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Proinflammatory cytokines (TNF-α and IL-6) in Egyptian patients with SLE: Its correlation with disease activity

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Abstract

Background: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by a wide variety of autoantibodies, some of which are pathogenic. In recent years it has become more evident that the polyclonal B cell activation in SLE is T-cell dependent. The stimulation of the autoantibody producing B cells is likely mediated by the TH2 subtype of T cells producing IL-4, IL-5, IL-6 and IL- 10, whereas the TH1 subtype secreting IL-2 and IFN-γ predominates in cell-mediated immune response. Tumor Necrosis Factor (TNF-α) is both a proinflammatory and an immunoregulatory cytokine. TNF-α has differential effects on B cells, on T cells and on dendritic cells as well as on the process of programmed cell death. Understanding how the immune system integrates the pleiotropic properties of TNF-α is a challenge, particularly so in diseases like SLE. Meanwhile the role of IL-6 in the pathogenesis of SLE is controversial. *Objective:* To investigate whether serum levels of TNF-α and IL-6 is higher in Egyptian patients with SLE than healthy control volunteers and its correlation with the clinical activity in patients with different activity scores as measured by Systemic Lupus Erythmatosus Disease Activity Index (SLEADI). *Methods:* Sixty individuals (40 patients with Systemic lupus Erythmatosus and 20 healthy control volunteers) were the subject of this study, they were subjected to thorough clinical examination, laboratory investigations, their clinical disease activity was scored according to SLEDAI, and serum sampling was obtained for TNF-α and IL-6 levels assay. Renal biopsy was carried out and examined by light microscopy by a pathologist blinded with the clinical activity. *Results:* The mean level of TNF-α was (766.95 ± 357.82 Pg/ml) for patients with active disease while it was (314.01 ± 100.87 Pg/ml) for those with inactive disease and (172.7 ± 39.19 Pg/ml) for the healthy control group. The difference was statistically significant ($P = .002$). The mean level of IL-6 was (135.4 ± 54.23 Pg/ml) for patients with active disease while it was (47.33 ± 18.61 Pg/ml) for those with inactive disease and (21.15 ± 10.99 Pg/ml) for the healthy control group. The difference was statistically significant ($P = .002$). A significant correlations between TNF-α and IL-6 serum levels and the SLEDAI score was observed ($r = .743$ and .772, respectively). *Conclusion:* Serum TNF-α and IL-6 are sensitive markers of SLE disease activity. They may be useful independent markers for prediction of SLE disease activity and to differentiate normal subjects from those having SLE. Possible therapeutic implications in the treatment of SLE in the future deserve wide scale trials.

Keywords: SLE; Lupus nephritis; TNF; IL-6

1. Introduction

Systemic Lupus Erythematosus (SLE) is a rheumatic autoimmune disease characterized by multisystem organ involvement and by high titers of autoantibodies against

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several nuclear and cytoplasmic antigens [1]. Numerous abnormalities of the cytokine network have been described in patients suffering from SLE as well as in murine lupus models. Some of them were shown to play a pivotal physiopathological role in certain T-cell, B-cell or antigen-presenting cell dysfunctions characteristic of the disease, while others are more likely to be innocent bystanders [2].

While many authors concluded that serum TNF-α is clearly elevated and was found to correlate with SLE disease activity [3–5], others reported increased levels only in a minority of patients with active SLE that correlates only with thrombocytopenia [6].

The role of IL-6 in the pathogenesis of SLE is controversial [7], While the majority of reports demonstrated increased levels of IL-6 in patients with active SLE that do not correlate with acute phase proteins [8,9], some authors found elevated IL-6 levels only in cases with increased C-reactive protein, concluding that it is part of the acute phase response[9].

Conventional treatment of SLE with immunosuppressive drugs and corticosteroids affects the entire immune system in a nonspecific manner. Although the introduction of these drugs led to markedly improved outcome in SLE there are still patients not sufficiently responding to conventional therapies.

The increased knowledge of the pathophysiological mechanisms involved in SLE open the possibility for more specific immunological treatments, targeting, for example, cytokines or cell surface molecules.

Data about the role of TNF-α and IL-6 in Egyptian patients with SLE are lacking. In this work, we wanted to assess the levels of TNF-α and IL-6 in sera of 40 patients with SLE and Lupus nephritis compared with 20 healthy volunteers and to study their correlation with clinical disease activity.

2. Materials and methods

Forty patients with SLE and 20 healthy control volunteers served as the subjects of this study. The SLE patients were recruited from the outpatient clinic at Mansoura Urology and Nephrology Center. They presented to the outpatient complaining of symptoms suggestive of lupus nephritis—during the period from January 2003 till February 2004—and had been diagnosed according to the criteria of the American Rheumatism Association (ARA) [10]. All were adults >18 years, suffering from Lupus nephritis (Renal impairment, proteinuria, and or hematuria). They were subjected to; thorough clinical examination and their clinical disease activity was assessed by SLEDAI [11]. Twenty SLE patients with active disease (SLE disease activity index (SLEDAI) $\geqslant 10$) served as active group (18 female and 2 were male, Mean age 25.68 ± 7.78 year, mean disease duration 25.68 ± 7.78 month, 17 patients were hypertensive), while the remaining 20 consecutive SLE patients with inactive disease—defined as the persistent absence of disease activity (SLE disease activity index (SLEDAI) $\leqslant 10$) for at least a 4-month-period, either without or on a constant dose of immunomodulating drugs (18 female and 2 male, mean age 27.5 ± 8.99 year, average disease duration was 42.5 ± 21.43 month, eight patients were hypertensive) were included.

Laboratory evaluation includes: Urinalysis, 24 h urinary protein and venous blood samples were collected for analysis where: Serum creatinine, electrolytes, complete liver function test, virological analysis (HBsAg, HCV antibodies, CMV antibodies, HIV), changes in levels of anti-dsDNA autoantibodies and Erythrocyte Sedimentation Rate (ESR) were done.

Ultrasonic guided renal biopsies were carried out to all patients and examined by one pathologist blinded with the clinical scoring activity of the disease. All biopsies were scored according to histological activity and chronicity index according to WHO classification for lupus nephritis. Twenty healthy volunteers matched for age and sex served as control group where serum samples were obtained for TNF-α and IL-6 assay. All patients gave written informed consent to participate in this study.

2.1. Measurement of TNF-α and of IL-6

A blood sample was drawn from all participants after an overnight fast and allowed to clot at room temperature for 30 min. Sera were separated by centrifugation at 3000 round per minute for 15 min. Sera were separated as soon as possible from the clot of red cells after centrifugation to avoid TNF-α production by blood cells that falsely could increase its values. Separated sera were kept in aliquots at −80 °C until the time of assay. Sera were assayed for TNF-α using ELISA kit—according to Aderka et al. [12], and for IL-6 using ELISA kit according to Brailly et al. [13].

2.2. Statistical analysis

Data are presented as means ± SD. SPSS package 9.05 for windows was used. Differences in mean levels between the groups determined by ANOVA and by Chi-Square test. A P-value $\leqslant 0.05$ was considered statistically significant. Spearman's rank correlation test was used to analyze the correlations between various laboratory measures and the SLEDAI score.

3. Results

SLE patients and healthy control volunteers were matched for age and sex. No statistically significant difference were observed between those with active and inactive disease regarding age, sex, body weight, and disease duration. Meanwhile 17 patients in the active group were hypertensive compared to only eight patients in the inactive group, this difference was statistically significant ($P = .001$). The demographic characteristics of the active and inactive SLE groups are given in Table 1.