

## CD26 mRNA expression in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease which involves several organs. CD26 is a multifunctional molecule that has an extracellular domain with dipeptidyl peptidase IV activity which digests crucial inflammatory molecules. CD26 plays an important role in T-cell activation and enhances immune responses. This study was carried out to evaluate the level of CD26 gene expression in SLE patients. Forty-six SLE patients and 44 healthy controls voluntarily participated in this study. Based on the SLE disease activity index (SLEDAI), the patients were divided into two subgroups, those with active disease (n=24) and those with inactive disease (n=22). Patients were also subgrouped according to renal involvement, as those with lupus nephritis (n=17) and those without lupus nephritis (n=29). Their CD26 mRNA levels in peripheral blood cells were analyzed by quantitative RT-PCR. CD26 mRNA expression increased 3.6-fold in SLE patients in comparison with the controls ( $p<0.0001$ ). No difference was found in the level of CD26 mRNA among the subgroups of the SLE patients with the active or inactive form of the disease ( $p>0.05$ ). Although CD26 mRNA expression in patients with lupus nephritis was 2.76-fold higher than those without nephritis, the difference was not statistically significant ( $p>0.05$ ). CD26 gene expression in peripheral blood cells of SLE patients significantly increased over that of the controls. This increase was not affected by the disease activity nor did it show any significant correlation with complications in organs.

**Keywords:** dipeptidyl peptidase IV, gene expression, systemic lupus erythematosus.

### Introduction

Systemic lupus erythematosus (SLE) is a severe autoimmune disease that has many pathological implications for most organs [1]. Innate immune cells, such as dendritic cells (DCs) and neutrophils, along with adaptive immune B and T immune cells are involved in the pathogenesis of lupus [2]. Classic SLE was considered to be a B-cell based disease. It has been shown recently that T cells also play crucial roles in the pathogenesis of SLE. They offer considerable helps to B cells to enhance differentiation, maturation and autoantibody production. These findings have changed the classic perspective so that SLE now is considered to be a T cell-associated disease. The study of molecules expressed by T cells will, thus, improve understanding of the pathogenesis of SLE.

CD26 is a membrane bound ecto-enzyme which is expressed on the cell surface of T lymphocytes to induce co-stimulatory signals through direct interaction with adenosine deaminase (ADA) and caveolin-1 molecules expressed on antigen-presenting cells (APCs) [3-5]. CD26 is up-regulated on the cell surface of B lymphocytes, natural killer cells, monocytes and DCs. A soluble form of CD26 (sCD26) that with dipeptidyl peptidase IV (DPP

IV, EC3.4.14.5) activity has been also traced in human serum, cleaves a variety of proteins, for instance, chemokines and cytokines, to reduce inflammation. [4,6]. It is clear that CD26 as a moonlighting protein plays a dual role in immune systems as a co-stimulatory molecule in promoting inflammation and as an ecto-enzyme in degrading interferon- $\gamma$ -induced chemokine and inflammatory cytokines.

The CD26 expression level upon activation. In addition to antigen stimulation, interferon alpha (IFN- $\alpha$ ) can increase CD26 expression. IFN- $\alpha$  is a major factor in the onset and development of SLE [7,8]. This cytokine stimulates the expression of genes which have a GAS element (TTCCCCGAA) instead of a TATA box in their promoter. It has been shown that CD26 has a GAS element in the promoter region and could be a direct target for IFN- $\alpha$  signaling [9]. The results of earlier studies have revealed that DPP IV enzyme activity in the serum of SLE patients is significantly reduced [10-13]. Additionally, we previously showed that circulating concentrations of CD26 in SLE patients were similar to those of the controls and did not correlate with the IFN- $\alpha$  level [14]. This study was conducted in order to evaluate

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the level of CD26 gene expression in the blood cells of those patients.

