SERUM LEVEL OF TUMOUR NECROSIS FACTOR–alpha IN EGYPTIAN PATIENTS WITH SYSTEMIC SCLEROSIS AND SYSTEMIC LUPUS ERYTHEMATOSUS: Correlation with Various Disease Parameters

Thesis
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Introduction

Tumor necrosis factor-alpha (TNFα) is a pluripotent cytokine with wide spectrum of biologic effects including growth promotion, B cells differentiation, neutrophils and macrophages activation, and hematopoiesis stimulation. In addition, it produces a broad range of effects on non-hematopoietic cell types. It also induces expression of many other cytokines and mediators that promote inflammation and is therefore known as a pro-inflammatory cytokine ***(Oppenheim & Ruscetti, 2001).***

Its main importance for immunity lies in its ability to enhance the activation of CD4+ T helper (***Th***) lymphocytes by antigen-presenting cells (APCs), being secreted by APCs on contact with a ***Th*** cell that has the appropriate antigen and major histocompatibility complex (MHC) specificity. They then act in an autocrine manner to induce or increase expression of various adhesion molecules, IFNγ receptors, and class II MHC proteins on the APC surface, and so increase the efficiency with which the APC can bind and activate ***Th*** cells They also act in a paracrine fashion on the ***Th*** cell, augmenting secretion of IL-2, expression of surface receptors for IL-2 and IFNγ, and other events leading to clonal T cell proliferation. As a result, TNFα is considered to help initiation of both humoral and cellular immune responses ***(Oppenheim & Ruscetti, 2001).***

The gene for TNFα is located on the short arm of chromosome 6, thus it lies within or near the major histocompatibility complex (MHC) gene. ***Ruuls & Sedgwick (1999)*** reviewed studies that had analyzed the contribution of TNF and related genes to susceptibility to a variety of human diseases, including sepsis, cerebral malaria, and autoimuune diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Systemic sclerosis (SSc), as well as cancer, and they discussed how the presence of the TNF gene within the MHC may potentially complicate the interpretation of studies in animal models in which the TNF gene is experimentally manipulated. Susceptibility for many of these diseases is thought to have a genetic basis, and the TNF gene is considered a candidate predisposing gene. However, unraveling the importance of genetic variation in the TNF gene to disease susceptibility or severity is complicated by its location within the MHC, a highly polymorphic region that encodes numerous genes involved in immunologic responses. They reviewed the problem of unlinking TNF biology from that of the MHC.

Dysregulation and, in particular, overproduction of TNF have been implicated in the pathogenesis of these and other diseases. It has been considered to play an important role in the inflammatory and immune response in recent years ***(O'Shea et al., 2000; Oppenheim & Ruscetti, 2001).***

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease of unknown etiology. T cell abnormalities, B cell hyperactivity, and abnormal cytokine production have been implicated to be of pathogenic importance in SLE patients ***(Grondal et al., 2000).*** TNFα among other cytokines has been suggested to play an important role in the immune dysregulation observed in SLE patients ***(Kollias et al., 1999).***

Systemic Sclerosis (SSc) is a generalized disorder of connective tissue characterized clinically by thickening and fibrosis of the skin (scleroderma) and by distinctive forms of involvement of internal organs. A diverse array of fibrogenic cytokines among which TNFα is released and may be capable of inducing or modulating the scleroderma fibroblast phenotype through mediating irreversible alterations in connective tissue inducing fibrosis of multiple organs that characterize the disease, thus sharing in its pathogenesis ***(Seibold, 2001).***

It is likely that multiple cytokines contribute to the scleroderma process, and that key cytokines may vary with the stage of the disease. TNFα has been proposed as one of the contributors to the pathogenesis of SSc ***(LeRoy et al., 1988).*** Regulation of fibroblast activity by cytokines appears to be important in the fibrotic phase of SSc, and an imbalance of fibroblast inhibitors or stimulating cytokines may result in varying degrees of fibrosis ***(Belsito, 1997).***

High levels of serum TNFα have been reported in several autoimmune diseases, and may be implicated in the etiopathogenesis of these two autoimmune diseases as well as others.

Aim of the Work

The aim of the current work is to measure the serum level of tumour necrosis factor-alpha (TNFα) in a group of Egyptian patients with systemic sclerosis (SSc) and another group with systemic lupus erythematosus (SLE), and to correlate it with various parameters of the previously mentioned two diseases.

