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Review

Decreased nerve growth factor levels in hyperthyroid Graves' ophthalmopathy highlighting the role of neuroprotective factor in autoimmune thyroid diseases

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Abstract

Nerve growth factor (NGF), which is a neurotrophic factor, is involved in autoimmune and inflammatory processes. Serum NGF levels were investigated in 131 patients with autoimmune (95 with Graves' disease, of whom 57 had ophthalmopathy, 19 with Hashimoto's thyroiditis) and nonimmune thyroid diseases (17 with toxic nodular goitre), and 20 controls. NGF levels were measured via enzyme-linked immunosorbent assay. Twenty-nine positive cases for NGF were detected: 21 cases in Graves' disease, 7 cases in Hashimoto's thyroiditis, no case in toxic nodular goitre and one case in controls. NGF levels were higher in patients with Graves' disease and particularly with Hashimoto's thyroiditis compared with controls (1786.47 ± 34.79 pg/ml and 1996.27 ± 77.71 pg/ml vs 1579.16 ± 57.45 pg/ml, $P < 0.049$ and $P < 0.0001$, respectively). Increased NGF levels associated with Graves' hyperthyroidism and correlated with FT_3 ($P < 0.01$). Patients with the presence of antibodies against TSH receptor showed higher NGF levels than those with no antibodies (1938.61 ± 56.44 pg/ml vs 1712.12 ± 54.22 pg/ml, $P < 0.009$). Decreased NGF levels were demonstrated in hyperthyroid Graves' ophthalmopathy compared with those without eye symptoms (1746.65 ± 51.98 pg/ml vs 1910.47 ± 55.62 pg/ml, $P < 0.036$).

NGF may be involved in the pathomechanism of autoimmune thyroid diseases. Decreased NGF levels in hyperthyroid Graves' ophthalmopathy highlight the importance of NGF in the neuroprotection of orbital tissues.

Keywords: Nerve growth factor; Autoimmunity; Graves' disease; Ophthalmopathy; Thyroid diseases

1. Introduction

Nerve growth factor (NGF) is identified as a neurotrophic factor for peripheral sympathetic, sensory neurons and cholinergic neuronal populations [1]. NGF belongs to neurotrophin family, which consists of four members: NGF, BDNF (brain derived neurotrophic factor), NT-3 (neurotrophin-3) and NT-4 (neurotrophin-4). They are essential for the survival and differentiation of developing neurons [2,3]. NGF, as a pleiotropic molecule, is involved in a multitude of biological functions, such as in inflammatory and immune processes, in the activation of pituitary-adrenocortical axis, in skin physiology, in peripheral tissue regeneration, and can act as a modulator in neuro-endocrine regulations [4,5].

A variety of cell sources have been shown to synthesise and secrete NGF in vitro. These are cells from the immune system, including mast cells, eosinophils, lymphocytes, monocytes and macrophages as well as structural cells, such as fibroblasts, epithelial cells and smooth muscle cells [6–9]. NGF mRNA and protein secretion were demonstrated in white and brown adipose tissues reflecting a link between NGF, as an adipokine and sympathetic nervous system in obesity, thermogenesis and tissue reparation [10,11].

Autoimmune Graves' disease is mediated by autoantibodies that bind to the thyrotropin receptor and

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characterized by the stimulation of thyroid hormone production (thyroxine, triiodothyronine). Thyroid-associated ophthalmopathy (TAO) could manifest in 20% of Graves' patients on the initial examination and its prevalence could be estimated in 80% of all cases [12]. Different subtypes of Th cells are activated in thyroid and orbital immune responses and the polarization of Th cells can be influenced by several factors, including dentritic cells, cytokines, and several growth factors [13,14]. The histological features in the orbits are characterized by lymphocytic infiltration in eye muscles and orbital fat tissue with the enlargement of eye muscles and expansion of the orbital fat compartment [15]. The primary autoantigen is unclear and eye muscle fibers are relatively spared in TAO suggesting that extraocular muscle fibers do not represent the primary target [16].

Thyroid hormones are essential in the development of the fetal central nervous system. Thyroid hormones are required for the normal timing of oligodendrocyte precursor cell differentiation and maturation as well as for the injured axonal remyelination [17]. The administration of thyroxine is effective in axonal pathology and in the acute phase of experimental allergic encephalomyelitis [18,19].

