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ORIGINAL ARTICLE

Tumor Necrosis Factor-Related Apoptosis Inducing Ligand in Systemic Lupus Erythematosus Patients; Potential Role in Disease Activity, Neutropenia and Renal Impairment

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Key words:
SLE; TRAIL; neutropenia and renal impairment

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ABSTRACT

Background: *The role of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in immunopathogenesis of systemic lupus erythematosus (SLE) was previously documented. Neutropenia and nephritis are common features in SLE patients and have been hypothesized to be due to accelerated apoptosis induced by binding of death receptor ligands like TRAIL to their cognate receptors on sensitive cells.* ***Objectives:*** *The aim of this study was to determine the serum concentration of soluble TRAIL (sTRAIL), the expression level of TRAIL mRNA in the peripheral blood mononuclear cells (PBMC) and expression level of TRAIL receptor-1 (TRAIL-R1, death receptor-4/DR-4/CD261) on polymorphonuclear leucocytes and to clarify their relation with disease activity, neutropenia and renal impairment in SLE patients.* ***Methodology:*** *The study enrolled 25 patients with active SLE, 25 patients with mild or no disease activity, 25 patients with Rheumatoid arthritis and 15 age and gender-matched healthy volunteers as a control group. Serum level of circulating TRAIL was measured by ELISA, the expression level of TRAIL mRNA on PBMC was determined by Quantitative Real-Time reverse transcription-polymerase chain reaction and flow cytometry was applied to evaluate the expression level of TRAIL R1 on polymorphonuclear leucocytes among the study population.* ***Results:*** *Serum level of sTRAIL was significantly (P<0.001) higher in patients with active SLE than those with no activity, Rheumatoid arthritis and healthy controls. Up-regulation of TRAIL mRNA expression in the PBMN cells and TRAIL R1 expression on neutrophils was detected in active lupus patients with a statistically significant difference (P<0.001) compared to other participants. A statistically significant correlation was detected between sTRAIL, TRAIL mRNA and TRAIL R1 expression and SLE activity, neutropenia and nephritis (P<0.001).The sensitivity, specificity, PPV, NPV and accuracy of TRAIL mRNA were 80%, 60%, 66.7%, 75% and 70% respectively.* ***Conclusion:*** *Concentration of circulating TRAIL and TRAIL mRNA levels could be potential markers for SLE activity assessment and predictors of lupus-associated neutropenia and renal affection.*

INTRODUCTION

Systemic lupus erythematosus is an autoimmune disease characterized by wide range of clinical, haematological and immunological abnormalities affecting various organs involving the skin, central and peripheral nervous system, joints, kidneys and the liver[1].

The etiology of the disease remains by far unknown. There is, however, increasing evidence that, the presence and accumulation of apoptotic cells debris contributes to autoimmunity and that the appearance of different groups of autoantibodies, particularly those directed against double stranded DNA, is characteristic of SLE. The disease has variable course, with frequent relapses and remissions [2].

It is recently suggested that, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is involved in the pathogenesis of SLE. TRAIL is a type II transmembrane protein that exhibits sequence homology to TNF-α and Fas ligand (Fas L). It induces apoptosis in

a variety of transformed cells and has been documented to be involved in monocytes apoptosis induced by T-cells in SLE as well. TRAIL modulates the apoptotic response by binding to its specific receptors on the target cells [3].

TRAIL has the ability to interact with 2 death receptors (DRs) namely; DR4 (TRAIL receptor 1) and DR5 (TRAIL receptor 2) and 2 decoy receptors (DcRs) namely; DcR1 (TRAIL receptor 3) and DcR2 (TRAIL receptor 4). TRAIL receptors 1 and 2 possess an intracellular motif called the “death domain” that activates caspase-8, and the caspase cascade subsequently stimulates apoptosis. In contrast, TRAIL receptors 3 and 4 have either truncated or missing intracellular domains and are therefore unable to transduce the death signal. That is why decoy receptors are proposed to compete for ligand-binding and considered as antiapoptotic receptors [4].

Lupus nephritis is a characteristic feature of SLE and one of the most important prognostic factors. The expression of TRAIL receptors is altered in patients with proliferative lupus nephritis. TRAIL/TRAIL-R interaction is also modulated in lupus patients with renal impairment [5].