Discussion

Systemic lupus erythematosus (SLE) is a disease with a complex set of immunologic abnormalities that appear to involve multiple mechanisms of dysregulation and that may be linked to more than 20 different genetic determinants. It lacks a single, unifying pathognomonic marker ***(Hahn et al., 2005).***

SLE is envisioned to arise from hyperactivated helper T cells that cause polyclonal B cell secretion of pathogenic autoantibodies and formation of immune complexes ***(Balow, 2000).***

Cellular defects in T cell signaling, cytokine production and apoptosis play a vital role in the pathogenesis of SLE. Cytokines have been suggested to play an important role in the immune dysregulation observed in SLE patients. Multiple cytokine-mediated defects have been demonstrated in SLE patients; aberrant increased or decreased production of IL-1, TNFα, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-18, and IFNγ has been reported in SLE patients ***(Sullivan, 2000).***

Altered cytokine homeostasis characterizes active SLE ***(Hahn et al., 2005).***The presence of IL-1, TNFα, IL-6, and IFNγ in diseased kidneys suggests a local pathogenic effect. Furthermore, it was reported that the clinical course, renal involvement, and survival in SLE can be significantly altered by cytokines and their inhibitors ***(Sullivan, 2000).***

Tumor necrosis factor-alpha (TNFα) is a pro-inflammatory cytokine. It is a potent activator of macrophages and is a growth factor for T and B cells. In addition, it plays an important role in the expression of vascular adhesion molecules and the development of secondary lymphoid tissue ***(Matsimoto et al., 1996; Cuff et al., 1998).***

Furthermore, the finding that high levels of soluble TNFα were found in the sera of SLE patients suggest that TNFα may participate in the initiation or perpetuation of the disease process ***(Meijer et al., 1993; Yokoyama et al., 1997; Alvarado et al., 1998; Herrera-Esparza et al., 1998; Sullivan, 2000).***

Systemic Sclerosis (SSc), synonym scleroderma is a generalized disorder of connective tissue characterized clinically by thickening and fibrosis of the skin (scleroderma) and by distinctive forms of involvement

of internal organs, notably the heart, lungs, kidneys, and gastrointestinal tract ***(Seibold, 2005).***

Early events of mononuclear cell activation that occurs in SSc process are thought to be followed by cytokine-driven proliferation of mesenchymal cells, and the deposition of extracellular matrix (ECM). A diverse array of fibrogenic cytokines and growth factors namely TNFα and IL-1β, IL-2, IL-2R, TGFβ, PDGF, and FGF are released and may be capable of inducing or modulating the scleroderma fibroblast phenotype (enhanced proliferation and synthetic function) through mediating irreversible alterations in connective tissue inducing fibrosis of multiple organs that characterize the disease, thus sharing in its pathogenesis ***(Kantor et al., 1992).*** The microenvironment of the scleroderma tissue lesion contains a variety of cellular populations capable of local release of cytokines. TNFα comes among the cytokines released from mast cells or monocytes in the scleroderma tissue lesion ***(Seibold, 2005).***

Tumor necrosis factor-alpha (TNFα) posseses pleiotropic biologic functions including, fibroblast mitogenicity, and, both the stimulation and the inhibition of matrix synthesis ***(LeRoy et al., 1989).*** It may be involved in the pathogenesis of SSc through its ability to inhibit endothelial cell growth, and in combination with interferon gamma (IFNγ), to promote cytolysis. Besides, TNFα can induce endothelial cell adhesion molecules for lymphocytes, thus contributing to vascular damage ***(Kahaleh et al., 1988; Smith & LeRoy, 1998).***

Miyazaki and associates (1995) showed that the expression of TNF gene under control of the human surfactant protein (SP) in transgenic mice causes a fibrosing alveolitis resembling systemic sclerosis lung disease.

Alekperov and coworkers (2003) have shown that TNFα participates in activation of vascular endothelium, regulation of immune response and metabolism of the connective tissue by modulation of fibroblastic function. SSc patients exhibit a systemic and local rise of TNFα content. This rise contributes to SSc progression, and development of fibrosing alveolitis.

Circulating concentrations of TNF receptors I and II, and those receptors on skin mononuclear cells and endothelial cells are increased in SSc. These concentrations correlate with laboratory signs of inflammation and disease progression ***(Grushwitz et al., 1997).***

Spontaneous production of TNFα by PBMCs has been demonstrated to be significantly greater in SSc than in individuals without the condition ***(Smith, 2003).***

The aim of our study was to investigate the serum levels of TNFα in Egyptian patients with systemic lupus erythematosus (SLE), and patients with systemic sclerosis (SSc), and to correlate these levels with various clinical and laboratory parameters of the two diseases.

In this present study, we studied sixty patients (thirty SLE patients and thirty SSc patients) and fifteen healthy age-matched subjects as control group. All patients and controls were females.

Serum samples were stored at -80°C immediately after centrifugation, and were defrosted only once immediately before the assay.

Serum level of TNFα was assessed using **BIOSOURCE TNFα EASIA Kit** (Biosource Europe S.A., Belgium). These assays employ the solid phase Enzyme Amplified Sensitivity Immunoassay techniques (EASIA). The EASIA Kit for TNFα assay has been shown to detect free cytokines as well as those bound to soluble receptors ***(Grau, 1995).*** Serum TNFα measurement was done to patients and controls.

