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Original Article

Correlation between Serum Levels of Soluble Fas (CD95/Apo-1) with Disease Activity in Systemic Lupus Erythematosus Patients in Khorasan, Iran

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Background: Soluble Fas (sFas) is a marker of apoptosis that appears to increase in the serum of systemic lupus erythematosus patients and may have a correlation with disease activity. The exact role of sFas in apoptosis is not clear. The purpose of this study is to assess the correlation between serum levels of soluble Fas (Apo/1-CD95) and the activity of systemic lupus erythematosus.

Patients and Methods: Our study was performed on 114 systemic lupus erythematosus patients who were compared with 50 randomly selected sex, age and race-matched healthy controls. Disease activity was defined according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2K). All physical exams and laboratory parameters were collected to determine the SLEDAI. sFas levels were determined using a commercially available ELISA kit.

Results: There was a significant difference between serum levels of sFas in the case and control groups (*P*=0.001). A significant correlation coefficient existed between the sFas and SLEDAI2K variables (*P*=0.001, r=0.494). Significant statistical difference was found between serum levels of sFas in the active and inactive phases of disease according to SLEDAI≤ 9 or ≥10, (*P*=0.002). The sFas levels were 270 – 300 pg/mL for SLEDAI≤9 and 355-502 pg/mL for SLEDAI≥10, with a confidence interval of 95%.

Conclusion: This study shows a significant elevation of sFas levels in the sera of systemic lupus erythematosus patients with active disease; therefore it can be used as an appropriate marker for evaluation of disease activity.

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Keywords: Disease Activity Index • SLEDAI • Systemic Lupus Erythematosus • Soluble Fas (CD95/Apo-1)

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of antinuclear antibodies (ANAs). Recent research on human and murine lupus suggests that disease susceptibility results from impairing the clearance of apoptotic cells, and increasing apoptosis as well as genetic polymorphisms regulating immune responses. Because the products of dead cells, including nucleic acids, have immunologic activity this situation can promote antigen driven ANA responses.[1]

Fas/APO-1 (CD95) belongs to the tumor necrosis factor/nerve growth factor super family. Fas and Fas ligand are essential factors in activated induced cell death, and any dysfunction in this system will lead to a breakdown in peripheral tolerance and induction of an autoimmune phenomenon.[2]

There are different hypotheses regarding the role of sFas in activated induced cell death and apoptosis. Some investigators report that during

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active apoptosis, released sFas may interact with FasL, inhibit cellular Fas/FasL interaction and therefore inhibit apoptosis, but others postulate that sFas is released to exert a pro-apoptogen effect. Proussakova and coworkers recently reported that sFas antigen can also protect cells against Fas-mediated apoptosis. sFas, generated by alternative splicing of the intact exon 6, is capable of inducing death in transformed cells by "reverse" apoptotic signaling via a transmembrane Fas ligand.[3] Other researchers showed that high levels of sFas in SLE patients correlate with leucopenia, tissue and organ damage. High serum concentrations of sFas are present in an oligomeric form, which demonstrate cytotoxicity in a lymphocyte primary culture and in transformed cells, while the non-toxic recombinant Fas-ligand partially blocks this effect.[4] On the other hand, most researchers agree with the anti-apoptotic effects of sFas.[5,6] It is known that defects in Fas mediated apoptosis in mice lead to autoimmune diseases such as SLE and the role of imbalances in apoptosis programs in the pathogenesis of SLE is an important hypothesis.[6–9] Some investigators have found a relationship between lupus activity and serum sFas levels, whereas others did not reach this conclusion.[5,10–12] Therefore, the current study has been designed to define the relationship between serum sFas levels and the lupus activity index, lupus laboratory tests, and to determine if there is a correlation between apoptosis markers, particularly sFas levels and SLE disease activity.

Patients and Methods

Patients

In this case control study, 114 SLE patients (105 women, 9 men), followed at the Rheumatology Department in Ghaem and Imam Reza Hospitals, Mashhad University of Medical Sciences, were enrolled from May 2006 to June 2007. The patients were at different stages of the disease and diagnosed according to the American College of Rheumatology criteria. Using the SLE Disease Activity Index (SLEDAI-2K) score,[13] patients were divided into two groups: active (58 patients) and inactive (56 patients).[14,15]