Neutropenia is a common laboratory finding in SLE and continues to complicate the course of 50%-60% of all lupus patients. Clinically, the reduced count of neutrophils contributes to increased susceptibility to infections which is a major cause of morbidity and mortality in SLE [6]. The molecular mechanism of SLE neutropenia has not been fully explained. Many theories suggest that, death ligands like TRAIL are able to shorten neutrophils lifespan at early time points. Recently, it was reported that TRAIL could potentially accelerate neutrophils apoptosis [4].

Since most of the researches reported the importance of TRAIL in the pathogenesis of SLE, the current study aimed to determine the expression level of TRAIL mRNA in the PBMC, serum concentrations of sTRAIL and expression level of TRAIL R1 on polymorphonuclear leucocytes and to assess their potential relationship with disease activity, neutropenia as well as renal impairment in SLE patients.

METHODOLOGY

Study Population

The study was conducted at Microbiology and Immunology Department in collaboration with Internal Medicine Department, Faculty of Medicine, Menoufia University, Egypt during the period from November 2016 to May 2017 enrolling 50 patients with SLE fulfilling Systemic Lupus International Collaborating Clinics (SLICC) criteria [7], 25 patients with Rheumatoid arthritis fulfilling the classification criteria of the disease [8] and 15 age and sex-matched healthy volunteers as a control group. Patients with SLE were classified into two groups; group I involved 25 patients (male to female, 2:23 with a mean age of 32.2±6.8) with active disease and group II involved 25 patients (male to female, 2:23 with a mean age of 35.0±8.4) with mild or no disease activity.SLE activity was assessed by systemic lupus disease activity index (SLEDAI)[9]. Written informed consents were obtained from all participants and the study protocol was approved by the Ethical Committee of Faculty of Medicine.

Methods:

All patients and controls included in the study were subjected to full history taking and physical examination. Laboratory data regarding serum levels of anti-double-stranded DNA antibody (anti-dsDNA) titers, anti-nuclear antibody (ANA) titres, complement components C3 and C4, hemoglobin level, ESR, peripheral blood leukocytic count, neutrophils and platelets counts, serum urea and creatinine levels were obtained from patient’s files. Blood samples were collected from all participants and then, sera were stored at -20˚C until assayed for estimation of serum level of sTRAIL, TRAIL mRNA expression level in the PBMC and TRAIL R1 expression on neutrophils as follows:

Estimation of serum level of soluble TRAIL by ELISA

Boster’s Human TRAIL ELISA kit (USA) is based on standard sandwich enzyme-linked immune-sorbent assay technology[10]. A monoclonal antibody from mouse specific for TRAIL has been precoated onto 96-wells of the microtitre plates. Serum samples were stored at -20˚C until assayed for sTRAIL concentrations. Linear calibration curves were made using standard TRAIL (16.5–1000 pg/ml), provided with the kit. The amount of sTRAIL in the serum samples was determined by extrapolating OD values to TRAIL concentrations using the calibration curves.

Estimation of TRAIL mRNA expression in PBMC by Quantitative Real-Time reverse transcription-polymerase chain reaction (RT-PCR)

TRAIL mRNA expression levels in PBMC samples from all participants were quantitatively estimated by RT-PCR. Total RNA was obtained by the RNeasy mini kit (Qiagen, Germany) according to the manufacturer’s instructions and used for cDNA synthesis with an oligo (dT) primer [11]. Primers for TRAIL were:
Forward
5‘-CTGAAGCAGATGCAGGACAAGTAC-3’ and
reverse 5’-GAAATGGTTTCCTCAGAGGTTCTCA-3’.
Primers for GAPDH (internal control) were:
Forward 5'-GAAGGTGAAGGTCGGAGTC-3'' and
reverse 5’-GAAGATGGTGATGGGATTTC-3.

RT-PCR reaction was performed in a final volume of 25µl containing; 12.5µl QuantiTect Probe PCR Master Mix, 0.5 µl of TRAIL TaqMan probe (Qiagen,

Germany), 1 µl of forward and 1 µl of reverse primers, 5 µl of cDNA, and 5µl of water, according to the manufacturer's instruction. The PCR assay involved an initial activation step at 95°C for 15 minutes (Hot Star Taq DNA Polymerase is activated by this heating step) followed by 2nd step of denaturation at 94°C for 15 sec, then the final step involved combined annealing/ extension at 60°C for 60 sec. A total of 45 cycles were performed using a Light Cycler (7500 Fast Real time PCR system, Germany). Quantitative real-time RT-PCR curves were analyzed by Light Cycler 3.5 software (Roche Diagnostics).