## Materials and Methods

### Study groups

In the current case-control study, the blood samples of 46 SLE patients and 44 age- and sex-matched healthy volunteer controls which had participated in the previous study were used [14]. Patients were selected using the convenience sampling method from those who had referred to Loghman Hakim Educational Hospital during 2014-2015. Only patients who fulfilled the 1982 revised criteria for classification of SLE were included [15]. The SLEDAI score was calculated according to the American Rheumatology Society criteria using clinical and paraclinical findings [16]. Based on renal involvement, patients were divided into two subgroups: patients with lupus nephritis (n=17) and those without it (n=29). For this purpose, the renal biopsy reports in the patient files were used. The second subgroup was formed according to their SLEDAI scores and included active SLE (n=24) and inactive SLE (n=22) patients. Patients with SLEDAI scores of less than 10 were considered to have active disease. More patients received 200-400 mg hydroxychloroquine daily. The laboratory data, such as white blood cell count (WBC), erythrocyte sedimentation rate (ESR), C reactive protein (CRP), C3 and C4 levels, antinuclear antibodies (ANA), and anti-dsDNA antibody titers were obtained from patient clinical history files. Forty-four age-and sex-matched individuals joined the study as the healthy control group, giving their own permission and consent. Individuals who had the following complications were excluded: subjects with a body mass index of more than 30, smokers and those who had been diagnosed with recent infectious diseases. The institutional ethics board of Shahid Beheshti University of Medical Sciences approved the study. All participants were well-informed and participated voluntarily in the study with their own consent.

### CD26 mRNA expression analysis

RNA extraction and qRT-PCR were performed as previously described [17]. Briefly, total RNA extraction from 200 µl of whole blood was performed using QIAamp RNA Blood Mini Kits (Qiagen; Germany) according to manufacturer protocol. The extracted RNA was converted back into complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis Kit (Thermo; Lithuania). The relative expression level of CD26 mRNA was measured by quantitative RT-PCR using a TaqMan probe. Two sets of exon spanning primers and TaqMan probes specific to CD26 and GAPDH genes as the normalizer were designed using Beacon Designer software (version 7; USA; Table 1). The PCR reaction mix was prepared as follows: 1.5 µl of single strand cDNA was mixed with 10 µl of DFS master mix (Bioron; Germany), 2 µl of forward and of reverse primer mix (10 pMol) and 0.5 µl of 20 pMol TaqMan probe. The final volume was set to 20 µl by adding 6 µl RNase free water.

Amplification was done on a Rotor-Gene Q real-time PCR machine (Qiagen; Germany) using the following PCR thermal cycle conditions: initial hold at 94°C for 2 min, followed by 40 cycles, including denaturation for 60 sec at 95°C and annealing at 58°C for 15 sec. The relative gene expression was calculated using the relative expression software tool (REST) and included the efficiency of every PCR reaction [18]. All samples were analyzed in duplicate.

### Statistical analysis

The Shapiro-Wilk test was used to examine the normality distribution of each continuous variable. Due to the skewed distribution of the data, the Mann-Whitney U test was used to analyze differences between groups. Moreover, Spearman's correlation test was employed to assess the correlation between variables. A p-value of less than 0.05 was considered to be statistically significant. Statistical analysis was done by SPSS statistical software (version 21; IBM).

**Table 1.** Primers and probes sequences. The sequences of CD26 and GAPDH genes were extracted from the NCBI data bank. Four exon-exon spanning primers and two probes were designed. Probes were labeled with FAM as a green reporter fluorescent day at their 5' and BHQ1 as quencher at 3' side.

Name	Sequences (5' > 3')	Tm	Product length	Accession number
CD26 forward primer	CTGACTGGGTTTATGAAGA	51.47		
CD26 reverse primer	CAGAGTAGAAGGAGTATTCAA	51.76	132	NM_001935.3
CD26 probe	FAM-CTACTCTGCTCTGTGGTGGTCTC-BHQ1	-		
GAPDH F Forward primer	CCAGGTGGTCTCCTCTGACTTCAACAG	66.25		
GAPDH reverse primer	AGGGTCTCTCTCTCCTCTTGTGCTCT	66.12	224	NM_002046.5
GAPDH probe	FAM-ATGATTGCCGCCCGCTGGACAC-BHQ1	-		

## Results

### *Clinical and laboratory findings*

The demographic data of the study groups is summarized in Table 2. Twenty-four of the 46 patients had active disease at the time of blood sampling. The laboratory findings included ESR C3, C4, WBC, ANA, and anti-dsDNA antibodies. Only ESR showed a significant statistical difference between the active and inactive SLE patients ( $p=0.004$ ; Table 3).

