

Neurotrophins: Are They Meaningful in Chronic Spontaneous Urticaria?

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SUMMARY Plasma neurotrophin levels are elevated in patients with allergic and autoimmune diseases. The present study was designed to investigate the serum neurotrophin levels in 42 patients displaying chronic spontaneous urticaria, as well as 22 healthy control subjects. Blood samples were obtained from subjects during their first visit to the clinic, and then again after one month of desloratadine therapy. No significant difference was found between patient and control groups in terms of basal serum neurotrophin levels. However, basal nerve growth factor levels in patients whose symptoms persisted despite treatment were significantly lower than those of the drug-responsive patients and the control group. In treatment-responsive patients, nerve growth factor increased after suppression of the symptoms. Our study suggests that chronic spontaneous urticaria is linked with changes serum nerve growth factor levels, and that the deregulation of neurotrophins may contribute to urticaria pathophysiology.

Chronic urticaria (CU) is a common skin disorder that is characterized by recurrent and spontaneously occurring wheals and flares associated with pruritus.¹⁻³ CU predominately affects adults and is approximately twice as common in women as in men. The etiology of chronic urticaria is unknown. In the majority of patients, the causes of the disease cannot be determined and the condition is commonly referred to as chronic spontaneous urticaria (CSU). At least one-third of CSU patients contain functional autoantibodies against the high-affinity IgE receptor (FcεRI), or less commonly IgE itself, which leads to a positive result on autolog serum skin tests (ASSTs).⁴ Incidences of autoimmune thyroid disease are high in CSU patients. Other studies have demonstrated links between CSU and stress, anxiety and depression.⁵ CSU is believed to be triggered by psychological factors and it persists in a vicious circle.

The neurotrophins comprise a family of structurally and functionally related proteins comprising nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), NT-6 and NT-7.⁶ These neurotrophins promote growth, survival and differentiation of developing neurons in the central and peripheral nervous systems. Numerous recent studies have indicated that neurotrophins are also involved in inflammation and play an important role in the pathogenesis of various inflammatory and autoimmune diseases.^{7,8} Plasma neurotrophin levels are elevated in patients with allergic diseases, asthma and

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atopic dermatitis (AD).^{6,9} A 2007 study by Namura *et al.*⁶ proposed that serum BDNF may serve as a useful marker in AD. Toyoda *et al.*¹⁰ reported that plasma NGF levels were markedly increased in patients with AD when compared to non-atopic controls.

While neurotrophins have been shown to play a critical role in the development and maintenance of cutaneous innervation⁸, there is evidence for non-neurotrophic functions of these compounds in the skin. These include the regulation of epidermal proliferation and apoptosis, control of hair follicle development and cycling, and melanogenesis. Moreover, autoimmune inflammatory diseases lead to altered concentrations of circulating NGF, which are associated with changes in cytokine synthesis and/or release.⁸

Together, these findings along with the extensive mast cell degranulation which occurs in urticaria, the following hypotheses were stated: 1) neurotrophin levels may be elevated in patients with active CSU due to mast cell degranulation, 2) CSU, regarded mostly as an autoimmune disease, may cause elevated circulating neurotrophins as seen in other autoimmune diseases, and finally 3) deregulation of neurotrophins may be responsible for mast cell degranulation if CSU is indeed triggered by stress and the subsequent neural effects. The aim of the present study was to investigate circulating levels of neurotrophins (NGF, BDNF and NT-3) in chronic spontaneous urticaria patients.

MATERIALS AND METHODS

Subjects

Forty-two patients suffering from active chronic spontaneous urticaria and 22 healthy subjects were included in the study. The healthy volunteers were selected from medical students and hospital staff to serve as the control group. The ethics committee of Istanbul Faculty of Medicine approved this work and signed consent forms were obtained from each subject involved in the study.

Chronic spontaneous urticaria diagnosis

Diagnosis was based on the elimination of

all the probable causes of chronic urticaria in patients reporting recurrent pruritus, wheals and flares for more than 6 weeks. Exposure to allergenic drugs, foods, insect stings or chemicals was determined by obtaining a detailed history from each patient. After physical examinations, peripheral blood counts, erythrocyte sedimentation rates, liver function tests, urine and stool analyses, thyroid hormone measurements, anti-nuclear antibody detection, and autolog serum skin tests (ASSTs) were performed to ascertain whether patients were suffering from pre-existing infectious or chronic autoimmune diseases. Patients not fulfilling diagnostic criteria for a causal illness or Type I sensitivities were considered to be suffering from CSU, whether or not they produced positive ASSTs.⁸ Among these, patients with daily recurrent hives but not receiving treatment were retained in the study. Patients were requested to rate the severity of their disease using a visual analogue scale, from 0 to 3 (0, none; 1, mild; 2, moderate; and 3, severe).