Estimation of TRAIL- R1 (DR-4/CD261) on Polymorphonuclear Leucocytes by Flowcytometry

For each sample two tubes were prepared; the first one for simultaneous detection of CD65 and CD261 expression using FITC- labeled anti CD65 Ab and PE-labeled anti CD261 Ab (BioscieceInc. SanDiego CA, USA). The second tube: auto control was used to exclude the cell auto-fluorescence or instrument noise. 5µl of monoclonal antihuman CD65Ab + 5µl of monoclonal antihuman CD261 Ab was added to 100µl of the peripheral blood, mix well, and incubated at 2-8 for 30 minutes in the dark. Then the cells were lysed by 3ml of lysing reagent for 3 min. The cells were washed 3 times in 2ml of PBS. Finally the cells were suspended in 200 µl of PBS for final flowcytometry analysis. Data were acquired on a FACS caliber flow cytometer (Becton Dickinson immune Cytometry systems, San Jose, CA) [12].

RESULTS

Demographic and clinical characteristics of the study population are shown in table (1). The incidence of SLE was significantly higher in females than males especially those with positive family history (P=0.02). The clinical features of SLE including fever, malar rash, ocular ulcers, arthritis, serositis, nephritis and neuropsychiatric manifestations were significantly prominent in active lupus patients compared to other groups (P<0.001).

Table 1: Demographic and clinical characteristics of the studied population

Socio-demographic and clinical characteristics	Group I (No.=25)		Group II (No.=25)		Group III (No.=25)		Group IV (No.=15)		Test of significance	P value Post hoc test
Age (years) Mean±SD Range	32.2±6.8 17 – 45		35.0±8.4 25 – 55		38.4±8.2 22 – 54		37.7±5.2 29 – 44		**ANOVA test**=3.33 P=0.02*	I versus III P=0.004* I versus IV P3=0.03*
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**χ^2 test**	**P value**
Gender Male Female	 2 23	 8.0 92.0	 2 23	 8.0 92.0	 2 23	 8.0 92.0	 2 13	 13.3 86.7	6.46	0.09
Family history of SLE Positive Negative	 4 21	 16.0 84.0	 6 19	 24.0 76.0	 0 25	 0.0 100	 0 15	 0.0 100	9.81	0.02*
Fever Positive Negative	 10 15	 40.0 60.0	 5 20	 20.0 80.0	 0 25	 0.0 100	 0 15	 0.0 100	18.0	<0.001**
Malar rash Positive Negative	 13 12	 52.0 48.0	 3 22	 12.0 88.0	 0 25	 0.0 100	 0 15	 0.0 100	29.25	<0.001**
Oral ulcer Positive Negative	 9 16	 36.0 64.0	 4 21	 16.0 84.0	 0 25	 0.0 100	 0 15	 0.0 100	16.20	0.001**
Alopecia Positive Negative	 3 22	 12.0 88.0	 1 24	 4.0 96.0	 0 25	 0.0 100	 0 15	 0.0 100	5.23	0.16
Arthritis Positive Negative	 6 19	 24.0 76.0	 7 18	 28.0 72.0	 0 25	 0.0 100	 0 15	 0.0 100	12.32	0.006*
Serositis Positive Negative	 7 18	 28.0 72.0	 0 25	 0.0 100	 0 25	 0.0 100	 0 15	 0.0 100	19.74	<0.001**
Nephritis Positive Negative	 12 13	 48.0 52.0	 1 24	 4.0 96.0	 0 25	 0.0 100	 0 15	 0.0 100	31.74	<0.001**
Neuropsychiatric Positive Negative	 6 19	 24.0 76.0	 2 23	 8.0 92.0	 0 25	 0.0 100	 0 15	 0.0 100	10.98	0.01*
Duration of treatment (month) Mean±SD Range	26.7±10.9 8 - 48		30.3±10.8 18 - 54		-		-		**Mann Whitney test**=0.89	0.37
Infection Positive – Pneumonia - UTI - GIT Negative	 3 1 0 21	 12.0 4.0 0.0 84.0	 0 1 2 22	 0.0 4.0 8.0 88.0	-	-	-	-	**χ^2 test** = 5.02	0.17
Mortality Death - Renal failure - Pneumonia Negative	 1 1 23	 4.0 4.0 92.0	 0 0 25	 0.0 0.0 100	-	-	-	-	**χ^2 test** =2.08	0.35