Comparison of the blood pressure of patients revealed that blood pressure was significantly higher in lupus patients than the control subjects ( $p=0.011$ ). Furthermore, there was a significant, but weak, correlation between

blood pressure and the dosage of prescribed prednisolone in the patient group ( $p=0.04$ ;  $r=0.49$ ).

The prescribed dosage of prednisolone for the lupus nephritis SLE group was significantly higher than for the SLE group without lupus nephritis ( $p=0.009$ ). Nevertheless, blood pressure was not significantly different between the group with lupus nephritis and the group without it. The lymphocyte count showed a significant decrease in the group with lupus nephritis over than the group without it ( $p=0.033$ ). There was no significant difference between lymphocyte count between the active and inactive groups.

**Table 2.** Demographic characteristic of the study participants

	SLE patients (n=46)	Healthy controls (n=44)
Women	43	41
Mean age (range), years	33.41 (16-62)	30.18 (23-50)
Mean of systolic Blood pressure	11.85±2.44*	11.26±0.90

Data are means±SD. \*  $p\geq 0.05$

**Table 3.** A number of clinical and paraclinical findings of SLE patients

	Disease activity		Renal involvement		All patients (N=46)
	Active (n=24)	Inactive (n=22)	With nephritis (n=17)	Without nephritis (n=29)	
Woman	22	21	16	27	43
Age (years)	33.62±10.42	33.18±10.17	33.64±11.35	33.27±9.66	33.41±10.19
Onset (years)	4.42±3.47	4.59±4.04	5.56±4.18	3.84±3.33	4.51±3.73
SLEDAI	22.20±13.22	3.63±2.68	20.00±14.99	9.41±10.99	13.44±13.32
ESR (mm/h)	42.00±29.21**	25.25±33.59	37.56±31.12	31.84±33.13	34.02±32.11
ANA	51.42±30.48	19.59±10.72	54.36±32.98	18.51±1070	39.09±20.60
Anti dsDNA Ab	379.71±662.52	319.28±790.19	334.84±637.40	353.81±761.47	348.68±721.39
C3 (mg/dL)	91.63±43.28	104.12±38.90	89.75±44.42	101.02±18.51	97.36±41.24
C4 (mg/dL)	14.64±10.54	19.05±1058	14.78±1026	17.52±1092	16.66±1064
WBC/ml	7180±5100	6523±1879	8760±5469	5871±2009	6836±3748
Lymphocyte/ml	3083±1147	3676±1292	2650±1212*	3730±11.30	3403±1248
Prednisolone	18.52±20.25*	8.92±12.43	21.09±21.90**	9.54±12.64	13.83±17.38
Systolic BP (mmHg)	11.72±3.28	12.00±0.80	11.87±3.86	11.83±0.99	11.84±2.43

Data are means±SD obtained from the file of 46 SLE patients.

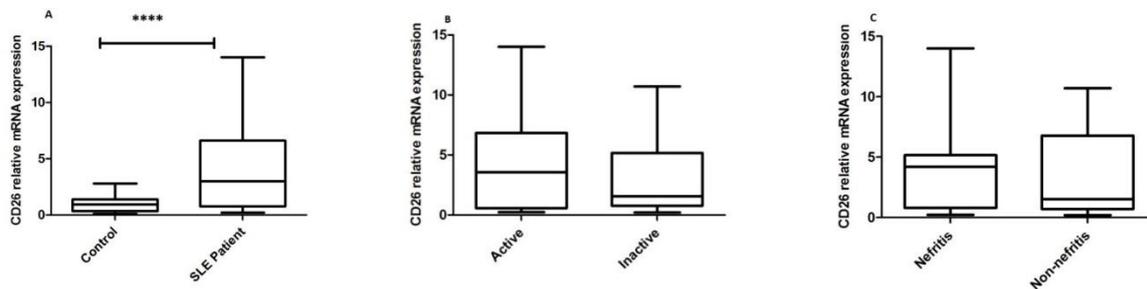
\* and \*\*  $p\geq 0.05$  and  $p\geq 0.01$ , respectively. ESR: Erythrocyte Sedimentation Rate, ANA: Anti-Nuclear Antibodies.

