Association between APRIL Gene Expression and Systemic Lupus Erythematosus

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Abstract: Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a high mortality risk if not properly treated. B cell hyperplasia has been implicated in the pathogenesis of the disease. B cells produce large quantities of autoantibodies, eventually leading to multiorgan complications. Treatments targeting B cell products appear particularly promising. APRIL is a TNF-like subclass ligand with yet unresolved implications in SLE. Some studies have accused its expression in the pathogenesis of SLE, while others negated it. **Aim of work:** This is to assess APRIL gene expression in SLE patients versus healthy controls and to correlate its presence with organ affection, and disease activity index. **Materials and methods:** This is a cross sectional study involving 40 SLE patients (35 females and 5 males), presenting in the Internal Medicine Department Cairo University. Ten healthy matched controls for age and sex were also involved. Age was 27.45 ± 8.85years. All subjects had a complete history and physical examination. Laboratory tests included CBC, ESR, urea, creatinine, ANA, antiDNA, SLEDAI, as well as APRIL gene expression. **Results:** We found 22 SLE patients were expressors (55%), while 18 patients (45%) were non expressers. None of the controls expressed the gene. We found astatistically significant association between APRIL gene expression in SLE patients and presence of neuropsychiatric (p<0.001) complications (fig.2), vasculitis (p< 0.05), and photosensitivity (p<0.007). There was a significantly positive correlationbetween APRIL gene expression and ACL IgG (r=0.6, p< 0.005), but not IgM or SLEDAI. **Conclusion**APRIL gene expression is significantly elevated in SLE patients with vasculitis, cerebritis, and photosensitivity. It has a strong positive correlation to ACL IgG levelshence possibly correlated to antiphospholipid syndrome. This implies the future possible implementation of APRIL gene expression in prognosis and therapy targets for SLE patients.

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Key words:SLE, nephritis, vasculitis, APRIL gene, antiphospholipid syndrome.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a high mortality risk if improperly treated [(1)]. SLE pathogenesis has been linked to B cell hyperproliferation in patients. These B cells generate large quantities of IgG autoantibodies that can form immune complexes, which are ultimately implicated in lupus nephritis, cerebritis, vasculitis as well as other complications [(2, 3)]. Treatments that target B cells appear particularly promising [(4)].

APRIL is a TNF-like subclass ligand that has recently been implicated in many diseases especially SLE. Conflicting results have been presented regarding its effect in SLE patients. Additional work lies ahead to expand our understandingof the normal function of APRIL, as well as its pathologicalrole in disease and treatment of SLE.

Aim of work

This is to assess APRIL gene expression in patients with SLE versus healthy controls and to correlate its presence with organ affection, and disease activity index.

2. Materials and methods

This cross sectional study was conducted on 40 SLE patients, chosen from the Rheumatology& Rehabilitation department and out-patient clinic over a period of 12 months. They were 35 females and 5 males. Their ages ranged between 16 and 52 years. Thirty four patients were receiving corticosteroid therapy and 6 patients were newly diagnosed. A control group of 10 normal, healthy, sex and age-matched subjects were chosen. None had family history of autoimmune diseases and all had normal ESR&CBC.

All patients fulfilled four or more of the eleven criteria designated by the American College of Rheumatology (ACR) for the diagnosis of SLE [(5)].

All patients were subjected to: Complete history taking and physical examination.Chest x –ray and other radiological examination were done as needed.Patients were also subjected to the following laboratory investigations: Complete haemogram, erythrocyte sedimentation rate (ESR), complete urine analysis,liver function tests in the form of (ALT), serum (AST) level,and albumin. Kidney function

tests in the form of serum ureaand serum creatinine, as well as 24 hour urinary protein were also done. Antinuclear antibodies (ANA),anti-ds DNA (detected by indirect immunofluorescence technique), Anticardiolipin(ACL) antibodies IgG and IgM, systemic lupus erythematosus disease activity index (SLEDAI) as well as serum complement level (C3and C4) were also measured .

Detection of APRIL:

Ready-To-Go RT-PCR beads utilize Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase and Taq to generate PCR product from an RNA template.

Statistics

All statistical analyses were done using GraphPad Prism software version 4.00.results were given as mean and standard deviation.For comparison of paired samples during the study, a student T pair test was used**.**A *p* value <0.05 was considered statistically significant.

