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ORIGINAL ARTICLE

CD26 mRNA Expression Level In Systemic lupus Erythematosus Patients Attending Benha University Hospital

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ABSTRACT

Background*: CD26 has a role in the pathogenesis of inflammation and participates in degrading interferon-γ-induced chemokine and inflammatory cytokines which have a role in Systemic lupus Erythematosus (SLE) pathogenesis.* ***Objectives:*** *This work was carried out to evaluate the level of CD26 mRNA expression in the peripheral blood leucocytes of SLE patients by quantitative RT-PCR and correlates this level with the disease activity and lupus nephritis.* ***Methodology:*** *thirty SLE patients' blood samples were obtained, leucocytes were isolated and the level of CD26 mRNA expression was evaluated by RT-PCR. Twenty healthy subjects matched for age and sex were chosen as a control group. All cases were subjected to full history taking, thorough clinical exanimation and laboratory investigations. The disease activity was evaluated with systemic lupus erythematosus disease activity index (SLEDAI).. Patients were subdivided into: 12 SLE patients with lupus nephritis and 18 SLE patients without lupus nephritis.* ***Results****: CD26 mRNA expression increased 1.28 fold in SLE patients compared to the controls (p<0.05) with no significant correlation between CD26 mRNA expression and SLEDAI score. No significant difference (p>0.05) was found among patients with and those without lupus nephritis.* ***Conclusion****: Our results support the hypothesis that CD26 mRNA plays a role in the pathogenesis of SLE; however it is not a good predictor of SLE disease activity or lupus nephritis.*

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease with diverse clinical manifestations as skin rashes, oral ulcers, hematological abnormalities, arthritis, renal abnormalities, and neurological troubles [1]. The nature of SLE disease and its pathogenesis are vague but the most reasonable thought is the interaction of hormonal ,genetic and environmental factors [2] .

CD26 is a 110-kD cell surface type II transmembrane protein with a co-stimulatory function in the immune system[3]. Its expression is great in different tissues, and it is expressed strongly in activated T cells, B cells, and natural killer (NK) cells. It also has an important function in regulation of immunity and pathogenesis of SLE, as it acts as a co-stimulator, CD26 participates in T-cell activation and differentiation by its interaction with other molecules with fundamental cellular functions in T-cell responses, as adenosine deaminase, CARMA1 and caveolin-1 [4]. CD26 can exist in a soluble monomeric form which has been notified in plasma and other body fluids which has an augmentation effect on T- cell proliferation.[5]

This soluble form with dipeptidyl peptidase IV activity has been existed in human serum and is important for chemokines and cytokines to decrease inflammation as it cleaves a variety of proteins engaged in it [6].

The aim of this work is to evaluate the level of CD26 mRNA expression in the leucocytes of systemic lupus erythematosus patients by quantitative RT-PCR and to correlate this level with the disease activity and lupus nephritis.

METHODOLOGY

The present case-control study included 30 SLE patients fulfilling SLICC Classification Criteria for SLE[7]. These patients were selected from the Inpatients and the Outpatients' Clinics of the Rheumatology, Rehabilitation and Physical medicine Department, Benha university Hospitals, during November 2018: September 2019.

A total of 20 age and sex matched apparently healthy volunteers serve as a control group .The practical part of this study was carried out in Medical Microbiology and Immunology Department, Faculty of medicine, Benha University.

All patients were subjected to full history taking, thorough clinical exanimation and laboratory

investigations such as white blood cell count (WBC), HB, Platelets ,erythrocyte sedimentation rate (ESR), C reactive protein (CRP), C3 and C4 levels, antinuclear antibodies (ANA), and anti-dsDNA antibody titers, serum Creatinine, blood urea and Protein in 24h urine was collected .

Assessment of disease activity was done by SLEDAI score. Patients with SLEDAI scores of ≥10 were considered to have active disease [8].

All subjects provided an informed written consent prior to participation in this study. Patients with Age < 16 years, body mass index(BMI) of more than 30,smokers,with systemic inflammatory autoimmune diseases such as rheumatoid arthritis and patients with recent infectious diseases or had diabetes were excluded.

Approval for the study design was obtained from the ethics committee, Faculty of medicine, Benha university.

