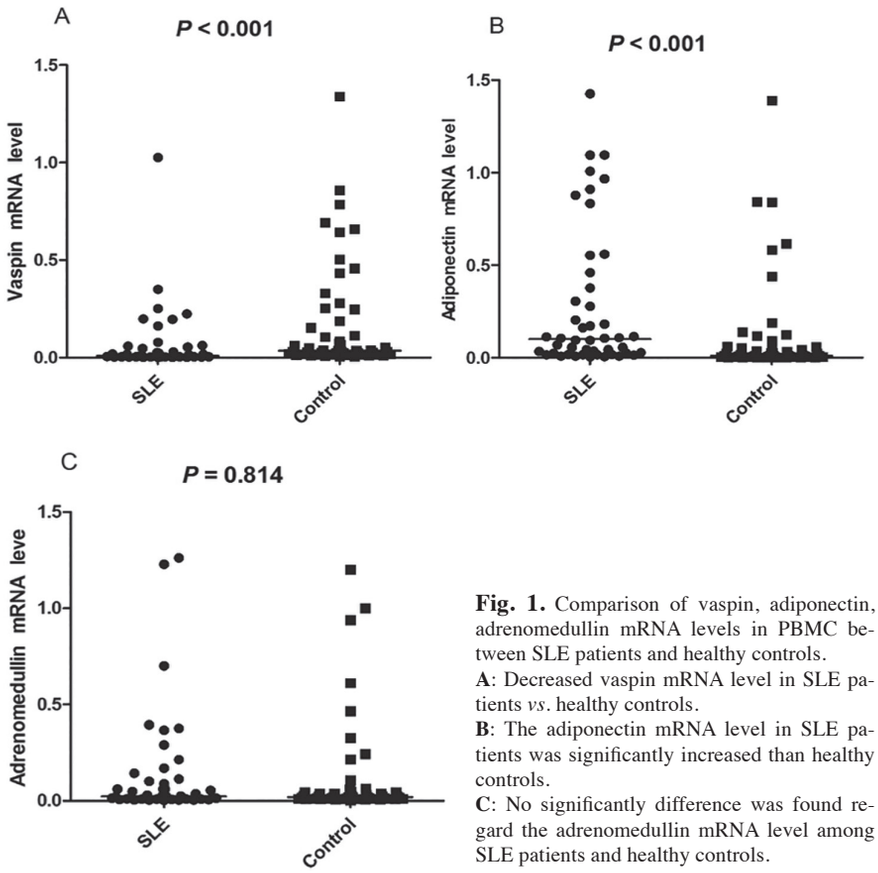

Fig. 1. Comparison of vaspin, adiponectin, adrenomedullin mRNA levels in PBMC between SLE patients and healthy controls.
A: Decreased vaspin mRNA level in SLE patients *vs*. healthy controls.
B: The adiponectin mRNA level in SLE patients was significantly increased than healthy controls.
C: No significantly difference was found regard the adrenomedullin mRNA level among SLE patients and healthy controls.