SLEDAI2K is a disease activity index for lupus patients consisting of 24 items such as: central nervous system, psychological and visual disturbances, kidney and skin involvement, fever, vasculitis, myositis, serositis, hematologic manifestations, and serologic findings such as the levels of anti-dsDNA antibodies and complements. Definitions of each item were provided on the form. Items that present ten days preceding the evaluation are noted, and scoring is calculated by the sum of the predetermined scores. For items that are life threatening, a higher score is noted. Possible scores with the SLEDAI2K system vary from 0 to 105.[13]

For each patient, a clinical data questionnaire and past medical histories were first completed. Excluded were all pregnant or postpartum patients, those with a past or concurrent history of malignancy, concurrent infection, smoking or addiction, overlapping syndromes, chronic renal failure, and other systemic problems not relevant to lupus such as a history of hepatitis or liver disease. Additionally, we excluded all patients with creatinine levels greater than 2 mg/dL or those who were on dialysis since a study has shown an increase in sFas serum levels during chronic renal failure and its negative correlation with creatinine clearance.[16]

For the control group, 50 healthy age, sex, and race-matched volunteer nurses and medical students (46 women and 4 men) were selected after a health assessment for their present and past medical histories and concomitant medications. Volunteers with any health problems were excluded from our study. This study was approved by the Ethics Committee of Mashhad University of Medical Science. All participants signed a written consent prior to their enrollment in the study.

Methods

Appropriate samples were collected for SLEDAI related laboratory tests. Briefly, 10 mL samples of venous blood were obtained and used for a complete blood count, determination of ANA (immunoflorescence) , anti-dsDNA (ELISA), C3, and C4 (nephelometry),

C-reactive protein (CRP**)** (Latex agglutination) and ESR (1st hour). A random urinalysis and measurement of 24-hour urine protein collection were also performed. Aliquots of the serum samples were stored at -70°C for analysis of sFas. All samples were numbered and the laboratory personnel were blinded to case and control samples. sFas levels were determined using an sAPO-1/Fas BMS245 ELISA kit (Bender Medsystems^{TM}, Austria) according to the manufacturer's instructions. The coefficient of

variation (CV): intra-assay precision/inter-assay precision for this test was 4.5/3.1%.

Statistical analysis

Results were expressed as the mean value of data±2SD. A *P* value less than 0.05 was considered significant. In order to determine the normal distribution of quantitative parameters such as: age, C3 and C4, dsDNA, sFas levels, SLEDAI and proteinuria, the Kolomogrove-Smirnov test was used. For a *P* value<0.05, nonparametric tests such as the Mann Whitney and Kraskal Wallis were used. In normal distributed parameters, the *t*-student test was the test of choice, and for non quantitative variables the Chi-square test was used. Since sFas serum level distribution was not normal, the Spearman coefficient of correlation was used for analysis.

Results

Demographics

This study was performed on 164 individuals (114 patients and 50 healthy controls). The mean age of patients was 30.7±9.55 years, the youngest was 13 and the oldest was 62. The mean age of controls was 29.5±7.21 years, the youngest was 20 and the oldest was 50. Nine patients and four controls were men. The mean duration of disease among the patients was four years. There was no significant difference between age (*P*=0.8) and sex (*P*=1) in case and control groups. Patients were at different stages of disease activity with or without major organ involvement.

Overall laboratory parameters

Table 1 summarizes important laboratory findings such as: sFas levels, anti-ds DNA, C3, C4, and serum creatinine for total patients as well as active and inactive groups. C3 and C4 normal ranges are 70 – 170 mg/dL and 15 – 55 mg/dL, respectively. Thirty three patients had a new onset proteinuria or an increase in the range of proteinuria greater than 0.5 gr/24h (0.5 – 10 gr/24 h), the mean value was 1.814±1.8 gr/24h. Leucopenia was seen in 12 patients (10.5%), 37 (32.5%) had lymphopenia, and 13 (11.4%) had thrombocytopenia. Five patients (4%) had active myositis. Creatine phosphokinase and aldolase levels in these five patients were two times greater than the upper limit of the normal range.

sFas and SLEDAI

We compared sFas serum levels in the healthy group (190.38±127.77 pg/mL) and the SLE patients (372.20±228.35 pg/mL) and found a significant difference between the levels of these two groups (*P*=0.001) (Figure 1). There was no correlation between age and sFas level in the patient group (*P*=0.13).

The mean SLEDAI for our patients was 11.97±10.078. Patients were divided into two groups according to their SLEDAI; the non-active group (SLEDAI≤9) and the active group (SLEDAI≥10). The mean sFas serum levels in the active and inactive groups were found to be significantly different (*P*=0.001) (Table 1). The 95% confidence interval for disease activity in SLEDAI≤9 was 207 – 303 pg/mL. This range was 355 – 502 pg/mL for SLEDAI ≥10 (Figure 2).