CD26 mRNA expression analysis:

A total of 3 ml peripheral blood were obtained from each participant for RNA extraction and serum collection. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll. followed by RNA extraction using QIAamp RNA Blood Mini Kit (Qiagen, Germany) according to manufacturer protocol .Complementary DNA (cDNA) was prepared by using QuantiTect Reverse Transcription Kit according to manufacturer protocol. The relative expression level of CD26 mRNA was measured by quantitative RT-PCR using a QuaniTect SYBR Green PCR Kit according to manufacturer protocol. Invitrogen (Thermo Fisher custom primers)was applied for designing the exon-exon spanning primers for CD26 (Table 1)

The PCR reaction mix was prepared as follows: 2 µl of single strand cDNA was mixed with 12.5µl of QuaniTect SYBR Green master mix , 1.25 µl of forward and 1.25 µl of reverse primer and 8 µl RNase free water.

Table 1: Primers and probes sequences

Name	Sequences (5' > 3')	Tm	Primer length
CD26 forward primer	5′-CTG ACT GGG TTT ATG AAG A-3′	46.7	19
CD26 reverse primer	5′-CAG AGT AGA AGG AGT ATT CAA-3′	48.5	21
GAPDH F Forward primer	5′-CCA GGT GGT CTC CTC TGA CTT CAA CAG-3′	62.77	27
GAPDH reverse primer	5′-AGG GTC TCT CTC TTC CTC TTG TGC TCT -3′.	61.26	27

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 [9] **.**All samples were analyzed in duplicate (fig 1 and fig 2)