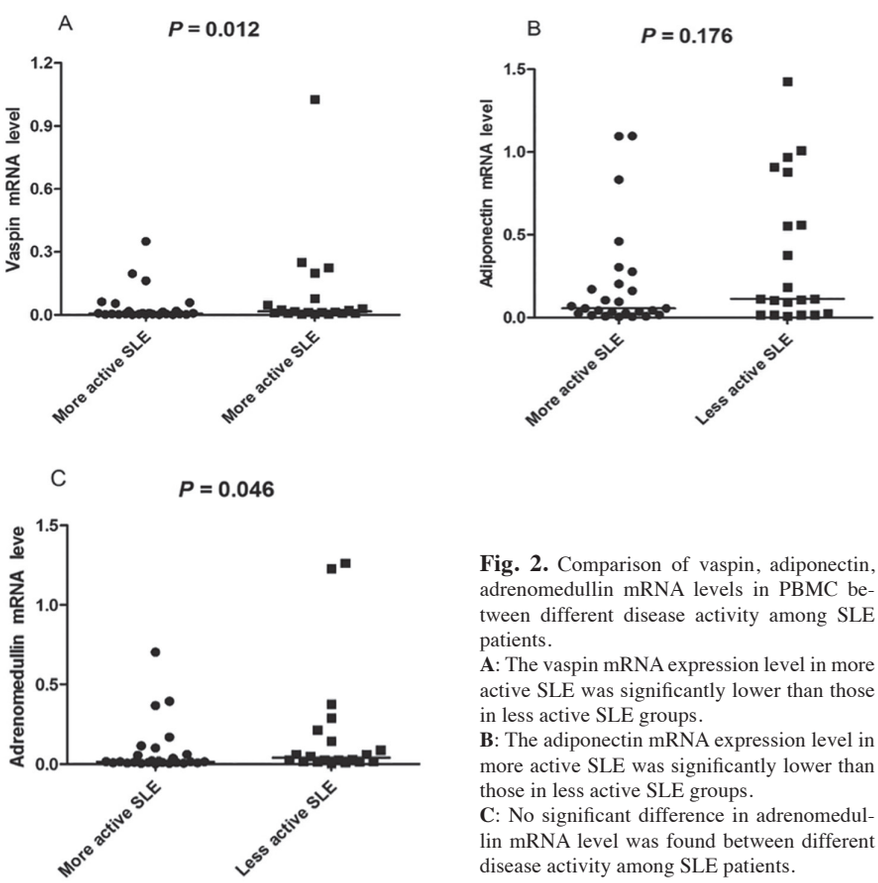

Fig. 2. Comparison of vaspin, adiponectin, adrenomedullin mRNA levels in PBMC between different disease activity among SLE patients.
A: The vaspin mRNA expression level in more active SLE was significantly lower than those in less active SLE groups.
B: The adiponectin mRNA expression level in more active SLE was significantly lower than those in less active SLE groups.
C: No significant difference in adrenomedullin mRNA level was found between different disease activity among SLE patients.

Table III. Associations of vaspin, adiponectin, adrenomedullin mRNA levels with clinical characteristics of SLE patients.

Group	Number	Vaspin mRNA level	*p*-value	Adiponectin mRNA level	*p*-value	Adrenomedullin mRNA level	*p*-value
Arthritis							
Yes	11	0.017(0.001,0.062)	0.919	0.055(0.017,0.305)	0.594	0.020(0.009,0.169)	0.859
No	35	0.009(0.004,0.029)		0.105(0.023,0.460)		0.025(0.011,0.102)	
Alopecia							
Yes	13	0.005(0.002,0.036)	0.289	0.043(0.015,0.291)	0.360	0.025(0.007,0.115)	0.442
No	33	0.010(0.005,0.053)		0.105(0.026,0.507)		0.020(0.014,0.108)	
Skin rash							
Yes	23	0.011(0.004,0.062)	0.606	0.069(0.023,0.305)	0.826	0.025(0.010,0.169)	0.886
No	23	0.009(0.004,0.026)		0.108(0.016,0.460)		0.020(0.010,0.088)	
Myositis							
Yes	5	0.017(0.004,0.058)	0.758	0.162(0.027,0.254)	0.945	0.025(0.018,0.112)	0.583
No	41	0.009(0.004,0.038)		0.096(0.020,0.507)		0.020(0.010,0.108)	
Fever							
Yes	13	0.007(0.001,0.034)	0.105	0.108(0.017,0.429)	0.864	0.019(0.008,0.192)	0.798
No	33	0.011(0.005,0.053)		0.094(0.024,0.419)		0.025(0.014,0.095)	
Oral ulcer							
Yes	8	0.005(0.001,0.018)	0.090	0.115(0.027,0.259)	0.898	0.015(0.007,0.060)	0.296
No	38	0.011(0.004,0.059)		0.101(0.017,0.555)		0.025(0.013,0.121)	
Prednisone (≥30mg/day)							
Yes	15	0.015(0.004,0.054)	0.972	0.094(0.014,0.278)	0.475	0.017(0.010,0.114)	0.824
No	31	0.009(0.004,0.047)		0.105(0.024,0.553)		0.025(0.010,0.102)	
Immunosuppressants							
Yes	17	0.007(0.003,0.035)	0.176	0.055(0.025,0.418)	0.927	0.036(0.012,0.095)	0.846
No	29	0.014(0.004,0.058)		0.105(0.017,0.416)		0.017(0.010,0.192)	

Median (interquartile range).