The present study is the first report which investigates the serum levels of NGF in patients with autoimmune and nonimmune thyroid diseases. Our results indicate that thyroxine could have an effect on NGF levels in hyperthyroid Graves' patients playing a role in thyroid and orbital neuronal pathology and tissue regenerations.

2. Patients and methods

2.1. Patients

Sera of 131 patients from four different groups and 20 sera of controls were investigated for NGF. The four patient groups were the following: (a) Graves' disease without eye symptoms, in total of 38 patients, 36 women and 2 men, mean age of 42 years. Thirty one patients were hyperthyroid, 5 patients euthyroid and 2 patients hypothyroid. Only euthyroid and hypothyroid cases received therapy at the time of the study. (b) Graves' ophthalmopathy, in total of 57 patients, 49 women and 8 men, mean age of 41 years. Twenty-nine patients were hyperthyroid, 19 patients euthyroid and 9 patients hypothyroid. Duration of ophthalmopathy was 17 ± 4.3 months. The classification of eye symptoms was performed by ophthalmologist and based on the recommendation of American Thyroid Association (ATA) [20]. Only the hyperthyroid cases did not receive therapy. The others were cured with β-blockers, pentoxifyllin, 13 cases received methimazole or propylthiouracil. Six cases were managed with radioiodine therapy. (c) Hashimoto's thyroiditis, in total of 19 patients, 18 women and one man, mean age of 44 years. Twelve patients were euthyroid and 7 patients hypothyroid. None of the patients were managed at the time of the study. (d) Toxic nodular goitre, in total of 17 patients, 16 women and one man, mean age of 58 years. Seven patients did not receive therapy at the time of the study. Radioiodine treatment was applied for management. Twenty healthy subjects, 15 women and 5 men served as controls, mean age of 42 years.

2.2. Methods

2.2.1. Detection of nerve growth factor (NGF)

The serum levels of NGF were measured with enzyme-linked immunosorbent assay (Promega, USA). 96-wells plates coated with anti-NGF polyclonal antibody were applied for the detection. After blocking the nonspecific binding, 100 μl/well of patient sera and standard samples with a dilution 1:2 were added to the plates and incubated with shaking for 6 h at room temperature. After washing, 100 μl/well anti-NGF monoclonal antibody were added and incubated overnight at 4 °C. 100 μl/well anti-rat IgG species-specific antibody conjugated to horseradish peroxidase was used as a tertiary reactant with shaking for 2.5 h at room temperature. After removing the unbound conjugate,100 μl/well TMB (3,3′,5,5′-tetramethylbenzidine) chromogenic substrate and its stop solution, 1 N hydrochloric acid were added to the plates and the colour change was measured at 450 nm. The results were given after representative NGF standard curve using the standard samples of NGF immunoassay systems (7.8–1000 pg/ml).

2.2.2. Detection of thyroid hormones and anti-thyroid antibodies

The levels of thyroid hormones and anti-thyroid antibodies were measured with commercial kits. The detection of FT_4, FT_3, TSH, anti-thyroid peroxidase (anti-TPO) and anti-human thyroglobulin (anti-Htg) antibodies were carried out with luminescence immunoassay (Immunlite, USA). The normal ranges of thyroid hormones, anti-TPO, anti-Htg were as follows: 12–22 pmol/l for FT_4, 2.8–7.1 pmol/l for FT_3, 0.27–4.2 mIU/l for TSH, 0–63 U/ml for anti-TPO, 0–115 U/ml for anti-Htg. Antibodies against TSH receptor (TRAK) were measured with radioimmunoassay (Brahms Diagnostics, Germany), values >14 U/l were regarded as positivity.

2.3. Statistics

The data were presented as means ± SE. The results among the patient groups were evaluated by one-way ANOVA variance analysis using Bonferroni multicomparison post-hoc test. Student's *t*-test was applied for comparison between two groups in relation of thyroid hormones, TSH receptor antibody levels and the presence or absence of ophthalmopathy. Correlation, linear regression and above mentioned statistical procedures were assessed by SPSS statistical program.

3. Results

The detailed levels of thyroid hormones, anti-thyroid antibodies and NGF are shown for each patient group in