A major difficulty in estimation of serum levels of cytokines lies mainly in the fact that cytokines have short half-life in the circulation, together with the presence of serum inhibitors for them. Thus, chronically elevated levels of serum cytokines could be taken as a sign of persistent immune cell activation ***(Kantor et al., 1992).***

As regards the **Systemic lupus erythematosus (SLE)** patients, their ages ranged between **16 - 50** years with a mean **26.73 ± 8.82** years. The age at which disease symptoms appeared for the first time ranged between **12 - 48** years with a mean of **22.63 ± 5.68** years. This is consistent with previous data that SLE is primarily a disease of women with a peak incidence between the ages of 15 and 50 years i.e. during the child-bearing period ***(Pisetsky, 1997).***

Our data were more compatible with a pathogenic, rather than a protective role of TNFα in human SLE. We have shown the presence of significant amounts of TNFα in the sera of SLE patients, together with

association of this elevated TNFα levels with a variety of other variables including disease activity, and various clinical and laboratory parameters.

In our study, serum TNFα yielded a mean of **94.9 ± 43.5** pg/ml, with a range from 36.3 -224.6 pg/ml for SLE patients, and a mean of **15.0 ± 2.6** pg/ml, with a range of 12.0 – 23.7 pg/ml, for the healthy controls with elevated mean serum TNFα in SLE patients compared to controls. There was a highly statistically significant difference between the two groups (P = 0.0024 HS). This elevated serum TNFα also correlated significantly with disease activity using the SLAM score (P=0.00012 HS).

In accordance to our results, ***Maury & Teppo (1989)*** found that patients with SLE had either normal or slightly elevated levels of TNF in the circulation. They measured serum TNF concentration using double-antibody radioimmunoassay (RIA), which measures the level of immunoreactive TNF in serum only, thus we do not know whether the TNF demonstrated in certain SLE patients is biologically active.

Also, ***Meijer and associates (1993)*** reported elevated levels of TNFα, but without any clear-cut correlation with disease activity or acute-phase response.

Besides, ***Steiner and colleagues (1995)*** made a comparison between serum TNFα levels in SLE and RA patients, and showed that TNFα levels were higher in SLE compared to RA.

Studnicka-Benke and associates (1996) investigated the involvement of TNFα and its soluble receptors (TNFR55 and TNFR75) in the pathophysiology of SLE. They found significantly elevated levels of TNFα in patients with SLE compared to healthy controls, together with a strong correlation between TNFα and its soluble receptors ($P < 0.0001$). Moreover TNFα and both TNFR strongly correlated with clinical and serological parameters of disease activity such as European Consensus Lupus Activity Measurement (ECLAM) score, anti-dsDNA antibodies, CRP, ESR, and anemia ($P < 0.0001$) for all comparisons suggesting a central role of the TNF system in the pathophysiology of SLE.

They used EASIA kit (the kit used in our work) that detects both free and receptor-bound TNFα. Such assays; the immunoassays have proven to be much more reproducible and more specific than bioassays.

Moreover, in contrast to bioassays, they are not influenced by therapeutically administered immunosuppressive drugs as corticosteroids (CS) or cyclophosphamide (CYC), which may be contained in patients' sera. Thus, although not measuring bioactivity per se, immunoassays are generally considered to be very useful for the determination of bioactive substances such as hormones, cytokines, or neurotransmitters in various body fluids ***(Studnicka-Benke et al., 1996)***.

Emilie and colleagues (1996) have demonstrated that serum levels of TNFα were increased in patients with active SLE, while CRP levels were normal unless there is serositis, synovitis, or concurrent infection despite the increase in TNFα which occurs in line with disease flares, suggesting an abnormal or 'underpowered' acute phase response in lupus.

In addition, ***Alvarado and coworkers (1998)*** found higher TNFα gene expression in bone marrow samples from patients with SLE compared to healthy controls.

Robak and coworkers (1998) have found elevated serum level of TNFα in patients with active SLE, and concluded that it may be a useful marker of SLE disease activity.

However, ***Gattorno and coworkers (1998)*** reported that serum concentrations of TNFα in pediatric SLE fell within the normal range, although mean serum levels of TNFα were slightly increased in high active compared to low active patients with SLE. Disease activity was evaluated using two sores; European Consensus Lupus Activity Measurement (ECLAM) score, and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Patients also displayed increased serum levels of sTNFR that correlated positively with the degree of disease activity. The overall pattern sTNFR and TNFα serum detection observed in the studied patients with pediatric SLE were similar to those reported for adult SLE and other autoimmune diseases.

