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Serum levels of nerve growth factor and tumor necrosis factor α in systemic lupus erythematosus and systemic sclerosis

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Abstract

Background: Nerve growth factor is a neurotrophic factor which is expressed both in the nervous system and in the peripheral organs. It is synthesized by cells of immune system and may be involved in autoimmune and inflammatory processes. Tumor necrosis factor α is a pro- inflammatory cytokine that plays a role in most of the inflammatory processes as well as in immune responses to infections and tumor antigens.

Objective: To investigate the serum level of nerve growth factor and tumor necrosis factor α in systemic lupus erythematosus and systemic sclerosis patients.

Patients and methods: The level of serum nerve growth factor and tumor necrosis factor α were measured in three groups enrolled in the study: Group A included 24 patients with systemic lupus erythematosus, group B included 19 patients with systemic sclerosis and group C included 20 healthy subjects as a control group.

Results: The serum levels of nerve growth factor and tumor necrosis factor α were significantly higher in group A and B when compared with the control group. Their levels correlated with the disease activity in systemic lupus patients and with the degree of disease dissemination in systemic sclerosis patients. Both markers were significantly correlated

Conclusion: Nerve growth factor and tumor necrosis factor α might be involved in the pathogenesis of systemic lupus erythematosus and systemic sclerosis and could be a sensitive marker of their activities. Tumor necrosis factor α reducing agents could have a good therapeutic impact.

Introduction

In systemic lupus erythematosus (SLE), B cell hyperactivity and the presence of auto-antibodies are documented together with abnormalities in the T cell immune response [1]. Numerous abnormalities of the cytokine network were shown to play a pivotal patho-physiological role in the T cells, B cells or antigen presenting cell dysfunctions characteristic of the disease. Of these cytokines is the nerve growth factor which is one of neurotrophin family [2,3].

Systemic sclerosis is a disease which affects both the microvasculature and the connective tissue [4]. The pathogenesis is characterized by immune abnormalities, endothelial injury and activation of fibroblasts with consequent collagen accumulation leading to fibrosis of the skin and internal organs [5]. Peripheral vascular involvement and dysregulation of vascular tone is a main feature in particular in the earliest phase of the disease [6].

Neurotrophins are reported as B cell anti-apoptotic and affecting T cells activity, which are implicated in auto immune diseases as SLE, Systemic sclerosis, rheumatoid arthritis [2] and primary Sjogren's syndrome [7]. Nerve growth factor (NGF) has a bio-regulatory effect on the nervous system [8] and endo, auto, paracrine and immuno-modulatory functions [6]. It is believed to play a role both in inflammatory responses and tissue repair [9].

Nerve growth factor is up-regulated in different inflammatory and autoimmune diseases such as rheumatoid arthritis[10], systemic sclerosis[11], SLE in children[12] and autoimmune thyroiditis [13]. Different cytokines such as TNFα , IL 2, INF and IL10 were suggested to affect NGF expression [14].

In systemic sclerosis, NGF induces fibroblast proliferation and collagen production and it was detected in the dermis [15]. It also induces an increase in the number of mast cells and histamine release from fully differentiated mast cells in patients with early systemic sclerosis [11].

Nerve growth factor acts through a specific tyrosine kinase receptor (TrKA) and a receptor belonging to TNFα family. Pro-inflammatory cytokines such as TNFα stimulates NGF production [16]. Nerve growth factor promotes differentiation, growth and survival of peripheral and central neurons. Its receptors are expressed by several immune cells. In addition, mast cells, lymphocytes and eosinophils can produce, store and release NGF [2].

Tumor necrosis factor α is a pro-inflammatory cytokine which is produced primarily by activated monocytes/ macrophages, activated T cells, B cells , mast cells, endothelial cells and fibroblasts. It has different effects on immune cells. It is a growth factor for B lymphocytes [17] and also constitutes an activation and maturation factor of dendritic cells which are essential in immune regulation [18]. The implication of TNFα as a principal player in several inflammatory and autoimmune diseases led to the potential effectiveness of TNFα blocking agents in the biological treatment of such diseases as psoriasis and rheumatoid arthritis [19].

It has been shown that TNFα participates in the activation of vascular endothelium,

regulation of immune response and metabolism of the connective tissue by modulation of fibroblastic function. Scleroderma patients exhibit both systemic and local increase of TNFα levels and consecutively these increases contribute to progression in scleroderma, development of fibrosing alveolitis and skin fibrosis [20,21,22].

Aim of the Study

The aim of this study is to measure the serum level of NGF and TNFα in SLE and SS to investigate their significance in the two diseases.

Subjects and Methods

Cases were collected from the inpatient departments and outpatient clinics of Dermatology & Venereology and Rheumatology & Rehabilitation departments. This study involved three groups:

Group (A): involved 24 patients of SLE which were diagnosed according to the American College of Rheumatology criteria for diagnosis of SLE [23]

They were divided into 13 active and 11 inactive cases according to SLAM score which is based on 24 clinical and 7 laboratory criteria [24]. It ranges from to 0 to 85. A score $\geq$ 20 was considered active.