3. Results

We included forty systemic lupus erythematosus patients; eight patients were newly diagnosed and 32 were under treatment. Their mean ageswas 27.45 ± 8.85years. Table1.shows the different laboratory data of SLE patients in the study. Figure 1, on the other hand, shows the frequency of distribution of the different clinical data in these patients. There was no correlation between APRIL gene expression and age, sex or duration of the disease.

The APRIL mRNA expression analysis by reverse transcription PCR (RT-PCR) showed gene expression in 22 patients (55%), while 18 patients (45%) were non expressers. Moreover, all patients expressed B-actin gene which serves as internal control. The entire control group was non expressers, but they expressed B-actin gene which serves as internal control. Their ANA and Anti –ds DNA were also negative.

Table 2. Shows statistically significant association of APRIL gene expression in SLE patients with neuropsychiatric complications (Fig.2), vasculitis, or photosensitivity.

Table 3. Shows no significant correlation between APRIL gene expression and the pattern of antinuclear antibodies, or presence of anti ds DNA at the time of the study. On the other hand, table 4 and figure 3 show a significant positive correlation between APRIL gene expression and anticardiolipin Ig G (ACL IgG), but not IgM or SLEDAI. This possibly denotes a positive correlation between APRIL gene expression and antiphospholipid syndrome.

Table 1: Descriptive statistics of laboratory data of SLE patients (n=40)

Variables	Minimum	Maximum	Mean	Std. Deviation
ESR	16.00	150.00	76.35	42.05
Hb (g/dl)	7.20	16.20	10.93	2.37
WBCx103	2.40	14.40	7.07	3.0
Platelets x106	117.00	522.00	311.75	107.03
Creatinine (mg/dl)	0.40	1.90	0.69	0.27
Urea (mg/dl)	6.00	120.00	39.20	21.84
AST(U/L)	11.00	66.00	26.98	13.63
ALT (U/L)	6.00	62.00	23.45	14.17
Albumin	2.20	4.60	3.57	0.58
Total protein	6.00	13.40	7.98	2.12
C3 (mg/dl)	0.20	1.70	0.78	0.38
C4 (mg/dl)	0.02	0.70	0.16	0.16
24 hr urinary protein (g/day)	0.03	2.80	1.0	1.0
Fasting blood sugar (mg/dl)	61.00	150.00	92.18	18.65
ACLIGG	0.00	59.00	20.65	15.34
ACLIGM	2.18	56.50	19.12	15.46
SLEDAI	10.00	48.00	24.68	9.77