In accordance to our results of elevated serum TNFα that showed significant positive correlation with disease activity using the Systemic Lupus Activity Measure (SLAM), ***Park and his colleagues (2000)*** found that TNFα reflected the changes in disease activity in patients with SLE using the Systemic lupus erythematosus Disease Activity Index (SLEDAI) and laboratory parameters including circulating immune complexes (CIC), C3, C4, anti-DNA antibody, IgG, IgM, IgA.

Sullivan (2000) reported that patients with active SLE have significantly higher levels of serum TNFα than patients with inactive disease. ***Dean and coworkers (2000)*** have also reported that the levels of TNFα are increased in patients with active SLE patients.

Miret and colleagues (2001), however, showed higher levels of TNFα in lupus patients as we showed, but with no relation to disease activity in contrast to our findings.

Smolen (2002) has proposed a critical role of TNFα in the final pathway of SLE disease, not only immunologically induced, but also the inflammation-induced tissue destruction. The central proinflammatory cytokines; TNFα and IL-1 are increased in SLE patients, and can be activated by immune complexes.

In contrast to our results, ***Aderka and colleagues (1993)*** failed to detect TNFα, while finding serum levels of both species of the soluble TNF receptor (sTNFR) to be significantly increased, and showed that the occurrence and the extent of the increase of the sTNFR correlate closely with disease activity. This failure to detect TNFα in the serum of most SLE patients might be attributable to antibodies against extracellular domains of the TNFR from which the sTNFR were derived, that could interfere with the binding of TNF to its receptors ***(Engelmann et al., 1990).***

Concerning the relation of TNFα to different clinical manifestations, the mean serum level of TNFα of our SLE patients showed significant higher levels in patients affected with certain clinical manifestations of SLE than those unaffected by the same clinical manifestation. These include weight loss (P = 0.0042), fatigue (P = 0.0113), Raynaud's phenomenon (P = 0.0108), discoid rash (P = 0.0320), gastrointestinal affection; mesenteric vasculitis, peritonitis, and/or organomegaly (P = 0.0237), dyspnea (P = 0.0101), renal affection; lupus nephritis (P = 0.0438) and twenty four hours urinary proteins (P = 0.0416), neurological affection; neuropsychiatric lupus erythematosus (NPLE) especially lupus cerebritis (P = 0.0316), psychosis (P = 0.0208), seizures (P = 0.0329), as well as erythrocyte sedimentation rate (ESR) (P = 0.0001)..

On the other hand, serum TNFα was negatively correlated with hemoglobin (Hb) levels, hematocrite (HCT %) levels, WBCs count,

platelet count (PLT). However, these correlations did not reach statistical significance ($P > 0.05$).

As regards the relation of TNFα to neuropsychiatric lupus erythematosus (NPLE), and in accordance to our results, ***El-Zorkany and his colleagues (2003)*** evaluated the relation of serum TNFα with different clinical and neuropsychiatric manifestations of SLE. They showed that mean TNFα level was significantly raised in SLE patients than controls ($P < 0.005$). Also NPLE patients had significantly higher mean TNFα serum levels than patients without NPLE manifestations ($P < 0.03$) supporting the hypothesis that TNFα could be involved in the pathogenesis of NPLE and hence it would be speculated that the evolving anti-TNF therapy can play a potential role in the management.

Furthermore, insignificant correlations existed between TNFα and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Nevertheless, a significant correlation existed between TNFα serum levels and first hour ESR, while insignificant correlations were found between serum TNFα levels and each of serum creatinine, complement 3 (C3), and complement 4(C4). Also, mean serum TNFα level was not significantly different in patients with positive and negative ANA, anti-DNA, and anticardiolipin antibodies (acL) ***(El-Zorkany et al., 2003).***

Early studies have reported increased levels of IL-1 IL-6, IFNγ in CSF of patients with NPLE ***(Hirohata & Miyamoto, 1990; Alcocer-Varela et al., 1992; Shiozawa et al., 1992)***, but without any detectable level of TNFα and IL-2 in CSF of NPLE patients ***(Alcocer-Varela et al., 1992).***

Patients with CNS involvement have significantly higher TNFα level compared to those without CNS involvement. TNFα was thought to play a significant role in pathogenesis of inflammatory demyelinating disease of CNS as has been demonstrated in experimental animal models with autoimmune encephalomyelitis: an established animal model for human multiple sclerosis ***(Selmaj et al., 1991)*** and in studies in humans ***(Hoffman et al., 1989; Sharieff & Hentages, 1991).***

On the contrary, ***Mohan and associates (2001)*** reported a series of patients who developed new-onset neurologic signs and symptoms, in most cases associated with demyelinating lesions of the CNS, while undergoing therapy with anti-TNF agents supporting the notion that TNF is not essential for induction and expression of inflammatory