Routine and specific laboratory data are shown in table (2). The haemoglobin level, total leukocytic count, serum urea and creatinine, anti-dsDNA and ANA titres were higher in active lupus patients than those in other groups with a statistically significantly difference (P<0.001, P<0.001, P=0.002, P<0.001, P<0.001 and P<0.001 respectively). On the other hand, C4 and C3 levels were significantly lower in active lupus cases (P=0.03 and P<0.001).

Table 2. Routine and specific laboratory findings of the studied population

Laboratory Parameters	Group I	Group II	Group III	Group IV	Kruskal Wallis Test and P value	Post hoc test
Hb (gm/dl) Mean±SD Range	8.1±0.93 6.5 – 9.9	10.1±0.78 8.8 – 11.7	11.3±1.3 9.5 – 14.9	12.0±1.1 10.8 – 14.4	Test=58.51# P<0.001**	P1<0.001** P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6=0.02*
ESR1st hour Mean±SD Range	113.2±12.6 90 – 135	106.8±9.9 90 – 120	27.8±11.6 3 – 55	11.2±6.1 6 – 25	Test=71.57 P<0.001**	P1=0.06 P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6<0.001**
Leucocytes (cell/cmm×10^3) Mean±SD Range	2.8±0.80 1.9 – 4	6.3±2.3 3.3 – 11	7.3±1.6 4.5 – 9.9	8.1±1.5 5.9 – 10.7	Test=56.04 P<0.001**	P1<0.001** P2<0.001** P3<0.001** P4=0.07 P5=0.008* P6=0.11
Neutrophils (%) Mean±SD Range	41.0±13.4 25 – 76	47.9±14.9 28 – 77	60.0±10.7 40 – 85	56.6±10.9 40 – 74	Test=10.67# P<0.001**	P1=0.06 P2<0.001** P3<0.001** P4=0.001** P5=0.04* P6=0.41**
Urea (mg/dl) Mean±SD Range	57.4±20.7 28 – 98	32.0±9.1 15 – 48	27.0±10.7 12 – 65	24.7±5.9 15 – 33	Test=44.56 P<0.001**	P1=0.002* P2<0.001** P3<0.001** P4=0.04* P5=0.01* P6=0.47
Creatinine (mg/dl) Mean±SD Range	1.7±0.72 0.7 – 3.2	0.96±0.24 0.6 – 1.6	0.97±0.29 0.6 – 2.2	0.81±0.17 0.6 – 1.1	Test=20.89 P<0.001**	P1=0.002* P2=0.002* P3<0.001** P4=0.96 P5=0.04* P6=0.04*
Complement C4 (mg/dl) Mean±SD Range	3.9±1.7 2 – 8	6.2±2.2 2.5 – 9.5	21.6±5.3 11 - 32	20.7±3.6 15 – 28	Test=162.54# P<0.001**	P1=0.03* P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6=0.41
Complement C3 (mg/dl) Mean±SD Range	28.9±6.6 17 – 39	57.8±25.8 23 – 90	124.4±17.3 90 – 160	124.0±20.2 99 – 170	Test=69.96 P<0.001**	P1<0.001** P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6=0.43
Anti-dsDNA (IU/ml) Mean±SD Range	479.4±218.7 230 – 970	137.1±116.9 32 – 450	20.6±4.1 12 – 28	14.6±5.1 4 – 23	Test=77.20 P<0.001**	P1<0.001** P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6=0.001**
ANA (IU/ml) Mean±SD Range	109.9±33.5 65 – 175	41.2±18.5 14 – 94	49.1±19.6 14 – 84	5.6±1.9 1 – 8	Test=69.22 P<0.001**	P1<0.001** P2<0.001** P3<0.001** P4=0.12 P5<0.001** P6<0.001**

Group I— active systemic lupus erythromatosus (SLE) , Group II— inactive SLE, Group III— other autoimmune inflammatory disease , Group IV— controls
*#ANOVA (F) test *significant difference **highly significant difference*
P1—Group I versus II P2—Group I versus III P3—Group I versus IV
P4—Group II versus III P5—Group II versus IV P6—Group III versus IV