Table IV. Associations of vaspin, adiponectin, adrenomedullin mRNA levels with laboratory parameters of SLE patients.

Group	Number	Vaspin mRNA level	*p*-value	Adiponectin mRNA level	*p*-value	Adrenomedullin mRNA level	*p*-value
Anti-disDNA↑							
Yes	20	0.007(0.004,0.052)	0.944	0.049(0.019,0.178)	0.211	0.017(0.008,0.136)	0.813
No	24	0.010(0.004,0.025)		0.113(0.019,0.495)		0.025(0.011,0.061)	
Anti-Sm							
Yes	18	0.014(0.006,0.099)	0.080	0.088(0.016,0.629)	0.703	0.025(0.012,0.180)	0.278
No	25	0.007(0.003,0.020)		0.094(0.021,0.193)		0.015(0.010,0.058)	
Anti-SSA							
Yes	29	0.008(0.004,0.025)	0.979	0.094(0.020,0.341)	0.826	0.025(0.014,0.075)	0.508
No	14	0.008(0.003,0.084)		0.101(0.015,0.361)		0.013(0.008,0.225)	
Anti-SSB							
Yes	5	0.023(0.004,0.151)	0.426	0.910(0.233,1.197)	0.063	0.088(0.031,0.672)	0.107
No	38	0.008(0.004,0.027)		0.081(0.017,0.187)		0.018(0.010,0.072)	
Anti-RNP							
Yes	14	0.010(0.003,0.108)	0.795	0.133(0.022,0.895)	0.300	0.058(0.011,0.233)	0.157
No	29	0.008(0.004,0.025)		0.069(0.017,0.193)		0.017(0.010,0.485)	
Proteinuria							
Yes	22	0.007(0.002,0.024)	0.084	0.095(0.025,0.344)	0.856	0.018(0.010,0.092)	0.874
No	23	0.012(0.005,0.054)		0.105(0.017,0.377)		0.025(0.010,0.143)	
Thrombocytopenia							
Yes	15	0.008(0.004,0.054)	0.847	0.162(0.027,0.460)	0.413	0.025(0.014,0.169)	0.311
No	30	0.011(0.004,0.031)		0.095(0.017,0.323)		0.016(0.010,0.068)	

Part of the study subjects of data missing; median (interquartile range).

adrenomedullin mRNA expression levels with major clinical and laboratory parameters of SLE patients.

In addition, the potential influences of the main medical therapies on these adipokine mRNA levels were also explored. However, there were no significant associations regarding these adipokine mRNA levels between SLE patients being treated with medium to high doses of prednisone (≥30 mg/day) and these patients being treated with low doses, as well as the patients being treated with immunosuppressants and without (Table III).

Table V. Correlation between vaspin, adiponectin, adrenomedullin mRNA levels and C3, C4, ESR, CRP of SLE patients.

Clinical parameters	Vaspin mRNA level		Adiponectin mRNA level		Adrenomedullin mRNA level	
	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value
C3	-0.083	0.596	-0.205	0.186	-0.217	0.163
C4	-0.165	0.336	-0.158	0.356	-0.180	0.293
ESR	0.215	0.166	0.195	0.211	0.124	0.428
CRP	-0.083	0.590	0.143	0.353	-0.123	0.425

r_s: Spearman's rank correlation coefficient.

Discussion

With the further discovery of adipokines, an increasing number of researches have focused on the contribution of adipokines to autoimmune diseases. These adipokines could act as the key players in the complex network of soluble mediators in the pathogenesis of SLE (29). Here, we aimed to analyse the mRNA expression levels of vaspin, adiponectin and adrenomedullin in PBMC from SLE patients. Our study identified reduced vaspin mRNA level, higher adiponectin mRNA level in SLE patients. However, there was no significant difference in the adrenomedullin mRNA level between SLE patients and normal controls.

