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ORIGINAL ARTICLE

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Tumor necrosis factor-alpha and neuropsychiatric lupus erythematosus: relation to single photon emission computed tomography findings

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Abstract This study was designed to highlight the relation of tumor necrosis factor-α (TNF-α) to neuropsychiatric lupus (NPLE) manifestations. The relation of TNF-α to the type of single photon emission computed tomography (SPECT) findings in this context was also studied. Twenty-one systemic lupus erythematosus (SLE) females, mean age 27.57 ± 9.89 years, and twenty age-matched normal females (controls), were subjected to TNF-α assessment. Different clinical and neuropsychiatric manifestations were evaluated. SPECT was carried out for all patients. The results showed that the mean TNF-α level (pg/ml) was significantly raised in patients compared with controls (167.8 ± 102.5 versus 64 ± 50.2, respectively, $P < 0.005$). Thirteen patients (69.1%) had NPLE manifestations. NPLE patients had a significantly higher mean TNF-α than patients without NPLE (203 ± 102.8 versus 109 ± 47.3, respectively, $P < 0.03$). Positive SPECT findings were found in 18 lupus patients (85.7%), including all 13 patients with NPLE (100% sensitivity), with a multiple focal pattern of hypoperfusion being the most frequent type (9/13), followed by diffuse (3/13), and then single focal pattern (1/13). The mean TNF-α was significantly higher in patients with multiple focal pattern ($P < 0.001$). In conclusion, results of this work support the hypothesis that TNF-α could be involved in the pathogenesis of NPLE, and hence, it could be speculated that the evolving anti-TNF therapy can play a potential role in the management of this disease.

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Introduction

Systemic lupus erythematosus (SLE) has frequent and potentially serious neuropsychiatric (NP) manifestations. The diverse clinical presentations, the relative paucity of information on clinicopathological correlations, the lack of uniform diagnostic criteria, and the uncertainities about optimal management are some of the multifaceted dilemmas facing the physician dealing with neuropsychiatric lupus erythematosus (NPLE).[1]

Explanations for neurological manifestations in SLE include damage to the nervous system mediated by autoantibodies and immune complexes.[2,3] There is also evidence that vascular lesions in patients with SLE, both cerebral and other types, are associated with the occurrence of antiphospholipid antibodies.[4] Another possible pathophysiological mechanism in NPLE is related to the direct or indirect effect of inflammatory mediators on the nervous system. Proinflammatory cytokines produced both outside and inside the central nervous system (CNS) are known to have dramatic effects on the nervous system, perhaps best described in infections.[5]

Earlier studies have reported increased levels of interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon alpha (IFN-α) in cerebrospinal fluid (CSF) of patients with NPLE.[6–8]

Tumor necrosis factor-α (TNF-α) is a pluripotent cytokine. It has cytotoxic and antiviral properties, but it exerts its main action through immunological mechanisms.[9] Certain SLE mechanisms are associated with differences in TNF production capacity, and it has been suggested that polymorphic elements within, or linked to, the HLA class II region determine TNF production capacity.[10] Rood et al.[11] found that TNF-308A and HLA-DR3 alleles are independent susceptibility factors for SLE.

Single photon emission computed tomography (SPECT) has proved to be highly sensitive (>90%) in detecting abnormalities in patients with NPLE,[12] including both major (psychosis, seizures, strokes) and minor (headache, mood swings, subjective congnitive disturbance) neuropsychiatric manifestations.[13]

This study was designed to highlight the relation of TNF-α to NPLE manifestations. The relation of TNF-α to the type of SPECT findings in this context was also studied.

Materials and methods

This study included 21 nonsmoker, female SLE patients. Their mean age was 27.57 ± 9.89 years (range 15–55 years), their mean duration of disease was 4.86 ± 6.59 years (range 0.5–38 years), and their mean age of disease onset was 22.2 ± 9.26 (range 4–51 years). The patients attended the Rheumatology and Rehabilitation Department, Cairo University Hospital. All patients fulfilled the American College of Rheumatology (ACR) criteria for the diagnosis of SLE.[14] Twenty healthy female subjects, with a mean age of 25.3 ± 13.7 years, were included as a control group.

