Search and identification of thyroid hormones receptors in ocular tissues

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Summary

Aim. To discover and identify thyroid receptors in ocular tissues by means of immunohistochemistry (IHC). **Materials and methods.** The objects of morphological studies were eyes enucleated for terminal painful glaucoma (n = 30). Thyroid receptors searching was performed in the retina and optic nerve, choroidal and optic nerve vessels. IHC reaction was considered as follows: negative – specific cellular staining is absent or less than 10% cells are stained; mild positive – 10-30% cells are stained (+); moderate positive – 30-75% cells are stained (++); highly positive – more than 75% are stained (+++). **Results:** Intensive (4+) IHC staining was revealed in the nuclei of inner and outer nuclear and ganglion cell layers. Mild positive (+) staining was detected in the inner segments of photoreceptors. Granular (3+) IHC staining was revealed in the nuclei of optic nerve glia. In choroidea, endotheliocytes nuclei and 20% stromal cells nuclei were stained. IHC reaction was detected in the cytoplasm of retinal pigment epithelium. **Conclusion.** The data obtained account for the mechanism of neurohumoral associations at the cellular level. According to these data, eye can be considered as a target for thyroid hormones. The causes of morphological and functional abnormalities of visual analyzer peripheral part in thyroid gland disorders are revealed as well.

Keywords: immunohistochemistry, thyroid receptors, neurohumoral association, retina.

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Thyroid hormones are considered to target all human tissues, however, the expression of specific high-affinity triiodothyronine (T3) and thyroxin (T4) -binding receptors is morphologically established only for liver, kidney, brain, testicles and pituitary gland [1-5]. The pattern of

thyroid receptors (THR) expression in pituitary gland, liver and heart is described in detail. Therein, eye and/or orbital structures are almost unexplored. Meanwhile, there can no doubt that thyroid gland pathology is strongly associated with ophthalmic disorders. The most striking examples are Graves' disease and autoimmune thyroiditis and related endocrine ophthalmopathy (EO). Additionally, thyroid gland diseases are considered as a risk factor for glaucoma [6-13]. Finally, primary hypothyroidism and/or thyrotoxicosis naturally result(s) in dysthyroid optic neuropathy that is often not associated with EO. It develops due to hemodynamic disturbances in ocular vessels owing to hormone imbalance [14-17]. We hypothesize the more close relationship on morphological level.

Aim

To discover and identify thyroid receptors in ocular tissues by means of immunohistochemistry (IHC).

Materials and methods

Postmortem eye donation presents difficulties, so the objects of morphological studies were eyes enucleated for terminal painful glaucoma (n = 30).

Thyroid receptors were searched in the retina and optic nerve, choroidal and optic nerve vessels.

The first step was mandatory morphological study of enucleated eyes to verify the diagnosis and to specify morphological abnormalities. The block of excised tissues was fixed with 10% neutral formalin for 3 days. Macro examination was performed following fixation. Blocks containing relevant tissue fragments were embedded in paraffin wax using standard protocol. Ten 4-5 micron sections of the tissue were cut from each block and stained with hematoxylin and eosin. Specimen examination and photorecording was performed using microscope OPTON (Carl Zeiss Meditec, Jena, Germany) with TV-camera at 40×, 125× and 400× magnifications.

The second step was IHC analysis performed on paraffin-embedded sections for standard morphological study. Primary antibodies were monoclonal antibodies against thyroid receptor (Diagnostic BioSystems, Pleasanton, CA), dilution 1:50.

Paraffin-embedded sections were deparaffinated and rehydrated using standard technique. Antigen retrieval was performed by sections heating on water bath in preheated citrate buffer (95-99°C) for 45 min. Specimens were cooled at room temperature for 15-20 min and transferred into phosphate buffer for 5 min. To block endogenous peroxidase, sections were incubated in the dark with 3% peroxide (prepared on distilled water) for 20 min and washed in phosphate buffer for 5 min. To block non-specific antigen binding, sections were incubated with 1% bovine serum albumin for 15 min. Incubation with primary antibodies was performed at 4°C for 40 min. Following this procedure, were washed twice for 5 min in phosphate buffer.