When patients were divided into low (SLEDAI≤9; 58 patients), medium (SLEDAI≥10 to ≤30; 50 patients) and high (SLEDAI>30; 6 patients) activity groups; the sFas mean level differed significantly in these three groups (*P*=0.001; Kruskal-Wallis test).

Next, we evaluated the correlation between sFas serum levels and SLEDAI score as a quantitative variable. There was a significant correlation between the two variables (*P*=0.001, r=0.494) (Figure 3).

The mean sFas in the control group was 200 pg/mL, which was close to the reported ranges of other references.[12,14] In the SLEDAI≤9 group, 40%, and in the SLEDAI≥10 group, 60% of the patients had increased sFas levels of greater than

Table 1. Comparison of important laboratory parameters in active and inactive groups of SLE patients according to SLEDAI2K

Laboratory parameters	Patient group Mean±2SD	Range	SLEDAI<9 group Mean±2SD	SLEDAI≥10 group Mean±2SD	*P*-value*
sFas (pg/mL)	372.20 ±228.35	1–3320	254.82±182.61	493.88±525.57	0.001
anti dsDNA(R**)	4.01± 4.6	1–15.7	3.07±4.5	4.8±4.3	0.002
C3 (mg/dL)	68.64 ±42.78	5–173	84.67±25	51.73±38	0.0001
C4 (mg/dL)	21.15 ±11.6	4–51	25.22±10	16.93±11	0.0001
Creatinine (mg/dL)	0.79± 0.25	0.4–1.5	0.76±0.22	0.83±0.29	0.2

**P* values represent the statistical difference between two groups (SLEDAI<9 and SLEDAI≥10) based on Mann-Witney test. **R is the ratio of the level of anti dsDNA in each patient to the upper limit of the normal range

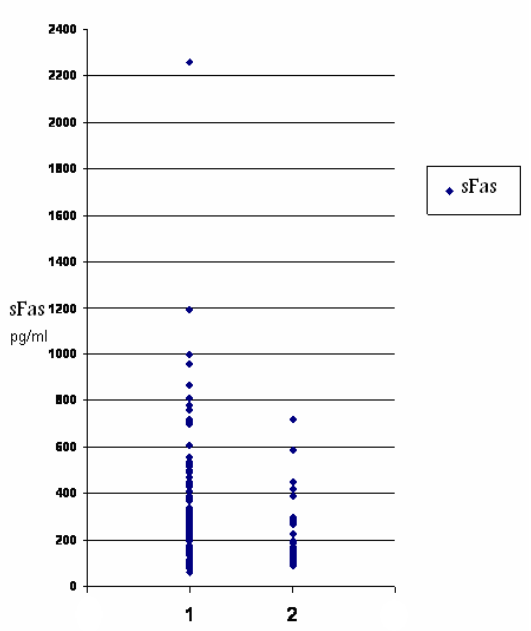

Figure 1. The distribution of soluble FAS level between patients and controls (1=patients, 2=controls)

200 pg/mL.

sFas and other laboratory parameters

The correlation coefficient between sFas and increased 1st hour ESR, was calculated (*P*=0.003, r=0.29). CRP was negative in 63% of the SLE patients and positive in 37%. The sFas serum level was significantly different in CRP positive and CRP negative patients (*P*=0.027). There was no significant correlation between sFas and C3 (*P*=0.511, r=0.06) and sFas and C4 (*P*=0.582, r=0.05). Also, sFas levels were not significantly different in patients with normal and decreased C3 (*P*=0.3102) or C4 (*P*=0.9565). We did not find any significant correlation between serum levels of creatinine and sFas (*P*=0.13). There was no