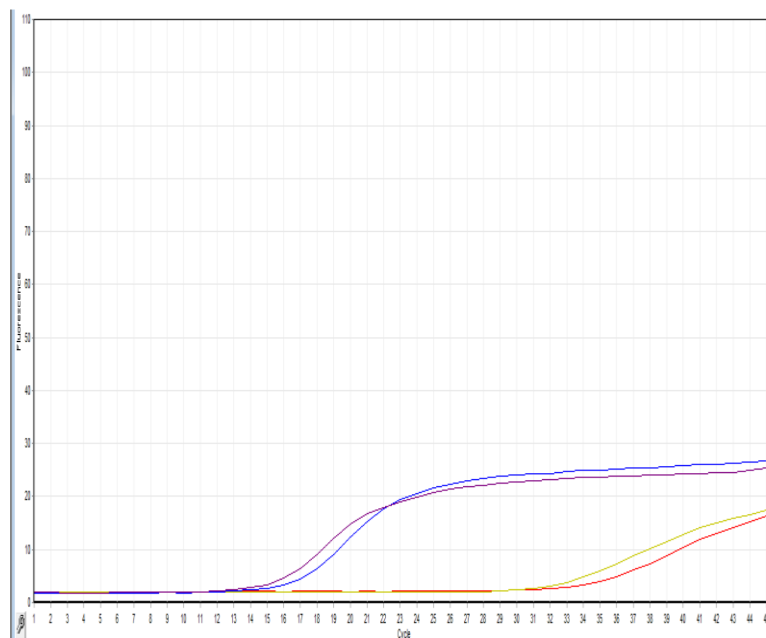

Fig. 1: Amplification curve using SYBR Green in real time PCR

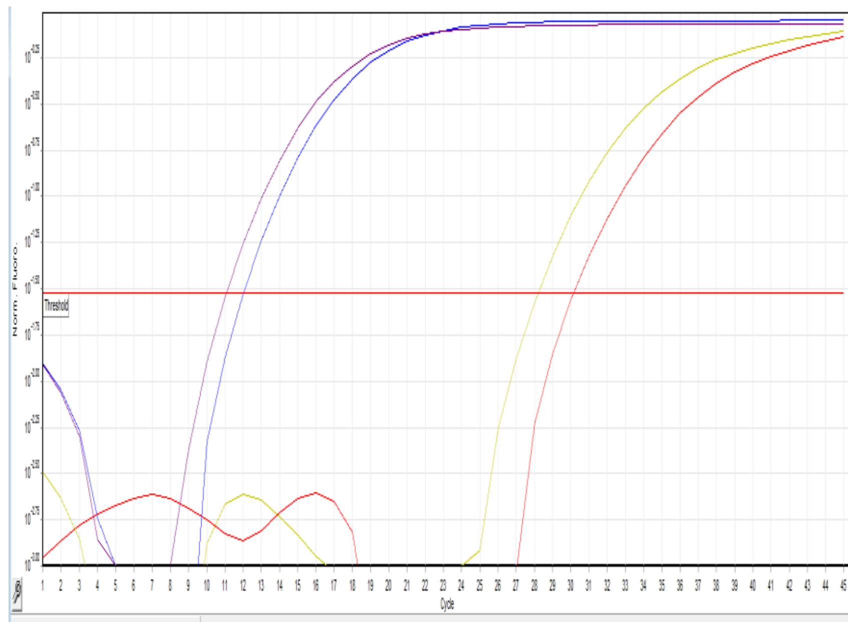

Fig. 2: The threshold line and Cycle threshold (C_T) of standard.

Statistical analysis:

The Mann-Whitney U test was used to analyse 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).

RESULTS

The present study included thirty SLE patients (30)**.**Their ages ranged between 16-46 years with mean of 28.8±5.04. They were 26 females (86.7%) and 4 males (13.3) with their disease duration ranged between 1-13years, together with twenty (20) apparently healthy subjects as a control group. Their ages ranged between 18-40 years with mean of 27.9±3.9. They were 17(85.0%) females 3 (15.0%) males with no statistical significant differences between the studied groups regarding age and sex (p >0.05).

CD26 gene expression in the leucocytes of the SLE group was 1.28 fold higher than in the control group with statistically significant difference between them (p<0.05) (Table 2)

Table 2: Comparison of C_T CD26 , C_T GAPDH and fold difference between cases and control groups.

	Cases	**Controls**	**test of significance**
C_T CD26	33.73(19.98-44.0) (30.37-37.78)	33.45(31.0-43.22) (31.49-40.89)	Z=0.23 P=0.82
C_T GAPDH	23.03(10.0-43.97) (13.34-28.28)	22.23(20.0-37.22) (20.5-33.5)	Z=0.47 P=0.64
ΔC_T	9.77(-22.07-21.54) (1.37-18.11)	10.77(6.0-11.9) (7.14-11.67)	Z=0.0 P=1.0
$\Delta\Delta C_T$	-0.09(-31.93 -11.68) (-8.49-8.25)	0.91(-3.86-2.04) (02.73-1.82)	Z=0.0 P=1.0
Fold difference in CD26 mRNA = $2^{-\Delta\Delta Ct}$ Median (IQR)	1.28(0.003-360.61)	0.539(0.295-11.05)	Z=1.98 P=0.049

Among the studied SLE cases, there were 6 active and 24 inactive cases regarding SLEDAI score.

In our study there was no significant difference between active and inactive SLE cases regarding CD26 expression (Table 3)

Table 3: comparison of CD26 expression between the active and inactive SLE groups

	inactive n=6	Active n=24	test of significance
CT CD26 Median (Min-Max) (Interquartile range	40.21 (30.15-44.0) (30.15-44.0)	33.49 (19.98-41.0) (30.54-36.27)	Z=1.22 P=0.22

Z:Mann Whitney U test

Significant differences were reported regarding C3, C4 levels between patients with and those without lupus nephritis (Table 4)

Table 4: Comparison of demographic, laboratory findings between cases with and without renal affection

	No renal affection n=18	Renal affection n=12	test of significance
Age/years mean ± SD	33.39±7.9	30.08±8.12	t=1.10 p=0.27
Sex Female Male	n(%) 17(94.4) 1(5.6)	n(%) 9(75.0) 3(25.0)	FET P=0.27
Duration/years Median (Min-Max) (Interquartile range)	5.0(1.0-13.0) (2.75-8.50)	3.0(1.0-10.0) (2.0-6.0)	Z=1.49 P=0.14
C3 Median (Min-Max) (Interquartile range)	70.0(38.0-130.0) (44.5-86.5)	12.0(20.0-92.0) (40.0-106.5)	Z=0.23 P=0.082
C4 Median (Min-Max) (Interquartile range)	14.5(7.0-58.0) (12.0-28.0)	9.0(5.0-32.0) (6.25-30.5)	Z=0.64 P=0.053
Proteinuria Median (Min-Max) (Interquartile range)	0.20(0.1-200.0) (0.1-0.4)	0.35(0.2-266.0) (0.23-8.50)	Z=1.98 P=0.04*
CT CD26 Median (Min-Max) (Interquartile range)	33.74(30.05-41.0) (31.56-37.78)	32.70(19.98-44.0) (26.74-39.05)	Z=0.62 P=0.54
CT GAPDH Median (Min-Max) (Interquartile range)	25.69(10.0-31.13) (13.34-28.28)	21.11(11.03-43.97) (13.27-32.16)	Z=0.0 P=1.0
SLEDAI score Median (Min-Max) (Interquartile range)	12.5(9.0-22.0) (11.75-15.0)	13.0(6.0-21.0) (8.25-16.75)	Z=0.32 P=0.75