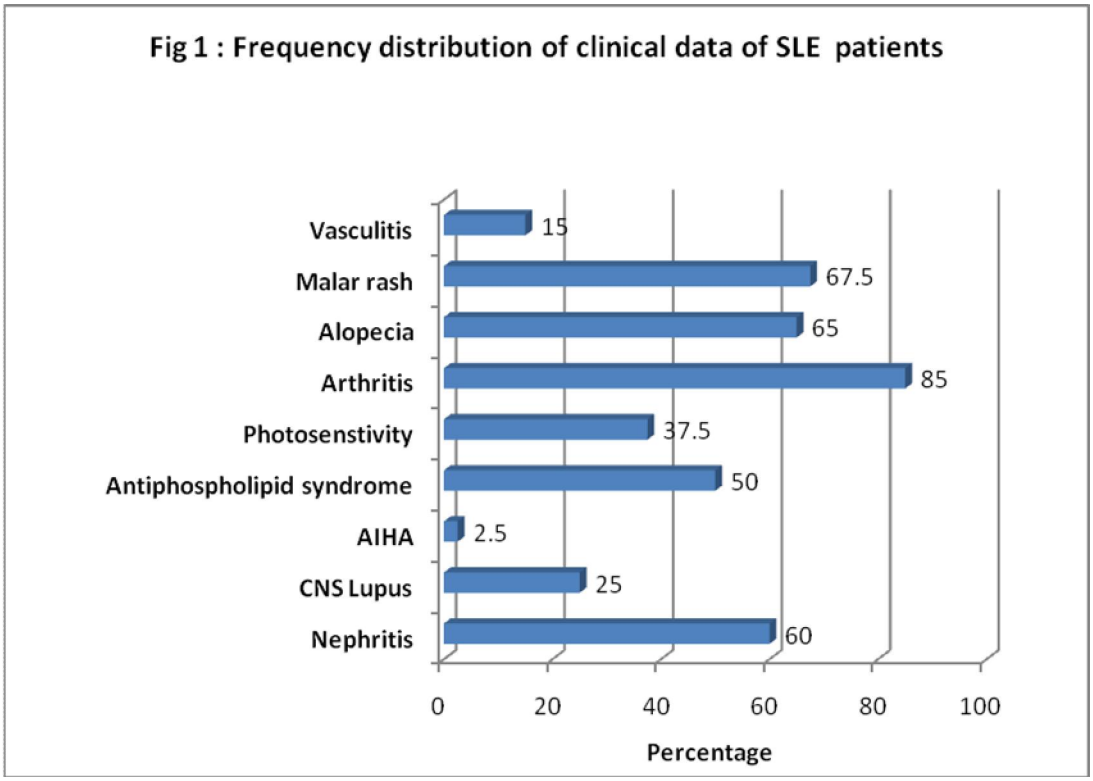


Table 2: Comparison between clinical data of SLE patients in relation to April gene expression

Variables	Negative expression		Positive expression		*P*-value
	N	%	N	%	
Nephritis:					
Negative	9	50	7	31.8	0.2
Positive	9	50	15	68.2	
CNS Lupus					
Negative	18	100	12	54.5	0.001
Positive	0	0	10	45.5	
AIHA:					
Negative	17	94.4	22	100	0.5
Positive	1	5.6	0	0	
Photosenstivity:					
Negative	7	38.9	18	81.8	0.007
Positive	11	61.1	4	18.2	
Arthritis					
Negative	3	16.7	3	13.6	0.6
Positive	15	83.3	19	86.4	
Alopecia:					
Negative	9	50	5	22.7	0.07
Positive	9	50	17	77.3	
Malar rash:					
Negative	6	33.3	7	31.8	0.6
Positive	12	66.7	15	68.2	
Vasculitis:					
Negative	13	72.2	21	92.2	0.05
Positive	5	27.8	1	4.5	

Table 3: Comparison between ANA pattern, andti DNA to April gene expression.

Variables	Negative expression		Positive expression		P-value
	N	%	N	%	
ANA Pattern					
1 homo	8	44.4	11	50	0.5
2 speck	4	22.2	4	18.2	
3 homo+rim	5	27.8	3	13.6	
4 rim	1	5.6	4	18.2	
Anti DNA:					
Negative	4	22.2	3	13.6	0.4
Positive	14	77.8	19	86.4	

Table 4: Comparison between laboratory data of SLE patients in relation to April gene expression

Variables	Negative expression		Positive expression		*P*-value
	Mean	Std. Deviation	Mean	Std. Deviation	
ACLIgG	14.51	±10.36	32.91	±17.00	**0.007**
ACLIgM	16.40	±5.89	20.38	±18.40	0.9
SLEDAI	10.00	±48.00	27.68	±10.98	0.07