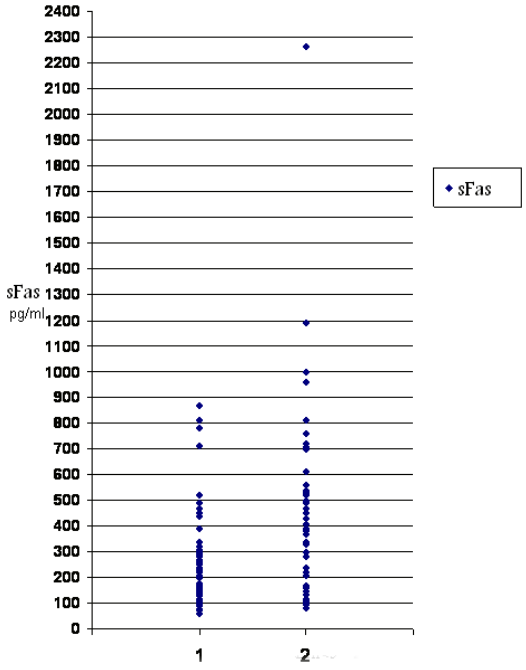

Figure 2. The distribution of soluble FAS in active and inactive lupus patients (1=inactive with SLEDAI<9, 2=active with SLEDAI≥10)

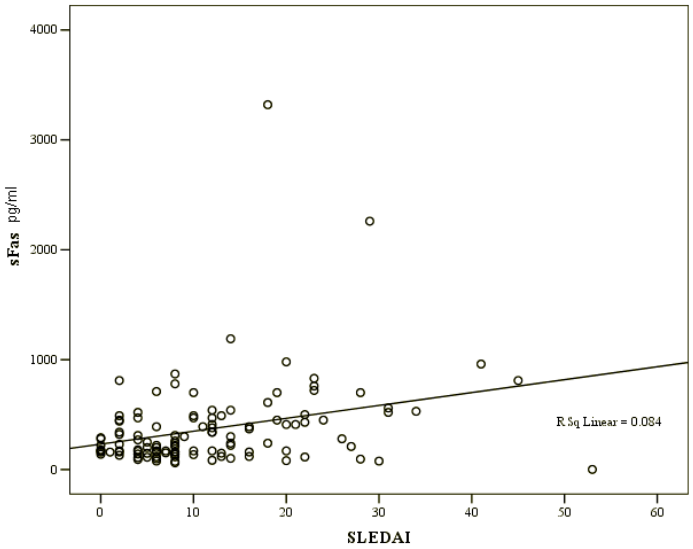

Figure 3. The regression line of correlation between soluble FAS and SLEDAI

significant correlation between sFas serum levels and serum anti ds-DNA, hematuria, sterile pyuria, lymphopenia, and thrombocytopenia in the patient group.

Major organ involvement

We evaluated the correlation between sFas serum levels and major organ involvement (according to SLEDAI score) at the time of the study, which has been summarized in Table 2.

sFas and drug history

Data for medication used was obtained for prednisolone according to the dose of the drug, and for cytotoxic drugs, according to the type of medication used. Among 114 patients, 82.5% were on prednisolone. In order to evaluate the correlation between sFas serum levels and the prednisolone dosage, we classified the patients into three groups; prednisolone doses less than 15 mg (71%), 16 to 60 mg (26%) and more than 60 mg (2.6%). The correlations between sFas levels and these three groups were not significant (P=0.16). Of all patients, 41% were under immunosuppressive therapy with the following medications: cyclophosphamide (14%), methotrexate (12%), azathioprine (10.5%), and mycofenalate mofetil (0.9%). The differences between the serum levels of sFas in the patients not using and using any type of cytotoxic medication was not significant (P=0.800).

Discussion

The role of sFas mediated apoptosis in immunity and elimination of autoreactive lymphocytes is clear,[17] but the function of sFas as a marker of apoptosis in autoimmune diseases, especially SLE is under investigation.[18–20] The

Table 2. Statistical significance of sFas levels in SLE patients with or without major organ involvement

Organ involvement	Number	Subtypes of each organ involvement	*P*-value*
Proteinuria**	33 (28.29%)	Proteinuria	0.04
Heart	8 (7%)	Pericarditis=7(6.1%)	0.05
		Pancarditis=1 (0.9%)	
CNS	21 (23.7%)	Seizure=10 (13.9%)	0.006
		Psychosis=5 (4.4%)	
		CVA=2 (1.8%)	
		Vasculitis=2 (1.8%)	
		Myasthenia gravis=2 (1.8%)	