Ten milliliters of venous blood were acquired from all subjects, and sera were isolated and stored at -70°C until further required. When necessary, patients were administered desloratadin at 5 mg/day for symptomatic therapy, and were requested to return to the clinic a month later. During these later visits, a second blood sample was obtained from each patient.

Neurotrophin detection

Levels of serum NGF, BDNF and NT3 were measured using an enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturers' instructions. The kit used to determine NGF levels was a DuoSet ELISA Development kit (R&D Systems, Minneapolis, MN, USA) while the kit used to measure BDNF and NT-3 was from Chemicon International (CA, USA).

Data analysis

The Student's *t* test was used for group comparisons between chronic urticaria and control subjects for NGF, BDNF and NT-3 levels. The Wilcoxon signed-rank test was used for comparisons between pretreatment and post treatment values. A two-tailed *p* value of < 0.05 was considered signifi-

cant. Pearson and Spearman correlation tests were applied to measure correlations between neurotrophin levels and age, gender, disease duration, disease severity scores, thyroid antibodies or ASSTs.

RESULTS

The mean age for the patient group was 37.4 ± 12.5 years (33 females, 9 males), and for the control group the mean age was 34 ± 9.5 years (17 females, 5 males)(Table 1). According to a self-scoring approach, symptom levels were mild in 8 patients, moderate in 32 patients and severe in 2 patients. Thyroid autoantibodies (antithyroid-peroxidase and anti-tyroglobulin antibodies) were positive in 12 patients, and 20 patients displayed positive ASSTs. There were no significant differences between the patient group and the control group in terms of basal serum NGF, BDNF and NT-3 levels (Fig. 1). The patient group was divided into two parts: those whose urticaria attacks were suppressed after the therapy (group 1, treatment-responsive group); and those who did not respond to drugs and remained symptomatic one month later (group 2, treatment-resistant group). Basal BDNF levels in group 2 were significantly higher, and NGF levels were significantly lower, when these groups were compared to either group 1 or the control group. However, no

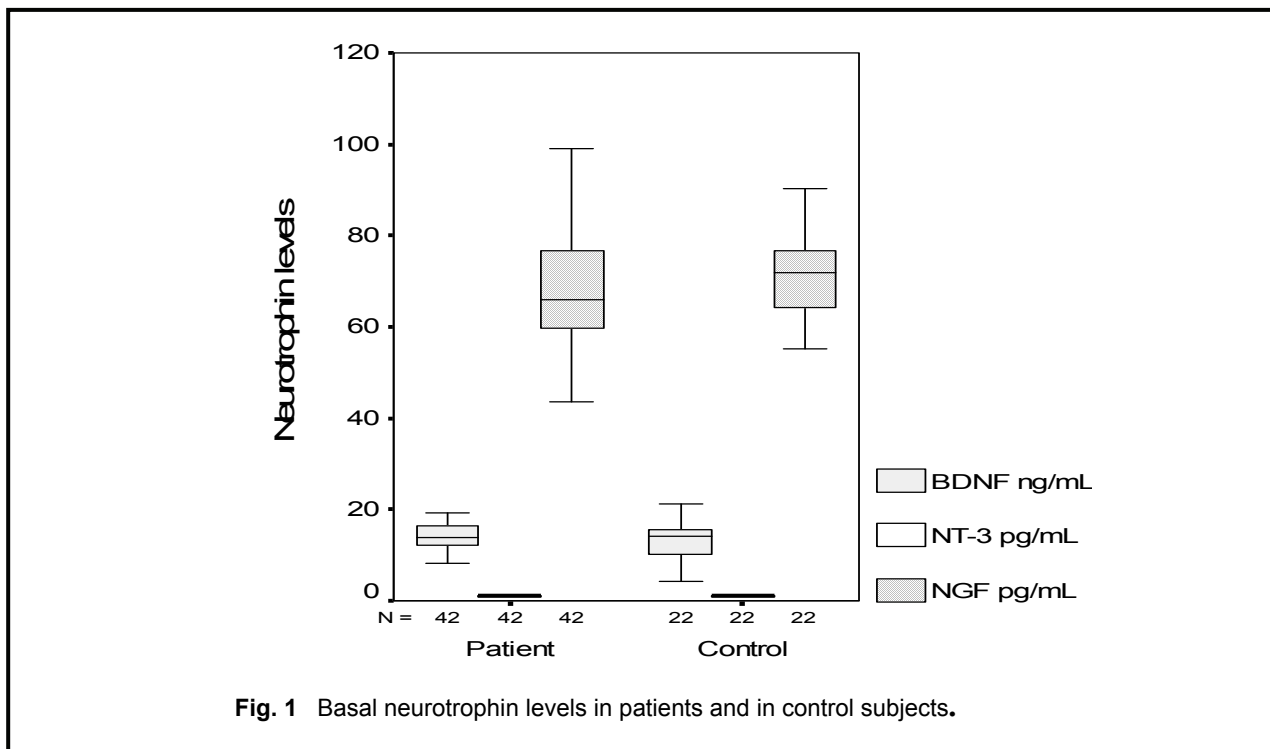
Table 1 Demographic data and disease severity of patients and healthy controls