Incubation with secondary antibodies (LSAB^{TM}+ Kit, DAKO, Denmark) was performed at room temperature for 20 min, then sections were washed twice for 5 min. Incubation with streptavidin-labeled antibodies (LSAB^{TM}+ Kit, DAKO, Denmark) was performed at room temperature for 20 min, then sections were washed thrice for 5 min. IHC reaction was visualized using DAB+ (BioGenex, Fremont, CA, USA). Reaction was performed for 5-10 min. Slides were stained with Mayer's hematoxylin and mounted into Canada balsam.

IHC reaction without primary antibodies included was a negative control.

Staining results were studied using Axiolab E re Microscope (Carl Zeiss Meditec, Jena, Germany) at 10×, 20× and 40× magnifications. The localization of IHC staining in a cell (nucleus, cytoplasm, membrane) was registered for each marker.

IHC reaction was considered as follows: negative – specific cellular staining is absent or less than 10% cells are stained; mild positive – 10-30% cells are stained (+); moderate positive – 30-75% cells are stained (++); highly positive – more than 75% are stained (+++).

In addition, staining intensity was considered as mild (+1), moderate (+2), strong (+3) and intense (+4).

Results and discussion

IHC revealed that different ocular structures express thyroid receptors. In particular, intensive (4+) IHC staining was detected in the nuclei of outer (1) and inner (2) nuclear and ganglion cell (3) layers. Mild positive (+) staining was detected in the inner segments of photoreceptors (4) (see Fig. 1).

Granular (3+) IHC staining was revealed in the nuclei of optic nerve glia (1) (see Fig. 2).

In choroidea, endotheliocytes nuclei (1) and 20% stromal cells nuclei (2) were stained. IHC reaction 3+ was detected in the cytoplasm of retinal pigment epithelium (3) (see Fig. 3).

Choroidal endothelium as well as nuclei of endothelial cells lining blood vessels that supply optic nerve (2) express thyroid receptors (see Fig. 2).

Our findings are of theoretical and practical importance for ophthalmologists, endocrinologists and morphologists. We failed to find any information in scientific literature that human eye can be considered as hormone-sensitive or target organ. Target organ is characterized by the ability to "read" the information encoding in the hormone via receptors. Therefore, it is the detection of thyroid receptors in orbital structures that defines their status as "target organs".

IHC analysis identified ocular structures and cells which metabolic homeostasis depends on thyroid hormones level and thyroid receptors expression. According to our data, peripheral part of visual analyzer (including retina and optic nerve) can be considered as a target for thyroid hormones. Active expression of thyroid receptors in outer and inner nuclear and ganglion cells layers, and inner segments of photoreceptors is responsible for dysthyroid optic neuropathy de-

velopment in thyroid gland disorders (i.e., hypothyroidism and/or thyrotoxicosis) in the absence of EO. These data support the integrity of neurohumoral regulation. “Neurohumoral regulation” is defined as a complex physiological process from the transmission of light photon on the first neuron of visual analyzer to image analysis.

Thyroid receptors expression in endothelial and smooth muscle cells of choroidal vessels and optic nerve indicates that thyroid hormones provide not only direct effect on retina and optic nerve but also indirect effect due to hemodynamic abnormalities as a result of thyroid gland disorder. It is known that thyroid hormones influence on hemodynamics by increasing circulating blood volume, regulating vascular tone and resistance as well as cardiac output. Blood flow in different compartments may increase or decrease depending on hormone excess or deficiency. Reduced blood flow results in organ ischemia and hypoxia. Our morphological findings indicate that human eye is not an exception to the rule. Electrophysiological methods verify optic neuropathy development in the absence of compression EO in primary hypothyroidism and thyrotoxicosis [8]. Direct correlation between the degree of visual dysfunction and the level of hemodynamic abnormalities in ocular and orbital vessels is confirmed as well. Our IHC findings explain the mechanism of this correlation and substantiate the causes of morphological functional abnormalities of visual analyzer peripheral part in thyroid gland disorders.