Z:Mann Whitney U test t:Student t test * statistically significant FET: Fischer exact test

Table 5, shows there was statistically insignificant correlations between C_T CD26 and C_TGAPDH, age, duration, SLEDAI, HB, TLC, Platelets, ESR, Urea, Creatinine, C3, C4, Protein in 24h urine

Table 5: Correlation between C_T CD26 and C_T GAPDH, age and laboratory findings among SLE cases.

SLE cases		C_T CD26
C_T GAPDH	rs	0.39
	p	0.08
Age	rs	0.152
	p	0.478
Duration	rs	0.305
	p	0.178
SLEDAI	rs	0.425
	p	0.062
HB	rs	0.217
	p	0.358
TLC	rs	0.191
	p	0.421
platelets	rs	0.071
	p	0.767
ESR	rs	0.253
	p	0.282
Urea	rs	0.002
	p	0.992
Creatinine	rs	0.409
	p	0.074
C3	rs	0.220
	p	0.351
C4	rs	0.119
	p	0.617
Protein in24h urine	rs	0.134
	p	0.574

rs: Spearman correlation co-efficient

DISCUSSION

As regard the level of CD26 mRNA expression in the leucocytes, this study revealed that the level was 1.28 fold higher in SLE group than in the healthy control group ($p< 0.05$). In agreement to our study Valizadeh et al.[10] founded that CD26 mRNA expression was higher in SLE group than in the healthy control group ($p< 0.05$) [10] .In contrast to our results, Wong et al.[11] found that cell surface expression of CD26 on CD4+T and iNKT lymphocytes was reduced significantly in SLE patients compared with controls (all $p < 0.05$), this may be due to absence of CD26+ lymphocytes and its decampment to target tissues such as the kidney to participate in immune responses [11]. Meanwhile Kruschinski et al[12] founded that there was a deficiency in CD26 and reduction in the T-cell recruitment to the airway which accompanied by decrease in the titer for allergen-specific Ig E.

In our study there was no significant difference in CD26 mRNA expression level between the active and inactive SLE groups (P=0.22).Also there was no significant correlation between CD26mRNA expression level and SLEADI activity index (P=0 .062).Also there was no significant correlation between CD26 mRNA expression and SLEDAI score. In agreement with our study Valizadeh et al[10] found non-significant correlation with disease activity and CD26 mRNA expression.

Other studies have shown that DPP IV activity and serum levels for sCD26 have been reduced significantly in SLE patients and also were inversely correlated with the SLEDAI score [13]. Moreover Kobayashi et al.[14] concluded involvement of sCD26 in the pathophysiology of SLE and being a disease activity measure for SLE. They also reported that Serum levels of sCD26 and its specific DPPIV activity were significantly reduced in SLE and were inversely correlated with SLE disease activity index score, but not with clinical variables or clinical subsets of SLE

In our study there was no significant correlations between CD26 mRNA level expression and disease duration, Hb, TLC, platelets, ESR, Urea,Creatinine,C3,C4 and Protein in 24h in urine.In agreement to our study Valizadeh et al[10] found no significant difference between CD26 mRNA level and C4, C3, ANA, anti dsDNA antibodies analysis and organ complications.

In the current study, insignificant difference was found regrading CD26mRNA level between SLE cases with and without LN which was similar to that reported by Valizadeh et al. [10].

CONCLUSION

Our results support the hypothesis that CD26 mRNA plays a role in the pathogenesis of SLE however it is not a good predictor of SLE disease activity. Therefore, the findings of this study need to be confirmed in a larger cohort of active patients and patients with LN and also adjustment of drugs used to control the disease. Also we recommend to perform this study on various ethnic groups and other autoimmune diseases.

Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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