All the patients studied were assessed by a full clinical evaluation and laboratory investigations (including antinuclear antibodies (ANA), anti-dsDNA, serum anticardiolipin (acL) antibodies (IgG and IgM), C_3 and C_4). Any neuropsychiatric manifestations were diagnosed and recorded according to the modified criteria proposed by Ainiala et al.[15] Disease activity was assessed by the SLE disease activity index (SLEDAI).[16]

Serum TNF-α was assessed by using the BIOSOURSE TNF-α EASIA Kit (BioSourse Europe, SA). These assays employ the solid-phase enzyme amplified sensitivity immunoassay techniques (EASIA). Serum TNF-α measurements were taken for patients and controls.

SPECT was carried out for all patients. Transverse, sagittal, and coronal images were obtained. The results were interpreted by an experienced nuclear medicine specialist with no knowledge of the patients' clinical status. Abnormal findings were described as single focal, multiple focal, and diffuse patterns of hypoperfusion.[13]

Statistical methods

Data were processed using the "spsswin" statistical package. Student's *t*-test and nonparametric tests were performed, and if there was no difference between these statistical results, Student's *t*-test result is reported. Analysis of variance (ANOVA) was performed when appropriate. A two-tailed analysis with a P value less than 0.05 was considered to be significant. The range, mean, and standard deviations are given. Correlation analysis was done using Kendall's correlation.

Results

The clinical manifestations of the study population are shown in Table 1. Thirteen out of the 21 patients (61.9%) had neuropsychiatric manifestations. The frequency distribution of the different NPLE manifestations are shown in Table 2.

The mean TNF-α level (pg/ml) was significantly raised in patients compared with controls (167.8 ± 102.5 versus 64 ± 50.2, respectively, $P < 0.005$) (Fig. 1). The cut-off value was set at 164.3, and 10 SLE patients showed high TNF-α levels above the cut-off value. Mean TNF-α levels showed insignificant differences in patients with and without different disease manifestations of SLE, except for patients with NPLE, who showed significantly higher mean TNF-α serum levels (Fig. 2) when compared with patients without NPLE manifestations (203 ± 102.8 versus 109 ± 47.3, respectively, $P < 0.03$). No statistical significant difference was found between the TNF-α serum levels of patients with clinical diffuse and focal neuropsychiatric manifestations, nor between major and minor CNS manifestations (data not shown). Furthermore, insignificant correlations existed between TNF-α and SLEDAI (data not shown). Nevertheless, a significant correlation existed between TNF-α serum

Table 1. Clinical manifestations, treatment, and disease activity score in the 21 patients studied

	Number	%
Malar rash	18	85.7
Photosensitivity	18	85.7
Oral ulcers	13	61.9
Neuropsychiatric manifestations	13	61.9
Systemic hypertension	10	47.6
Renal disease	10	47.6
Chest disease[a]	5	23.8
Heart disease[a]	5	23.8
Serositis[a]	3	14.3
Myositis	1	4.8
Discoid rash	1	4.8
Steroids	16	76.2
Antimalarials	12	57.1
Azathioprine	9	42.9
Cyclophosphamide	7	33.3
Median SLEDAI score (range)	10 (4–17)	–

SLEDAI, SLE disease activity index

[a] Including patients with pleuritis and pericarditis (also included in chest and heart disease, as three patients had pericardial and pleural effusions)

Table 2. Frequency distribution of NPLE manifestations (13/21)

	Number	% (of total)
Cognitive dysfunction	9	42.9
Seizures	6	28.6
Major depression	4	19
Psychosis	3	13.3
Peripheral neuritis	2	9.5
Strokes	1	4.8
Pseudotumor cerebri	1	4.8
Chorea	1	4.8

NPLE, neuropsychiatric lupus erythematosus

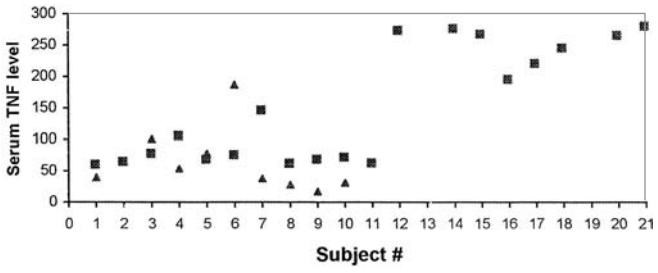

Fig. 1. Scatter diagram showing the distribution of TNF-α levels (pg/ml) in lupus patients and controls. *Squares*, patients; *triangles*, controls