They were 5 males and 19 females. The disease duration varied from 3 months to 7 years. Ten patients were receiving systemic steroid treatment with or without Azathioprine and 14 patients were not using any treatment. The age ranged from 18 to 46 years.

Group (B): involved 19 patients of systemic sclerosis which were diagnosed according to the criteria of the American Rheumatism Association [25]. They were subdivided into 8 limited and 11 disseminated [26]. Assessment of skin thickening was done using modified skin score of Kahaleh [27] with a scale of 0-3 (0=normal,1= slight thickening, 2= severe thickening, 3= extreme thickening) at 15 anatomic sites on both sides and the maximal score is 45. A score $\geq$ 15 is considered a disseminated case.

The patients were 4 males and 15 females and their age ranged from 24 to 50 years. Their disease duration ranged from 6 months to 11 years. Thirteen patients were using systemic steroids and 6 patients were not.

Group (C): involved 20 apparently healthy subjects, including 7 males and 13 females. Their age ranged from 20 to 48 years.

Patients were subjected to full history taking and clinical examination. Relevant laboratory tests were done; urine analysis, ESR, CBC, CRP, ANA, anti ds DNA. ESR, in addition to Chest x rays, barium swallow and assessment of pulmonary function using computerized pulmonary function apparatus were done for group (B).

The serum level of NGF was measured for the three groups using ELISA technique according to manufacturer instructions using kits provided by R and D systems Inc, USA.

The serum level of TNFα was measured for the three groups using ELISA technique according to manufacturer instructions using kits provided by Roche Diagnostics GmbH, Mannheim, Germany.

Statistical analysis:

Data were checked, entered and analyzed using SPSS version 11. Data were represented as mean ± standard deviation for quantitative variables and number and percentage for qualitative variables. ANOVA, Post hoc test, t test, Chi- square (X2) and Correlation Coefficient (r) were used when appropriate. $P<0.05$ was considered significant.

Results

The characteristics of the studied groups are shown in **table (1).**

NGF:

1-There is a significant difference between the levels of NGF in sera of group (A) patients and control group(C). **Table (2)**

Within the group (A) there is a significant difference in the level between active and inactive disease, being higher in the active cases. **Table (3)**. There is a significant positive correlation with SLAM score, the indicator of disease activity. **Table (6)**

2-There is a significant difference between the level of NGF in sera of group (B) patients and control group(C). **Table (2)**

Within the group (B) there is a significant difference in the level between disseminated and localized disease being higher in disseminated cases. **Table (3)** There is a positive correlation with the modified Kahaleh skin score, the indicator of skin disease dissemination. **Table (6)**

TNFα:

1-there is a significant difference between the level of TNFα in both group (A) and (B) when compared with the control group (C). **Table (4)**

Within group (A) the difference is significant between active and inactive cases, being higher in active cases. **Table (5)**. There is a significant positive correlation with SLAM score. **Table (6)**

2-There is a significant difference between the level of TNFα in sera of group (B) patients and control group(C). **Table (4)**

Within group (B) the difference is significant between disseminated and localized cases. **Table (5).** There is a positive correlation with the modified Kahaleh skin score. **Table (6)**

We found a positive significant correlation between both NGF and TNFα in each disease.

Correlation coefficient(r) in group (A) was 0.67 and P<0.001, (r) in group (B) was 0.7 and P<0.001.

	Group (A) N=24	Group (B) N=19	Group (C) N=20
Age (years)			
mean±SD	33.1±9.8	33.7±9.2	34.1±8.3
range	18-46	24-50	20-48
Gender			
Male	5 20.8	3 15.8	7 35.0
Female	19 79.2	16 84.2	13 65.0
Duration (months)			
mean±SD	4.47±2.5	4.9±3.4	
range	3-84	6-132	

Table (1): characteristics of the studied groups

	NGF Mean±SD	P
Group(A)	332.4±80.4	<0.001*
Group(B)	277.9±102.3	<0.001*
Group (C)	65.9±25.6	

Table (2): Serum level of NGF among studied groups

	No	NGF mean±SD	P
Group(A)			
active	13	367.3±73.96	
inactive	11	283.5±63.3	0.008*
Group (B)			
disseminated	8	216.2±66.1	
localized	11	322.7±102.5	0.019*

Table (3): Serum level of NGF among groups (A) and (B) subgroups

	TNF α Mean±SD	P
Group(A)	42.7±18.1	<0.001*
Group(B)	36.3±11.4	<0.001*
Group (C)	15.9±2.6	

Table (4): Serum level of TNFα among studied groups

	No	NGF mean±SD	P
Group(A)			
active	13	53.4±15.1	
inactive	11	27.6±8.6	0.05*
Group (B)			
disseminated	8	41.1±11.2	
localized	11	29.8±8.1	0.02*