	Patients (n = 42)	Controls (n = 22)
Gender (female/male)	33/9	17/5
Age ± SD (years)	37.4 ± 12.5	34 ± 9.5
Urticaria severity		
Mild	8	0
Moderate	32	0
Severe	2	0

significant differences in NT-3 levels were observed between any of the groups (Fig. 2). NGF levels in group 1 increased significantly following treatment (compared to pretreatment samples) while no changes were observed for group 2 (Table 2). Correlations could not be determined between neurotrophin levels and age, gender, disease duration, disease severity scores, thyroid antibodies or ASSTs.

DISCUSSION

Serum basal neurotrophins were found to be similar in both patient and control groups. Interestingly, however, NGF levels were significantly lower



in patients whose urticaria persisted despite intensive therapy using antihistamines. Moreover, NGF levels tended to increase following drug therapy in patients who responded well to treatment, while no change was observed in those patients who were resistant to treatment. These findings suggest that NGF play an important role in CSU. Thus far, only a single study has proposed that neurotrophins are elevated in allergic urticaria patients, as well as in allergic rhinoconjunctivitis and asthma patients.⁹ However, these authors did not report recurrence, intensity, duration and triggering of specific sensitivities in their study

on urticaria.

Over the past decade, substantial evidence has been published on the expression of NTs in non-neuronal tissues.^{11,12} A major source of NTs and their receptors outside the nervous system is believed to be cells within the haematopoietic/immune system and the skeletal/connective tissue system.^{11,12} Several studies have demonstrated that NGF is produced by fibroblasts, keratinocytes, mast cells, thymus cells and lymphocytes. These cells were shown not only to synthesize and release NGF under basal condi-

Table 2 Neurotrophin levels in groups 1 and 2 before and after treatment

Neurotrophins	Before treatment	After treatment	<i>p</i> value
BDNF ng/ml			
Group 1	12.6 ± 2.3	13.5 ± 2.4	NS
Group 2	15.1 ± 2.4	15.0 ± 0.8	NS
NT-3 pg/ml			
Group 1	1.06 ± 0.1	1.06 ± 0.1	NS
Group 2	0.9 ± 0.1	0.9 ± 0.05	NS
NGF pg/ml			
Group 1	75.1 ± 10.9	81.0 ± 10.5	0.023
Group 2	62.4 ± 12.5	63.2 ± 11.7	NS

Values are expressed as mean ± SD; NS = not significant.