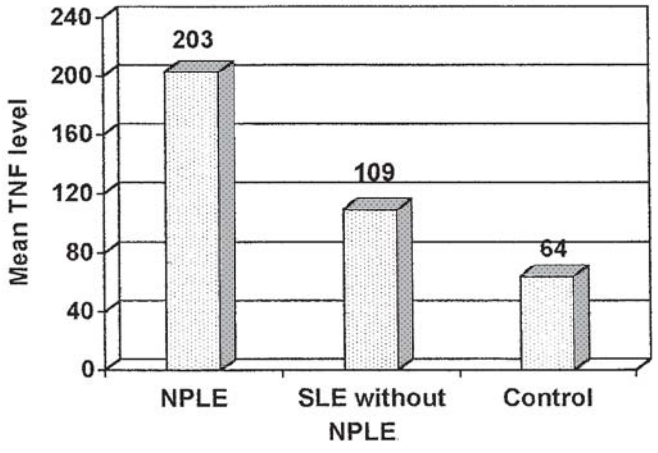

Fig. 2. Comparison between mean TNF-α level (pg/ml) in NPLE patients, and in SLE patients without NPLE and the control group

levels and first-hour erythrocyte sedimentation rate (ESR) ($r = 0.4$, $P < 0.01$), while insignificant correlations were found in each case between serum TNF-α levels and serum creatinine, C3, and C4. Also, the mean serum TNF-α levels were not significantly different in patients with positive and negative ANA, anti ds-DNA, and acL.

SPECT showed positive findings in 18 out of 21 SLE patients (85.7%), including all NPLE patients (i.e., 100% sensitivity). The multiple focal pattern of hypoperfusion (Fig. 3) was the most frequent pattern in patients with NPLE (9/13), followed by the diffuse pattern (3/13) (Fig. 4), and the single focal pattern (1/13) (Fig. 5). Five lupus patients without clinically overt NPLE features showed positive SPECT findings. Table 3 gives type of SPECT findings in relation to the presence of NPLE.

Analysing TNF-α levels in relation to the type of SPECT finding showed that mean the TNF-α level in patients with multiple focal pattern hypoperfusion (244.9 ± 72.3) was significantly higher than the level in the other patients (Table 4).

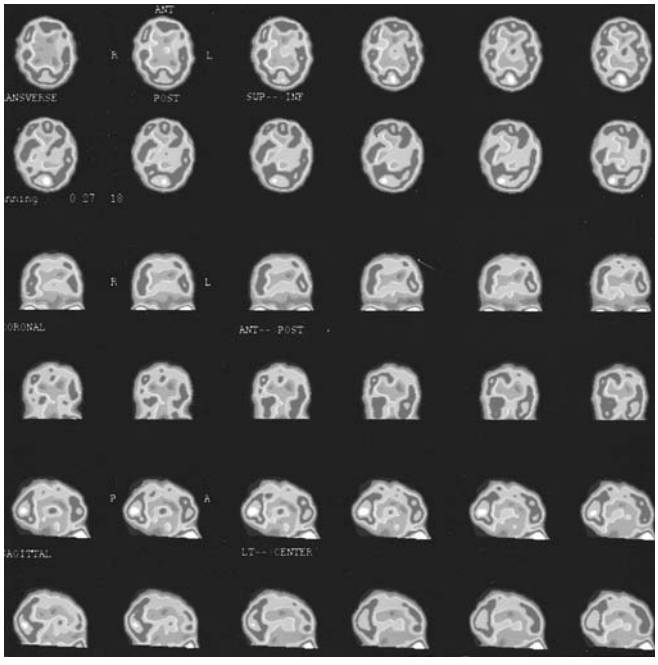

Fig. 3. Brain SPECT of an SLE patient showing the multiple-focal pattern of hypoperfusion