Studies have suggested that the expression of vaspin was correlated with glucose level, insulin sensitivity and body fat mass (14, 30), and vaspin might play anti-inflammatory actions due to it could suppresses the productions of TNF-α, pro-inflammatory adipokines (22). Senolt *et al*. reported that an elevated level of vaspin was observed in the synovial fluid from rheumatoid arthritis (RA) patients when compared with that in osteoarthritis (OA) patients, but no correlation between the synovial fluid vaspin levels and DAS28 in RA patients was observed (31). Moreover, vaspin mRNA levels in PBMCs might serve as a novel biomarker for some diseases (32). However, direct measurement of vaspin mRNA in PBMCs from SLE patients has not been performed. Our study provided the first evidence that vaspin mRNA level was reduced in PBMCs from SLE patients, Another significant discovery was that the mRNA expression level of vaspin in more active SLE was significantly lower than that in less active SLE groups. However, there was no association of the vaspin mRNA expression level with the most of the clinical manifestations and complications. The above findings together indicated that vaspin might be involved in the pathogenesis of SLE, although this adipokine might not have a direct effect on clinical manifestations.

Adiponectin has been considered as a potent anti-inflammatory factor that exerts multiple anti-inflammatory effects on metabolic pathways and vasculature, and an elevated adiponectin level has also been observed in several inflammatory diseases (21, 33). Studies have suggested that serum adiponectin level was significantly increased in SLE patients than controls, and there was no significant correlation between adiponectin level and fat mass measurements (34, 35). Consistent with the above studies, we observed significantly higher adiponectin mRNA levels of PBMC in SLE patients. However, no significant relationships were found between adiponectin mRNA expression level and clinical features, disease severity and BMI among SLE patients. A recent study showed that the elevated adiponectin level was associated with renal complications in SLE; specifically, urinary level of adiponectin in SLE patients with renal involvement was significantly increased when compared to other patients without renal involvement (35). However, the similar difference between adiponectin mRNA expression levels from SLE patients with LN and those patients without LN was not found, against a direct involvement in LN. The role of adiponectin in pathophysiology of renal involvement remains to be evaluated.

One previous study had demonstrated that the plasma adrenomedullin level was significantly increased in SLE patients compared with normal controls, and a positive association was found between the plasma adrenomedullin level and SLEDAI-2K (36). In addition, the LN patients with active nephritis had decreased adrenomedullin mRNA expression in PBMCs compared with those patients without active nephritis, and adrenomedullin suppressed IL-6 mRNA level in cultured PBMC of active LN patients (13). This study proposed that adrenomedullin might act as a suppressor of SLE progression through the inhibition of IL-6 production by PBMCs. The study also indicated that adrenomedullin mRNA level was correlated negatively with SLEDAI. This confirmed another study by Cheung *et al*. who found a significant correlation between plasma adrenomedullin level and SLEDAI (20). Similarly, our results showed that adrenomedullin mRNA in more active SLE was significantly lower than those in less active SLE groups. This supports the opinion that drenomedullin mRNA level in PBMC might be an indicator of the disease activity of SLE. However, there was no significant difference in adrenomedullin mRNA level between SLE patients and normal controls. These discrepancies might be due to the following factors: different treatment options such as prednisolone, immunosuppressive therapy, and very low degrees of cardiac and renal dysfunction which were not clinically apparent but could potentially affect adrenomedullin expression in SLE patients (13, 36).

However, several limitations in this study should be acknowledged. Firstly, the disease activity of SLE patients was assessed by SLEDAI-2K, and no all renal biopsies were taken to evaluate the degree of renal damage. Therefore, it is not possible to accurately determine the relationship between the renal activity index and adipokines mRNA levels. Secondly, this was a case-control study and we were unable to analyse the correlation between the adipokines mRNA levels and various clinical variables, disease severity, treatment of the subjects over a long period of time. Finally,

environmental characteristics, region and ethnicity may affect the mRNA expression levels of adipokine levels in SLE patients.

In conclusion, our study provides evidences for decreased vaspin mRNA level and increased adiponectin mRNA level in PBMCs of SLE patients, and vaspin and adrenomedullin levels were associated with disease activity in SLE patients. The above findings suggested that these adipokines might play a role in SLE. Functional studies will be required to further explore the exact roles of these adipokines in SLE.

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