Table (5): Serum level of TNFα among groups(A) and (B) subgroups

	R	P
SLAM		
with NGF	**0.48**	**<0.01***
with TNF α	**0.51**	**<0.05***
Modified Kahaleh score		
with NGF		
with TNF α	**0.53**	**<0.02***
	0.47	**<0.05***

*=P value significant

Table(6):Correlation between serum NGF and TNFα levels and SLAM in group (A) and modified Kahaleh score in group (B)

Discussion

NGF is considered one of Th2 cytokines suggested to be involved in the pathophysiology of SLE through its immuno-modulatory effects on different cells of immune system. It also has an autocrine survival factor for memory B cell [28]. The role played by NGF on cells of the immune system was strengthened by evidence demonstrating that cells normally present in inflammatory tissues such as mast cells and lymphocytes express NGF receptors and are receptive to its action in an autocrine manner [10].

In this study the serum level of NGF was significantly higher in SLE patients when compared with the controls. Furthermore, it was also statistically significantly higher in the SLE patients with active disease when compared with patients with inactive disease. Similar results have been reported in earlier studies [12,29,30].

The elevated levels of NGF in SLE and their correlation with the disease activity raise the question of what role NGF plays in this inflammatory disease. It is not clear whether NGF has a causal role in inflammatory processes or represents a part of defensive mechanisms. It has been shown to accumulate at the inflammation site and it is a potent attractant for neutrophils [31]. Moreover, there are reports that administration of neutralizing anti- NGF antibodies can inhibit development of inflammation [32]. On the other hand; NGF could protect against autoimmune encephalitis by exerting an anti-inflammatory effect [33]. NGF down regulated the immune response by regulating calcitonin gene related peptide (CGRP) synthesis. The increased levels of NGF might represent a physiological mechanism to dampen the inflammatory response [34].

However, it remains to be studied whether the use of NGF or anti- NGF antibodies may have some beneficial effects during the various stages of the disease.

Similarly, the level of NGF in serum was higher in SS patients than healthy subjects

and higher concentration correlated with the disease dissemination. This was found earlier and the level of NGF clearly was related to joint involvement and worsening of lung condition [6]. It has been suggested that NGF is a mediator of the acute phase response and it plays a part in the development of visceral involvement. It was also shown that NGF is expressed in high levels in the skin of patients with SS together with an increase in the number of mast cells thus contributing to the inflammatory process and potentially to disease pathogenesis [10].

The observation that the serum concentration of NGF is elevated in the two autoimmune diseases SLE and SS along with the early evidence that cells of the immune system are able to respond to and/or synthesize NGF contribute to the concept that this molecule might be involved in their pathogenesis.

In the present study we found a significant increase in serum level of TNFα in SLE patients in comparison with controls and higher levels were present in active SLE as compared with patients with inactive SLE. Significant positive correlation was found between TNFα and SLAM score indicating that the TNFα can be used as a sensitive marker for the SLE disease activity. This is in accordance with earlier studies [36,37,38]. Higher levels of TNFα were found especially in lupus nephritis [39].

On the contrary, TNFα levels were reported to be diminished as a function of disease activity [40]. The controversy of these data with our results can be explained by the hypothesis that SLE is a genetic disease and the assumption that the difference in the genetics of different populations may be responsible for the difference in clinical presentation [41].

In our group of SS patients we also found a significant increase of the serum level of TNFα compared with normal subjects. Similarly, the level of TNFα was higher in disseminated than in localized cases indicating its correlation with the spread and severity of the disease.

This confirms earlier studies where serum level of TNFα was found elevated in patients with systemic sclerosis associated with pulmonary fibrosis [42].

The TNFα conveyed induction of pro-inflammatory cytokines, leukocyte chemotaxis and angiogenesis all confirm the postulated role in autoimmune diseases [43]. The approach of treating such inflammatory diseases by blocking TNFα has been confirmed by success of TNFα blockers in cases of psoriasis, rheumatoid arthritis and juvenile idiopathic arthritis [44,45].

Experimental inhibition of TNFα with etanercept in bleomycin induced experimental scleroderma resulted in a significant reduction of the dermal sclerosis, collagen accumulation and infiltrating myofibroblastic cells indicating that TNFα antagonists may be useful in the management of systemic sclerosis [46]. Infliximab therapy was tried successfully in some cases with systemic sclerosis associated with lung fibrosis and pulmonary hypertension [47,48].

An important finding in the present study is the positive correlation between TNFα and NGF in either groups of patients (SLE and SS) which supports the possibility that TNFα is a potent inducer of NGF and that elevated TNFα levels may lead to continued

increase of NGF production [10].

In conclusion, the serum levels of NGF and TNFα were shown to be elevated in both SLE and SS and correlated with the disease activity and severity. This indicates that they are implicated in the pathogenic process of both autoimmune diseases and could be used as sensitive markers for monitoring the disease activity and progress. Moreover, the reduction of TNF α with biological agents could have an influence on the progress of the diseases.

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