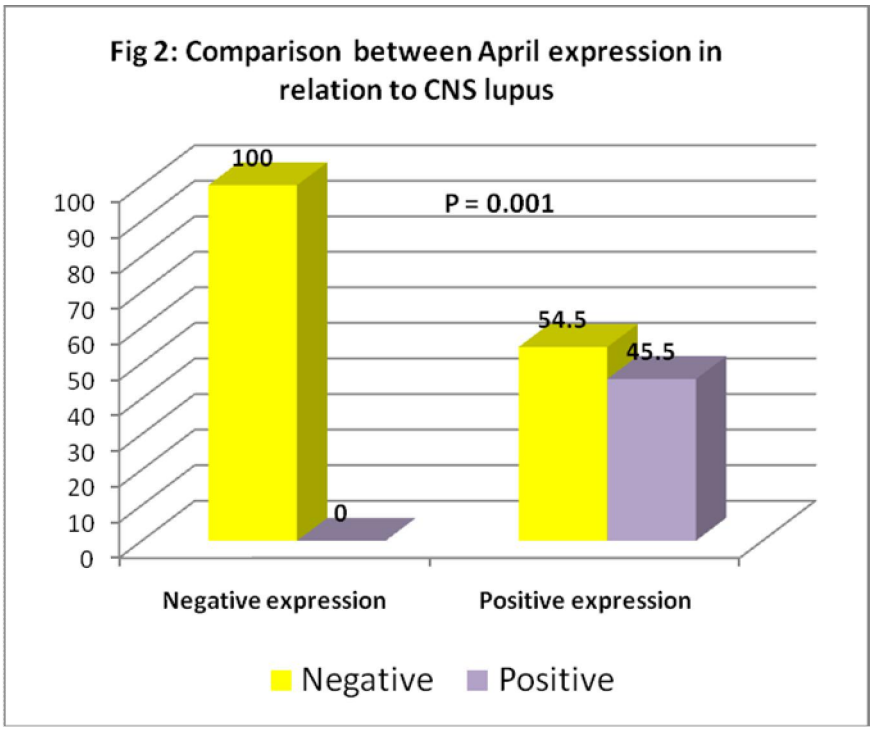
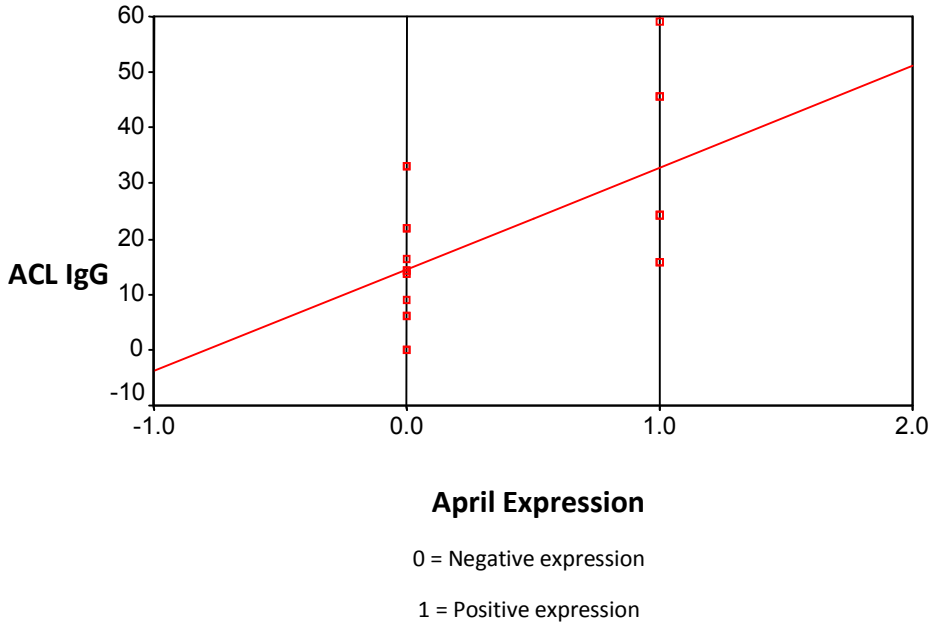


Fig 3:Correlation between ACL IgG and April expression r = 0.6, P = 0.005



4. Discussion

Systemic lupus is an autoimmune disease that has been linked to B cell hyperproliferation, generating large quantities of IgG autoantibodies and forming immune complexes [(3)]. This ultimately leads to lupus complications, such asnephritis, cerebritis, and vasculitis.

Furthermore, APRIL comprises one of the subfamily of tumour necrosis factor (TNF)-like ligands that are implicated in regulationof B cell development and immune responses. They are also associated with SLE pathology and its treatment[(4)].

Our study showed APRIL gene expression in 55% of SLE patients, while 45% were non expressers. All of our control groups were non expressers.APRIL gene results have been quite controversial in the literature. In fact there are conflicting data to the effects of TNF alpha as a proinflammatory and immunomodulatory regulator.

Morel *et al.*[(6)] found serum APRIL levels in 53% of the sera tested of SLE patients. Koyama *et al.*[(5)] found elevated serum APRIL levels in the majority of patients with SLE and 42% had levels above 100 ng/ml.On the other hand Stohl *et al.*[(7)] found that only 5% of the SLE sera displayed elevated serum APRIL levels in the study. In fact, Stohl et al.[(8)]found a negative correlation between APRIL and SLE patients.

We can currently only speculate about the reason for these discrepancies; one explanation could be the differences between the groups of patients with SLE analyzed. A comparison of the different studies is not possible, however, given the lack of the clinical information in the studies mentioned.

We also found that APRIL gene expression also showed significant association with SLE patients presenting with neurological symptoms.

In support of our results, recentlyChandy *et al.* [(9)] showed that SLE patients had elevated levels of APRIL in CSF that were more than 20-fold higher than those of healthy controls. Separate analyses of SLE patients with and without CNS involvement revealed that neuropsychiatric SLE patients had enhanced levels of APRIL in CSF. Moreover, CSF levels of APRIL correlated strongly to CNS manifestations in SLE patients.

We found a statistically significant associationbetween APRIL gene expression andvasculitis, as well as photosensitivity in SLE patients.APRIL is implicated in regulation of B cell development and immune responses. It is also claimed to be associated with SLE pathology [(10)].