The concentration of circulating sTRAIL, expression level of TRAIL mRNA and TRAIL R1 were significantly elevated in active lupus group than other groups (P<0.001, P=0.002 and P=0.04 respectively) as shown in table (3). Also there was a significant positive correlation between sTRAIL concentration, expression level of TRAIL mRNA and TRAIL R1 and SLE SLEDAI activity indices (r=0.721; P<0.001, r=547; P<0.001 and r=0.436; P=0.002 respectively) (fig. 1). Flow cytometric analysis of TRAIL R1 expression is shown in fig.2.

Table 3: Comparison between the studied groups regarding sTRAIL concentration, TRAIL mRNA and TRAIL R1 expression levels

Studied parameters	Group I (No.=25)	Group II (No.=25)	Group III (No.=25)	Group IV (No.=15)	Kruskal Wallis Test and P value	Post hoc test
TRAIL mRNA						P1<0.001**
Mean±SD						P2<0.001**
Range	2.7±0.49	2.1±0.69	0.89±0.23	1.0±0.00	Test=77.73#	P3<0.001**
	1.8 – 3.7	0.9 – 3.8	0.5 – 1.4		P<0.001**	P4<0.001**
						P5<0.001**
						P6=0.47
Soluble TRAIL						P1=0.002*
(pg/ml)						P2<0.001**
Mean±SD						P3<0.001**
Range	437.5±138.5	325.0±109.7	67.2±30.4	39.6±21.8	Test=70.70	P4<0.001**
	260 – 780	170 – 640	20 – 120	22 – 100	P<0.001**	P5<0.001**
						P6=0.003*
TRAIL R1 (MU)						P1=0.04*
Mean±SD	51.2±22.1	39.9±13.5	29.9±9.5	23.1±8.1	Test=37.46	P2<0.001**
Range	28 – 118	26 – 85	16 – 55	11 – 45	P<0.001**	P3<0.001**
						P4=0.003*
						P5<0.001**
						P6=0.02*

#ANOVA (F) test **significant difference* ***highly significant difference*
P1—Group I versus group II *P2—Group I versus group III*
P3—Group I versus group IV *P4—Group II versus group III*
P5—Group II versus group IV *P6—Group III versus group IV*

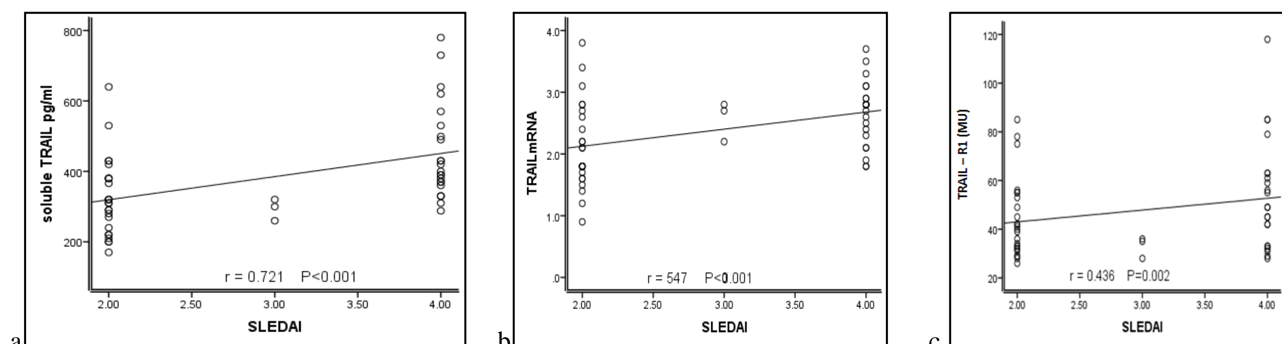

Fig. 1: Correlation between serum level of sTRAIL, TRAIL mRNA and TRAIL R1 expression levels and SLE activity indices (SLEDAI)