Conclusions

1. Expression of thyroid receptors in outer and inner nuclear and ganglion cells layers, and inner segments of photoreceptors demonstrates neurohumoral association on cellular level. Retina can be considered as a target for thyroid hormones.
2. Thyroid receptors expression in endothelial and smooth muscle cells of choroidal vessels and optic nerve indicates that thyroid hormones provide not only direct effect on retina and optic nerve but also indirect effect due to hemodynamic abnormalities as a result of thyroid gland disorder.
3. IHC findings explain the mechanism of eye-thyroid gland association and substantiate the causes of morphological functional abnormalities of visual analyzer peripheral part in thyroid gland disorders (hypothyroidism and/or thyrotoxicosis).

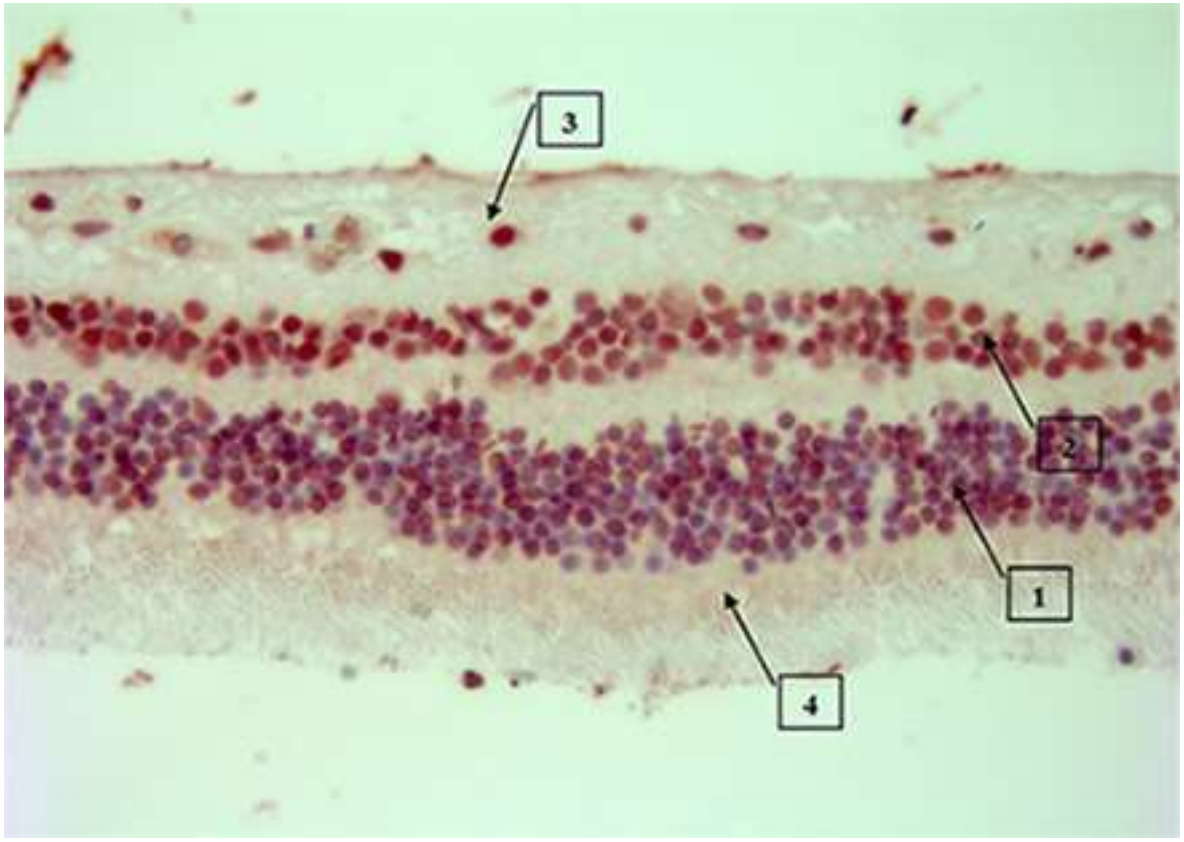

Fig. 1. IHC staining of retina with anti-THR monoclonal antibodies (1 – outer nuclear layer, 2 – inner nuclear layer, 3 – ganglion cells layer, 4 – inner segments of photoreceptors).