### *CD26 mRNA expression*

CD26 mRNA expression was 3.6-fold higher in SLE group than in the healthy control group ( $p=0.000$ ; Fig. 1A). Statistical analysis showed no difference in CD26 expression level between the active and inactive SLE groups ( $p>0.05$ ; Fig. 1B). CD26 mRNA expression in the lupus nephritis group was 2.76-fold higher than in the group without lupus nephritis, but there was no statistical

difference ( $p>0.05$ ; Fig. 1C). CD26 gene expression showed no correlation with the dosage of prednisolone prescribed ( $p>0.05$ ) nor with SLEDAI scores ( $p>0.05$ ). Complications in other organs, for example, skin rash, vasculitis, pericarditis, seizure and arthritis showed no significant effect on the level of CD26 gene expression ( $p>0.05$ ).

## CD26 gene expression in SLE



**Fig. 1.** The changes in CD26 relative mRNA expression levels were evaluated by quantitative RT-PCR. A) mRNA level of CD26 was significantly higher in SLE patients than healthy controls ( $p \leq 0.0001$ ). B) mRNA level of CD26 was not significantly differed in patients with active disease vs. inactive SLE ( $p \leq 0.05$ ). C) The mRNA level of CD26 in nephritis patients was similar to the level of CD26 expression in non-nephritis patients ( $p > 0.05$ ). \*\*\*\* represents  $p \leq 0.0001$ .

## Discussion

Auto-reactive lymphocytes play a central role in initiation and development of autoimmune responses in SLE; therefore, they are directly involved in the development of related organ pathology. Evidences suggest that CD26 is up-regulated in lymphocytes following antigenic stimulation. CD26 delivers a potent co-stimulatory signal to T cells and may have a pathologic role in SLE. The results of the current study showed that CD26 gene expression in the WBCs of the SLE group was 3.6-fold higher than in the control group. Contrary to this finding, Wang et al. reported that the expression of CD26 on the cell surface of CD4<sup>+</sup> lymphocytes and iNKT lymphocytes decreased significantly in SLE patients. To shed light on this discrepancy, it is important to note that the trans-endothelial migration capacity of CD4<sup>+</sup> T cells depends on high-level expression of CD26 [19]. Hence, the observed decrease in that study may be because CD26<sup>+</sup> lymphocytes were absent in the blood as they had migrated to target tissues such as the kidney to participate in immune responses. The results of a previous study in CD26 low and CD26-deficient rats support this opinion and showed that T-cell recruitment to the airway decreased and was associated with a significant decrease in the titer for allergen-specific IgE [20]. The current study show that there aren't any significant differences in CD26 gene expression in the blood samples of the group with lupus nephritis in comparison with those without this complication. In order to evaluate the correlation between CD26 expression and renal involvement, it should be noted that the use of a kidney biopsy might give better results.

Results of previous studies have shown that DPP IV activity and serum levels for sCD26 decreased significantly in SLE patients and also were inversely correlated with the SLEDAI score. Additionally, DPP IV

activity decreased in plasma and tissues of the New Zealand black mouse as a model for human SLE in comparison with the control mice [10]. In the current study, CD26 mRNA expression showed no significant correlation with disease activity. Similar to the findings of the present study, a previous study on rheumatoid arthritis (RA) showed that the density of CD26 on the membranes of T cells did not correlate with disease severity, organ complication or laboratory findings [21]. Furthermore, the results of the present study showed that the level of none of the laboratory findings for C4, C3, ANA, and anti dsDNA antibodies analysis correlated with CD26 mRNA level.

It was also observed that the expression level of CD26 mRNA in SLE patients did not correlate with the dosage of prednisolone prescribed, although the dosages of prednisolone in the active SLE group were significantly higher than for the inactive SLE group. Similar results have been seen in two previous studies on RA. Ellingsen et al. showed that, after 52 weeks of treatment by clinically-effective disease-modifying drugs, the density of CD26 on monocytes remained up-regulated [21]. In another study, the higher CD26 density on CD4<sup>+</sup> T lymphocytes was not affected by methotrexate treatment. On the contrary, CD26 density decreased on monocytes in the responder group and was associated with decreased disease activity [22].

Together with our previous findings, it can be suggested that an increase in mRNA expression of CD26 in WBCs may enhance immunopathogenesis by increasing lymphocyte activation and migration. On the other hand, in inflammatory responses, a decreased or stable sCD26 level led to disease progression and severity due to insufficient enzyme activity for destruction of inflammatory molecules in serum.

**Conflicts of interest**

The authors declare no conflict of interest.

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