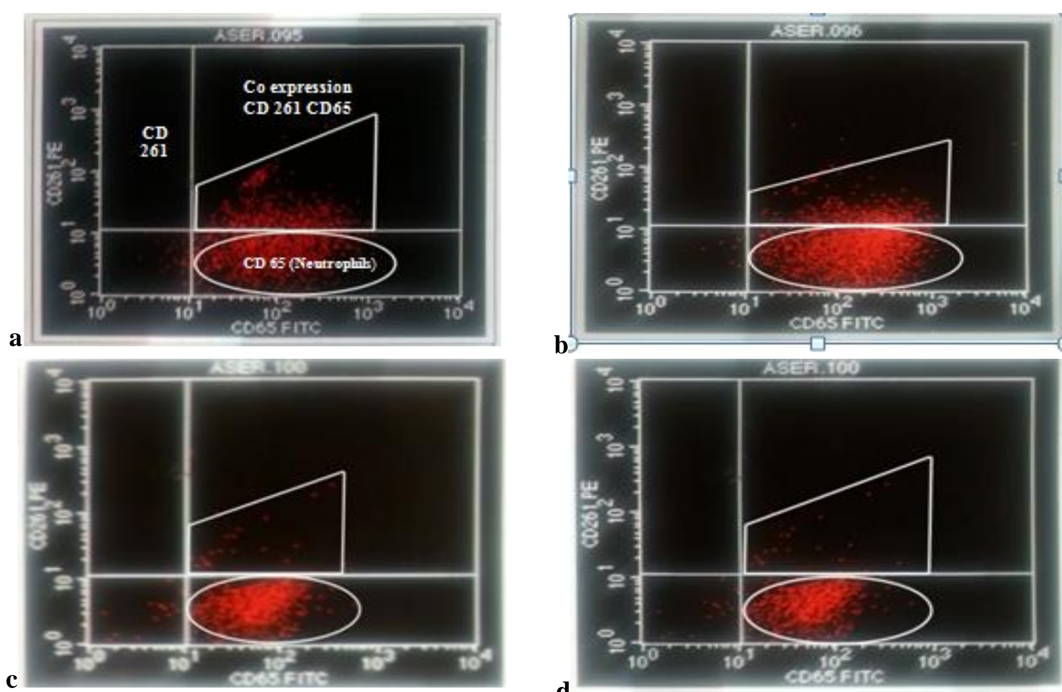

Fig. 2: Flow cytometric analysis for TRAILR1 (DR4/CD261) expression on polymorphonuclear leucocytes. A; represents patients with active SLE. B; represents patients with mild or no activity. C; represents patients with Rheumatoid arthritis. D; represents the control group

Lupus patients with neutropenia had a statistically significant higher level of sTRAIL, TRAIL mRNA and TRAIL R1 compared to those without neutropenia (P<0.001, P=0.001, P=0.001 respectively) as shown in table (4). Lupus patients with nephritis also had a statistically significant higher level of sTRAIL, TRAIL mRNA and TRAIL R1 compared to those without nephritis (P<0.001, P=0.004, P=0.01 respectively) as shown in table (5)

Table 4: Correlation between serum level of sTRAIL, TRAIL mRNA and TRAIL R1 expression levels and neutropenia among SLE patients

TRAIL	SLE patients		Test of significance	P value
	With neutropenia (n=24)	Without neutropenia (n=26)		
	Mean±SD	Mean±SD		
Soluble TRAIL (pg/ml)	462.8±144.3	306.0±69.8	Student t-test=4.83	<0.001**
TRAIL mRNA	2.7±0.57	2.1±0.62	Student t-test=3.46	0.001**
TRAIL R1 (MU)	58.3±22.6	37.7±8.2	Mann Whiney test =3.73	0.001**

***highly significant difference*

Table 5. Correlation between serum level of sTRAIL, TRAIL mRNA and TRAIL R1 expression levels and renal impairment among SLE patients

TRAIL	SLE patients		Test of significance	P value
	With nephritis (n=13)	Without nephritis (n=37)		
	Mean±SD	Mean±SD		
Soluble TRAIL (pg/ml)	530.7±154.3	328.8±79.9	Student t-test=4.51	<0.001**
TRAIL mRNA	2.8±0.49	2.2±0.64	Student t-test=3.07	0.004*
TRAIL R1 (MU)	61.8±25.8	42.6±14.1	Mann Whiney test =2.48	0.01*

**significant difference* ***highly significant difference*

Table 6. Evaluation of sTRAIL (pg/ml), TRAIL mRNA and TRAILR1 (MU) as novel markers for assessment of SLE activity