April mainly affects B1 cell activity, B-cell co-stimulation, plasma cell survival, ,humoral responses,Ig class switch, and enhanced B-cell antigen presenting cell (APC) activity. Recombinant APRIL stimulates B and T cell proliferation. *APRIL-*

transgenic mice showed increase of T cell survival and B1 cell expansion [3, 4, and 10].

This could provide a plausible explanation to the significant role of APRIL gene expression in the pathogenesis of disease formation and organ affection in SLE.

On the other hand, there was no statistically significant relation between APRIL gene expression in SLE patients with arthritis, alopecia, oral ulcers, fever, or hepatomegaly.

As regards nephritis, APRIL gene expression was present in 15 patients with nephritis and not expressed in 7 of them, yet this did not reach statistical significance. There was no statistically significant correlation between the expression of the APRIL gene and parameters of renal affection, including serum creatinine level and 24 hrs protein in urine.The small number of patients may have affected the statistical analysis.Furthermore, Morel *et al.*[6] found high serum APRIL levels tended to associate with a lack of renal involvement in patients with SLE.

Regarding the SLEDAI, a higher mean value of 27.68 was found among positive expressers of the APRIL gene, yet it did not reach statistical significance (p=0.07). Our findings could be explained that SLEDAI scoring system concentrates on assessing the whole sum of manifestations, both new and recurrent, yet not separate complications nor organ affection. April gene involvement, on the other hand, could be correlated to organ affection and complications.

There was no statistically significant correlation between the expression of the APRIL gene and the presence of anti- ds DNA at the time of the study.

Available data regarding this issue are contradictory. Morel *et al.*[6], found in a study of 43 SLE patients, APRIL levels to be elevated in patients with SLE compared to patients with osteoarthritis and healthy controls, but did not find a significant correlation with the SLE Disease Activity Index (SLEDAI). For patients with SLE with positive anti-dsDNA titer, Morel *et al.* found circulating APRIL was inversely correlated with anti-dsDNA antibody titers.Furthermore, Stohl*et al.*[7]described a modest inverse association of APRIL levels and disease activity in sixty eight patients with SLE. Serum APRIL levels modestly, but significantly, inversely correlated with serum anti-dsDNA titers in anti-dsDNA positive patients analyzed in aggregate.. He suggested that APRIL may serve as a downmodulator of serological and/or clinical autoimmunity in patients with SLE.

Conversely, in a study by Koyama *et al.*[5], serum APRIL was found to be significantly higher in systemic lupus disease patients when compared to healthy controls as well as osteoarthritispatients. They also found a tendency for APRIL levels to correlate with DNA titers as well as to musculoskeletal manifestations.

On the other hand, Vallerskog *et al.*[11] found no significant difference in circulating APRIL between patients with SLE and controls.

Interestingly, we found a significant positivecorrelation betweenAPRIL gene expression and ACL IgG. April causes promotion of plasmablast and plasma cell survival. It also causeshumoral responses andIg class switch. APRIL cooperates with interleukin - 4 to induce class switch DNA recombination from IgM to IgG [12].This could explain its correlation with the ACL Ig G in our study. Hence APRIL gene expression could be a main cause in the propagation and formation of immunoglobulin and thus, the immune complex responsible for the systemic nature and organ affection of SLE.

Davidson *et al.*[13] proposedthat many circulating auto-antibodies derive fromplasma cells are at least partially dependent on signalsfrom APRIL. Furthermore,because patients with active SLE have high numbers ofcirculating plasmablasts, the same authors speculatethat neutralizing APRIL could rapidly depletethese cells during an acute flare [4].

TACI is a member of the TNF receptor superfamily and serves as a key regulator of B cell function. Furthermore, TACI binds to, APRIL, with highaffinity [14-17]

Therapeutic strategies have been suggested, ranging from the application of APRIL antagonists[16]to the use of soluble TACI receptors, also antagonizing APRIL. In fact, there is abundant optimism that neutralizingAPRIL will prove to be an effective therapyfor patients suffering from a variety of autoimmune diseasesand cancers.

Conclusion

April gene expression is significantly elevated in SLE patients with vasculitis, cerebritis, and photosensitivity. It has a strong positive correlation to ACL IgG levels. This could be explained by the active participation of APRIL gene in B cell activation, plasma cell maturation and immunoglobulin formation. These are key factors in the pathogenesis oforgan affection in SLE patients. Larger studies are further needed to confirm our findings. These results may be further implicated in the future therapy for SLE patients through blockade of APRIL gene expression and /or TACI receptor blockade[16, 17].

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