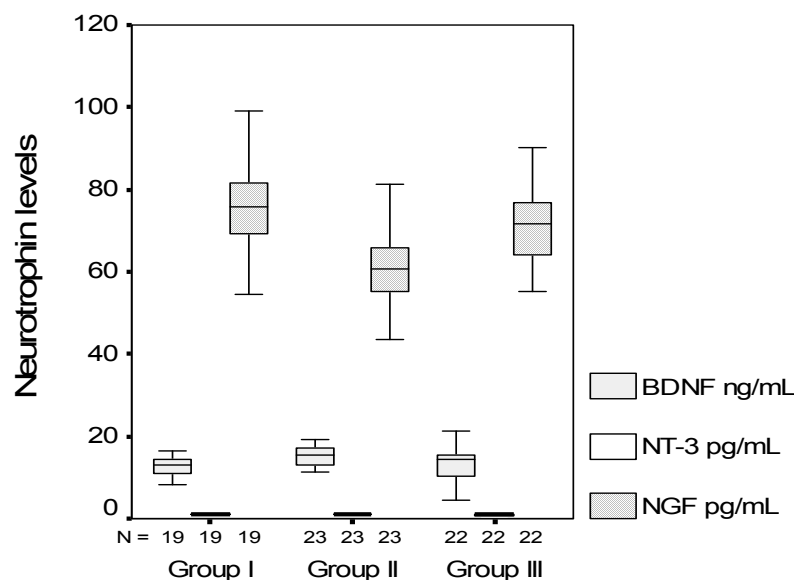


Fig. 2 Comparisons between basal neurotrophin levels and treatment-responsive (group I), treatment-resistant (group II), and the control (group III) groups. Basal BDNF: group 1 versus group 2, $p < 0.02$; group 2 versus control group, $p < 0.04$. Basal NT 3: group 1 versus group 2 versus control group, not significant. Basal NGF: group 1 versus group 2, $p < 0.001$; group 2 versus control group, $p < 0.007$.

tions, but also to express both the low-affinity NGF receptor (p75) and the high-affinity receptor (trkA). Bonini *et al.*⁹ demonstrated that large quantities of NGF are present in the bloodstream during human allergic reactions, and emphasized that the NGF released from degranulating MC's could contribute to enhanced circulating NGF levels. In addition, immune-haematopoietic cells are receptive to the action of NGF, and mast cells were the first non-neuronal cell types identified as NGF targets.¹¹ As an example, *in vitro* NGF exposure to peritoneal MCS induces noncytotoxic degranulation and histamine release, which is mediated through a receptor-ligand interaction. The characteristic clinical symptoms and signs of chronic urticaria comprising recurrent and spontaneous pruritus, wheals and flares are caused by mast cell degranulation. Therefore, high basal blood NGF levels can be expected in symptomatic CSU patients. However, our results as well as the findings of Bonini *et al.*⁹ do not strongly support this expectation, with the exception of BDNF which was slightly higher in the treatment-resistant group. In contrast, basal NGF levels were significantly lower in treatment-resistant patients as compared to treatment-responsive patients and the control subjects. Furthermore, NGF levels increased after urticaria symptoms disappeared in responsive patients. This discrepancy requires further investigation.

There is evidence that neurotrophins play important roles in the pathogenesis of autoimmune diseases.¹² NGF levels are altered in autoimmune inflammatory diseases such as multiple sclerosis, systemic lupus erythematosus and rheumatoid arthritis. The expression of both NGF and its high-affinity receptor (trkA) have been measured in arthritic synovium and chondrocytes, and a functional role of NTs and their receptors in immune-mediated inflammatory diseases has been suggested. Rihl *et al.*¹² reported that the expression of neurotrophins in peripheral synovitis may be either directly or indirectly related to the inflammatory disease process. Some studies have suggested that NGF plays a suppressive role in the development of autoimmune responses. Micera *et al.*¹³ demonstrated that NGF-deprived rats display an increased severity in the clinical signs of experimental allergic encephalomyelitis. Villoslada *et al.*¹⁴ found that NGF exerts its anti-inflammatory effects by down-regulating the production of interferon gamma by T cells infiltrating the CNS, and up-

regulating the production of interleukin-10. Although only 12 patients showed increased thyroid autoantibodies and 20 showed positive ASSTs in the present study, we were unable to definitively exclude autoimmunity as the cause of urticaria in the remaining patients. To this end, we suspect that lower serum NGF levels may be responsible for the resistance to treatment observed in 20 of the patients included in this study. Similarly, the increases in serum NGF following suppression of the symptoms seems to reflect the importance of this neurotrophin in leading to the disappearance of wheals and flares.

It is well known that CSU patients frequently exhibit psychiatric comorbidity.¹⁵ The most common psychiatric diagnoses observed in CSU patients were depression, anxiety and somatoform disorders.^{16,17} Whether psychiatric conditions predispose the onset of CSU or emerge during the course of CSU remains undetermined. However, a few studies have shown that stress and depression are linked to a decrease in serum BDNF levels, and that antidepressant treatment increases the expression of brain-derived neurotrophic factor.^{18,19} The complex interactions between stress, neurotrophins and skin may contribute to the development of chronic urticaria. In this context, alterations in serum neurotrophins may be a consequence of the basic disorder as well being involved in the pathogenesis. Skin biopsy studies from urticarial plaques may also be used to evaluate the roles of neurotrophins in urticaria.

In conclusion, the present study suggests that CSU is associated with alterations of serum neurotrophins, particularly NGF. Deregulation of these neurotrophins may contribute to the pathophysiology of urticaria. Further studies are necessary to clarify the precise roles of neurotrophins in this complex disorder to assist in the development of effective therapeutic strategies.

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