	AUC	Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
Soluble TRAIL (pg/ml)	0.753	345	72%	64%	66.7%	69.6%	68%
TRAIL mRNA	0.757	2.15	80%	60%	66.7%	75%	70%
TRAIL R1 (MU)	0.606	35.5	68%	40%	53.1%	55.6%	54%

Regarding evaluation of soluble TRAIL, TRAIL mRNA and TRAIL R1 as novel markers for assessment of SLE activity; the sensitivity, specificity, PPV, NPV and accuracy were 72%, 64%, 66.7%, 69.6 and 68% for sTRAIL and 80%, 60%, 66.7%, 75% and 70% for TRAIL mRNA while for TRAIL R1 they were 68%, 40%, 53.1%, 55.6% and 54% respectively (table 6).

DISCUSSION

Systemic lupus erythematosus is a generalized autoimmunity disease directed against various organs. B- cells hyperactivity, abnormally activated T- cells, autoantibodies, immune complex deposition and accelerated apoptosis are the key features involved in the immunopathogenesis of SLE [11]. Apoptosis in SLE could be enhanced through binding of death receptor ligands such as Fas and TRAIL to their specific receptors on the target cells. TRAIL could amplify the abnormal apoptotic process in SLE and has been previously documented to be involved in lupus- induced neutropenia and renal impairment [10].

In the present study, the mean value of sTRAIL level was significantly higher in patients with active SLE than those in other groups (P<0.001). Increased serum sTRAIL level could be disease-specific for SLE and could have a role in SLE pathophysiology [13]. This finding was in agreement with Lub-de Hooge et al. [10] who demonstrated higher sTRAIL concentrations in the sera of SLE patients compared with other participants (inactive SLE, other autoimmune diseases like Rheumatoid arthritis and Wegener's granulomatosis as well as healthy volunteers) (P<0.001). Mervat et al. [14] also found that, an increased level of circulating TRAIL was a characteristic feature for SLE and significantly correlated with disease activity, neutropenia and lupus nephritis. The elevated sTRAIL levels in lupus patients was explained by Rus et al. [15] who stated that, peripheral blood lymphocytes were abnormally activated in SLE patients and this T- cell activation was found to contribute to increased expression of TRAIL and other apoptotic ligands on lupus T- cells. These findings were supported by increased gene-expression of TRAIL in peripheral blood mononuclear cells from lupus patients. This over-expression could result in increased TRAIL concentrations.

In the current study, the mean values of TRAIL mRNA expression levels in the PBMC were significantly higher in active SLE patients than those of other groups (P<0.001). These results agreed with Komatsuda et al. [11], Safaa et al.[16] and Jing Gao et al.[17] who reported that, the expression levels of TRAIL mRNA in SLE patients were not only significantly higher than in controls but also correlated with SLE activity index. Kaplan et al.[18] also obtained the same results and documented that, individuals with SLE showed a significant increase in monocytes apoptosis induced by upregulated TRAIL mRNA expression. This process is mediated, at least in part, by an autoreactive T- cell subset that kills autologous monocytes in the absence of nominal Ag. They investigated the apoptotic pathways involved in this T cell-mediated process, expression of the apoptotic ligands like TRAIL on lupus T-cells and the role of these molecules in the monocytes apoptotic response. They concluded that, the apoptotic ligands like TRAIL mediated the autologous monocytes death induced by lupus T- cells and that this cytotoxicity was associated with elevated mRNA expression of these molecules on activated T-cells, rather than with an increased susceptibility of lupus monocytes to apoptosis induced by these ligands.

In this study the mean value of TRAIL R1 expression levels was 51.2±22.1 (MU) when using flow cytometric analysis with a highly significant difference for active SLE patients than those of other groups (P<0.001). These results agreed with Song and Lee[19] who documented increased expression of TRAIL R1 and TRAIL R2 in patient with SLE and Maria et al.[20] who reported that, TRAIL R1 had a similar pattern of expression to sTRAIL in lupus patients. The expression and distribution of TRAIL receptors were altered in lupus patients partly due to immuno-modulatory mechanisms and partly due to exposure to immunosuppressive drugs.