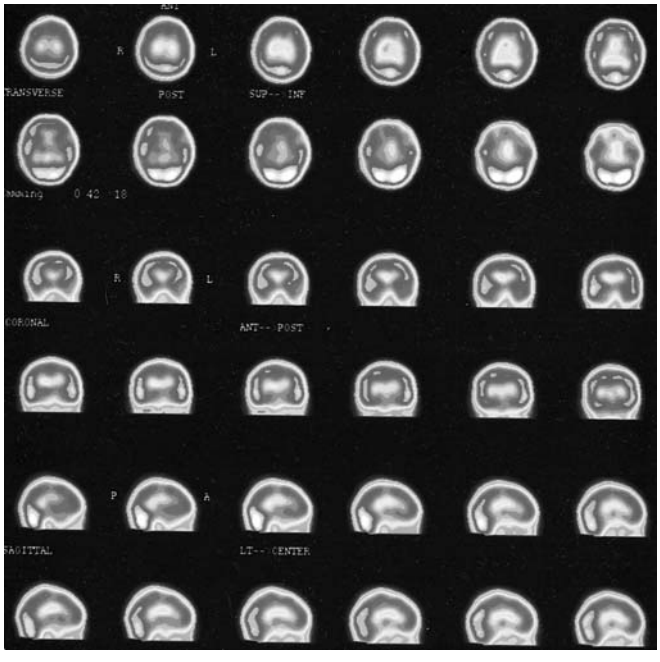

Fig. 4. Brain SPECT of an SLE patient showing the diffuse pattern of hypoperfusion

Discussion

TNF-α is a member of a growing family of mediators. Studies of TNF-α and SLE have so far led to some controversial results. TNF-α administered at high doses to lupus-prone mice before apparent disease manifestation seemed to have a protective effect,[17] whereas TNF-α administration in low amounts at a later stage led to the development of nephritis.[18] In other lupus-prone strains, the administration of TNF-α had neither a beneficial effect nor accelerated the disease.[19] Nevertheless, as lupus is a cytokine-driven disease, and over-expression of certain proinflammatory

cytokines has been reported in patients with neurodegeneration, TNF-α is thought to play a significant role in the pathogenesis of inflammatory demyelinating diseases of the CNS, as has been demonstrated in experimental autoimmune encephalomyelitis,[20] an established animal model for human multiple sclerosis, as well as in studies in humans.[21,22] Moreover, Mohan et al.[23] reported a series of patients who developed new-onset neurological signs and symptoms, which in most cases were associated with demyelinating lesions of the CNS, while undergoing therapy with anti-TNF agents. This supports the notion that TNF is not essential for the induction and expression of inflammatory and demyelinating lesions, and may have a neuroprotective function in CNS.[24]

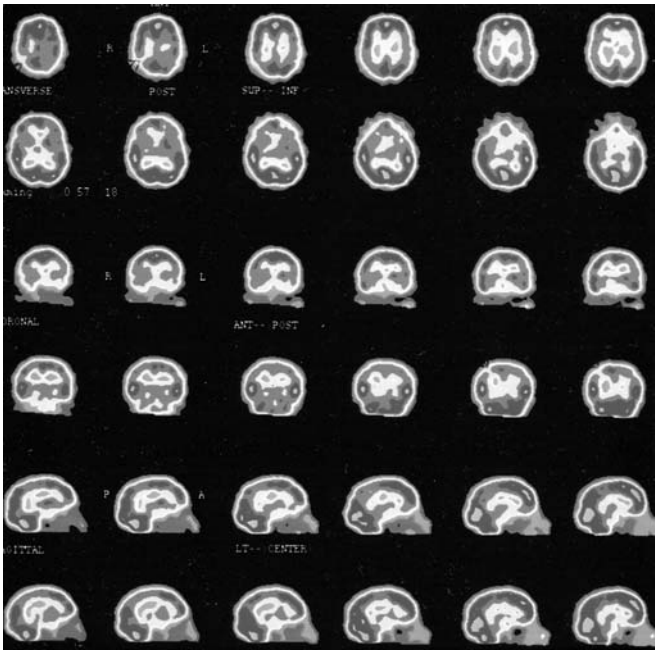

Fig. 5. Brain SPECT of an SLE patient showing the single focal pattern of hypoperfusion

Table 3. Type of SPECT findings in relation to the presence of NPLE

	+ve NPLE patients	−ve NPLE patients	Total
Multiple focal	9	1	10
Diffuse	3	1	4
Single focal	1	3	4
Total	13	5	18