*P values represent the statistical difference between two groups with and without organ involvement based on Mann-Witney test. **Renal involvement was found in 49 patients (based on presence of hematuria, sterile pyuria or proteinuria). Only patients with proteinuria were included in the analysis

main goal of this study was to determine the correlation between serum levels of sFas and disease activity according to SLEDAI. In the first step, we had to determine whether there was a significant difference in the serum levels of sFas between patients and healthy controls. To reach this purpose, a case control study was designed and a significant statistical difference between cases and controls was established. The mean sFas serum levels were 190.38±127.77 pg/mL and 372.20±228.35 pg/mL in controls and patients, respectively. The mean values of serum sFas levels in healthy individuals reported by different investigators were as follows: 220 pg/mL on 40 blood samples,[14] 260 pg/mL on 22 samples,[12] 618 pg/mL on 20 samples,[21] and 2353.4 pg/mL on 15 samples.[15] Different values that were reported for mean sFas serum levels might reflect both a difference in sample sizes and racial differences. Despite controversies on this issue, the normal value has been reported from 200 to 2000 pg/mL in a more extensive study by Cheng et al.[5,22] Therefore, this range possibly differs with various populations and defining an accurate range for normal sFas serum level in each geographical region would seem necessary in order to perform an accurate study.

This study showed a significant difference in the serum level of sFas between the patient and healthy groups which confirmed the data as reported by Jodo et al.,[14] Al-Maini et al.,[12] Courteny et al.,[21] and Bijl et al.[23]

We also suggest that sFas serum levels are a likely marker of the disease activity index. This has confirmed previous studies performed by Jodo et al.[14] and Bijl et al.[23] but contradicted data reported by Hao et al.,[15] Alecu,[24] and Al-Maini et al.[12] In a study by Courteny et al., the correlation between sFas and SLEDAI was reported significant only during the relapse phase but not for the entire course of the disease.[21] It is obvious that the SLE disease activity score system could be affected by different factors. The scores evaluate the patients' status for a limited time and thus these controversies might have originated from the low number of patients, medications, and duration of activity prior to sampling.

The coefficient of correlation between sFas and SLEDAI was statistically significant. There was no significant difference between sFas serum levels in males and females of both the patient and control groups. Although SLE patients have a particular age range; in this study we did not find any correlation between age and sFas serum levels in SLE patients.

Although we did not evaluate the SLE damage index, however based on the SLEDAI questionnaire we found a significant correlation between sFas serum levels and major CNS involvement. Additionally, we found a significant difference between the sFas levels in patients with and without active proteinuria. In a study by Hao et al., a correlation existed between renal involvement and sFas levels.[15]

No correlation was found between the serum levels of sFas and the use or dosage of prednisolone and single or combination immunosuppressive therapy. These results supported a Sahin et al.,[18] study that reported no significant difference between serum sFas levels in patients on glucocorticoid, immunosuppressive or immunomodulatory therapies that were diagnosed with different autoimmune diseases and controls, who were not treated with these drugs. Although, this may be due to the small number of patients who did not use prednisolone in our study, it also could be a reflection of sampling bias. These drugs may decrease the serum levels of sFas via apoptosis modulation before decreasing disease activity in patients prior to sampling. Based on these preliminary results, further investigations should be undertaken to discover if sFas levels can be used as an independent parameter in determination of disease activity regardless if the patient is under treatment or not.

sFas levels did not correlate with hematological abnormalities, anti dsDNA, and C3 and C4 levels, but did show a correlation with ESR and positive CRP. In a study by Alecu et al., a weak correlation has been found between anti dsDNA and sFas.[24] An Al-Maini et al.[12] study found no correlation between sFas and acute phase reactants while Dalboni et al.,[16] reported a significant correlation between sFas serum levels and serum CRP levels in uremic patients. These variations may be related to the differences in factors that make a high activity score, type of organ involvement and medications used prior to sampling.

Although several studies have been published concerning sFas and its role in apoptosis [1,3,4,6,25,26]; as discussed earlier, the details of this mechanism needs more investigation.

In summary, we have shown that sFas serum levels are higher in lupus patients when compared

to a normal age and sex matched control group, and these levels are also higher in active lupus patients when compared with less active patients. We have shown a correlation between serum levels of sFas, disease activity, and acute phase reactants (ESR, CRP). However, the association between these factors remains to be studied.

Further studies are needed to determine if sFas elevation is an enhancing factor in apoptosis or if sFas works as a suppressor factor that is elevated secondary to active apoptosis. Determination of sFas levels in new cases prior to treatment with prednisolone and immunosuppressive therapy would be a valuable study. These patients may be followed for sFas level assessment after medical therapy. Ultimately, determining the correlation between sFas and other apoptotic factors may reveal important aspects of apoptosis in lupus activity.

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Cuneiform script and a bas-relief of a lance-bearing soldier, Persepolis—Tachara Palace, southern view, ca. 500 BCE, Shiraz, Iran (Photo by M. H. Azizi MD)