The present study proved a significant positive correlation between serum concentration of sTRAIL (r=0.721), TRAIL mRNA expression level (r=547) and SLEDAI score. Our results agreed with Mervat et al.[14] who found that, the circulating TRAIL levels correlated with SLE activity. In another study by Komatsuda et al.[11] and Safaa et al.[16], they observed that, expression levels of TRAIL mRNA in SLE patients showed a

statistically significant association with the disease activity, while serum sTRAIL concentrations did not.

Regarding the role of TRAIL in the development and progression of neutropenia among SLE patients, the current study revealed that the mean values of sTRAIL, TRAIL mRNA and TRAIL R1 showed a highly significant statistical difference (P<0.001) in lupus patients with neutropenia than those without neutropenia. The same result was obtained by Matsuyama et al.[4] and Mervat et al.[14] who found that sTRAIL and mRNA levels in SLE patients with neutropenia were significantly higher than those without neutropenia (P<0.01). However, Matsuyama et al.[4] reported that TRAIL R1 expression values were the same in neutropenic lupus patients and controls and that, the expression of TRAIL R3 was significantly lower in SLE patients with neutropenia than in patients without neutropenia or in healthy volunteers. They explained the occurrence of neutropenia in lupus patients by the fact that, reduced expression of TRAIL R3 could potentiate the effect of TRAIL on neutrophils resulting in accelerated apoptosis and reduction of neutrophils count.

Carli et al.[21] also reported that, in SLE patients, neutrophils undergo rapid constitutive apoptosis, and because of their vast numbers, they represent an enormous apoptotic cell burden and that the dysregulated apoptosis of neutrophils induced by higher TRAIL concentrations may lead to the development of autoimmunity. Kaplan[22] also studied for lupus-induced neutropenia and mentioned that, circulating apoptotic neutrophils were not only increased in patients with adult-onset SLE, as compared with normal subjects and with disease controls, but also correlated with SLE activity and dsDNA concentrations. The higher frequencies of lupus-induced neutropenia might be explained by the fact that, extrinsic apoptotic pathway of neutrophils is enhanced by ligation of death receptors, such as TRAIL and after ligand binding, the FADD protein is recruited, which then recruits and activates caspase cascade leading to accelerated apoptosis of neutrophils.

Higher level of sTRAIL is a key feature of SLE and correlate with lupus nephritis [23].This fact was proved in this research where the mean values of sTRAIL, TRAIL mRNA and TRAIL R1 in SLE cases with nephritis were significantly higher than those without nephritis (P<0.05). Safaa et al.,[16] demonstrated that, all SLE patients with high expression of TRAIL mRNA had lupus nephritis but without significant difference. Nguyen et al.[23] reported that TRAIL R1 is exerting a proinflammatory effect that may contribute to local inflammation and injury in kidneys from patients with lupus nephritis. Song et al.[5] also reported that, the expression of TRAIL receptors was altered in patients with proliferative lupus nephritis and that the expression of TRAIL DR4 and DR5 is upregulated in patients with lupus nephritis compared to non-SLE renal diseases.

The evidence of lupus nephritis was achieved in this study by elevated creatinine and urea levels as well as appearance of proteinuria. These findings correlated with elevated sTRAIL and mRNA levels. These observations were in consistence with Mohamed et al.[24] who reported that, SLE patients during activity showed significant positive correlations between serum TRAIL values and serum creatinine and 24-h urinary protein excretion (P<0.05). On the other hand, serum TRAIL levels correlated inversely with the estimated creatinine clearance. They also performed renal biopsy of active lupus children and observed that sTRAIL concentrations were significantly higher in patients with classes III and IV nephritis compared to those with classes I and II nephritis (P < 0.01).

Regarding mortality rates among the studied populations, two cases with active lupus died from serious devastating complications; one patient died with renal impairment and the other with and pneumonia. These findings could suggest a significant association between TRAIL and clinical outcomes of SLE.

CONCLUSION & RECOMMENDATIONS

This study proved that, the elevated serum concentration of sTRAIL could be disease specific for SLE. The upregulated expression of TRAIL mRNA in the PBMC and TRAIL R1 on neutrophils from active lupus patients provides additional support that, TRAIL has an obvious role in the pathogenesis of SLE as well as the associated complications. However, the exact mechanism remains to be elucidated in further prospective studies with a larger population scale.

Competing Interests

Authors have declared that no competing interests exist.

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