SPECT, single photon emission computed tomography

The mean serum level of TNF-α in SLE patients, was significantly increased compared with that in controls. Although TNF-α serum levels showed no significant correlation with SLEDAI, they did show a significant correlation with ESR, but not to other laboratory markers. Such controversial observations have been reported in several studies where low or undetectable TNF-α levels were found in SLE patients,[25,26] and Meijer et al.[27] reported elevated TNF-α levels but without any clear-cut correlation with disease activity or acute-phase response. Nevertheless, Studnicka-Benke et al.[28] found increased levels of TNF-α in lupus patients, as well as a strong correlation with clinical and serological parameters of disease activity, suggesting a central role of the TNF system in the pathophysiology of SLE. Miret et al.[29] also reported higher levels of TNF-α in lupus patients with no relation to disease activity. Alvarado-de la Barrera et al.[30] found higher TNF-α gene expression in bone-marrow samples from SLE patients than from controls. However, Gattoron et al.[31] found that the serum concentration of TNF-α fell within the normal range in SLE patients irrespective of disease activity. Robak et al.[32] concluded that the increase in serum levels of TNF-α and IL-6 may be useful markers for SLE activity.

Concerning the relation of TNF-α to different SLE clinical manifestations, only patients with CNS involvement have significantly higher TNF-α levels than those without CNS involvement. Korner et al.[33] showed that TNF is essential for the normal initiation of a neurological deficit in CNS autoimmune inflammation.

In a recent study by Svenungsson et al.[34] to investigate the systemic and intrathecal production of proinflammatory cytokines and their relation to CSF nitric oxide (NO) release in patients with NPLE, they found that patients with NPLE not only have high levels of CSF NO metabolites and increased numbers of TNF-α-producing peripheral lymphocytes, but there is also a correlation between the number of cells expressing TNF-α mRNA and CSF NO metabolites on the one hand, and the severity of NPLE on the other, suggesting that TNF-α may be of pathogenic importance in NPLE. These authors also raised the possibility that peripheral inflammatory mediators such as TNF-α induce intrathecal inflammation. Therefore, induction of central nervous damage via an elevated peripheral TNF-α level might be supposed to occur through the stimulation of intrathecal production of several proinflammatory mediators. Smolen[35] proposed a critical role of TNF-α in the final

Table 4. Comparison between mean TNF-α levels in patients with different SPECT patterns, and in those with negative SPECT findings

	Multiple focal (10/21)	Diffuse (4/21)	Single focal (4/21)	Negative (3/21)	P^a
TNF-α (mean ± SD)	244.9 ± 72.3	99.8 ± 63.1	66.0 ± 4.40	137.0 ± 119.8	0.002

TNF-α, tumor necrosis factor-α
[a] With ANOVA where $F = 7.894$

pathway of SLE disease, not only immunologically induced, but also inflammation-induced tissue destruction.

SPECT is a sensitive tool for detecting the abnormalities in NPLE. Scan abnormalities are detected in up to 90% of patients with clinically overt NPLE.[36] This study showed positive scan abnormalities in all patients with NPLE, which gives a sensitivity figure of 100%. In this study, the most frequent pattern seen in patients with NPLE was multiple focal areas of hypoperfusion, which were also found in other studies to be the most frequent SPECT finding in patients with NPLE.[37] Therefore, such a finding, when combined with the finding that mean the TNF-α level is significantly higher in patients with multiple focal pattern hypoperfusion, should raise a question about the role of biological antagonists of TNF-α in the treatment of NPLE disease. Segal et al.[38] studied the effects of two therapeutic modalities that lower the TNF-α activity and clinical manifestations of SLE in mice. They concluded that the abrogation of TNF-α and IL-1 production in the early stages of experimental SLE by anti-TNF-α therapy improves the clinical state of mice afflicted with this autoimmune disease.

In conclusion, the serum TNF-α level is significantly raised in lupus patients in general, and in patients with NPLE in particular. There is also a significant increase in TNF-α levels in patients with multiple focal pattern hypoperfusion, which is the most frequent SPECT pattern in NPLE patients. Accordingly, the present study supports the hypothesis that TNF-α could play a role in the pathogenesis of NPLE, and hence, we speculate that the cytokine would be a candidate target for future treatment.

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