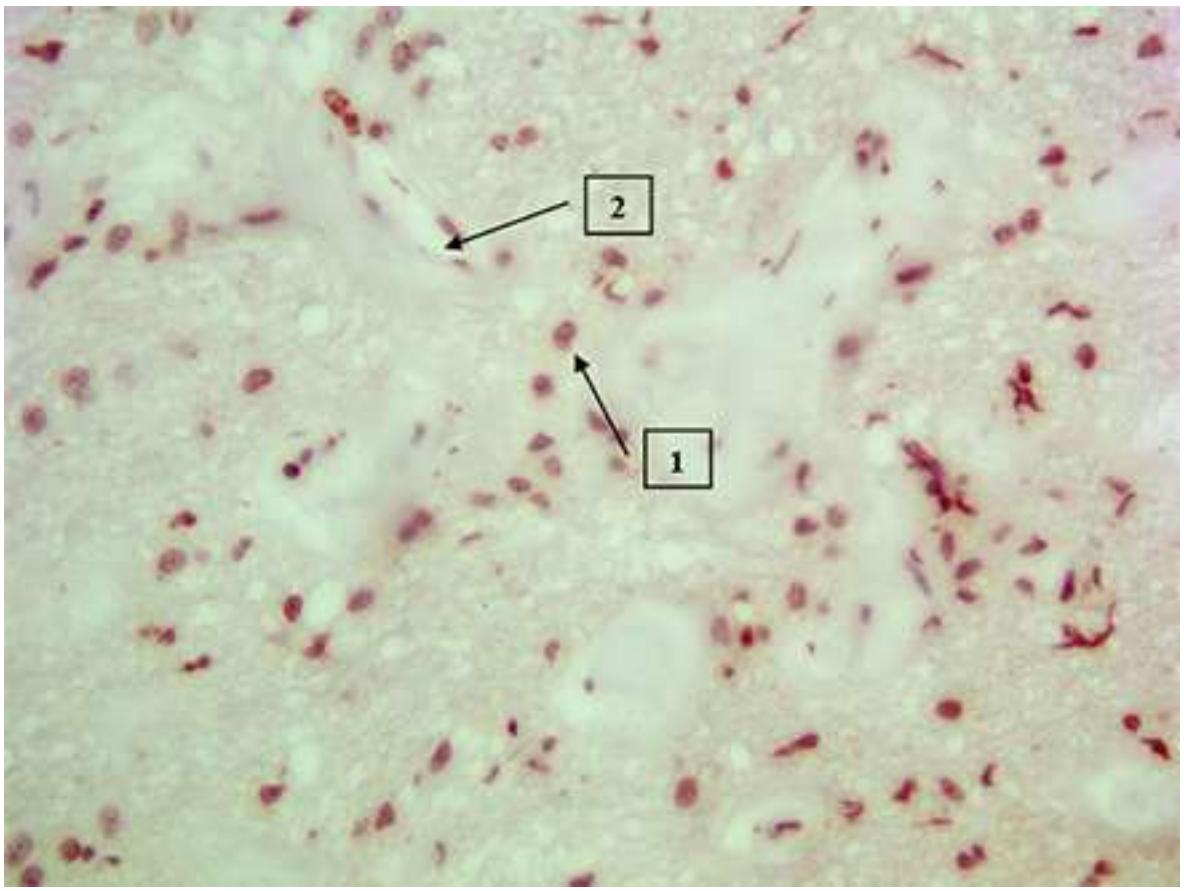

Fig. 2. IHC staining of optic nerve with anti-THR monoclonal antibodies (1 – nuclei of optic nerve glia, 2 – nuclei of endothelial cells of vessels supplying optic nerve).

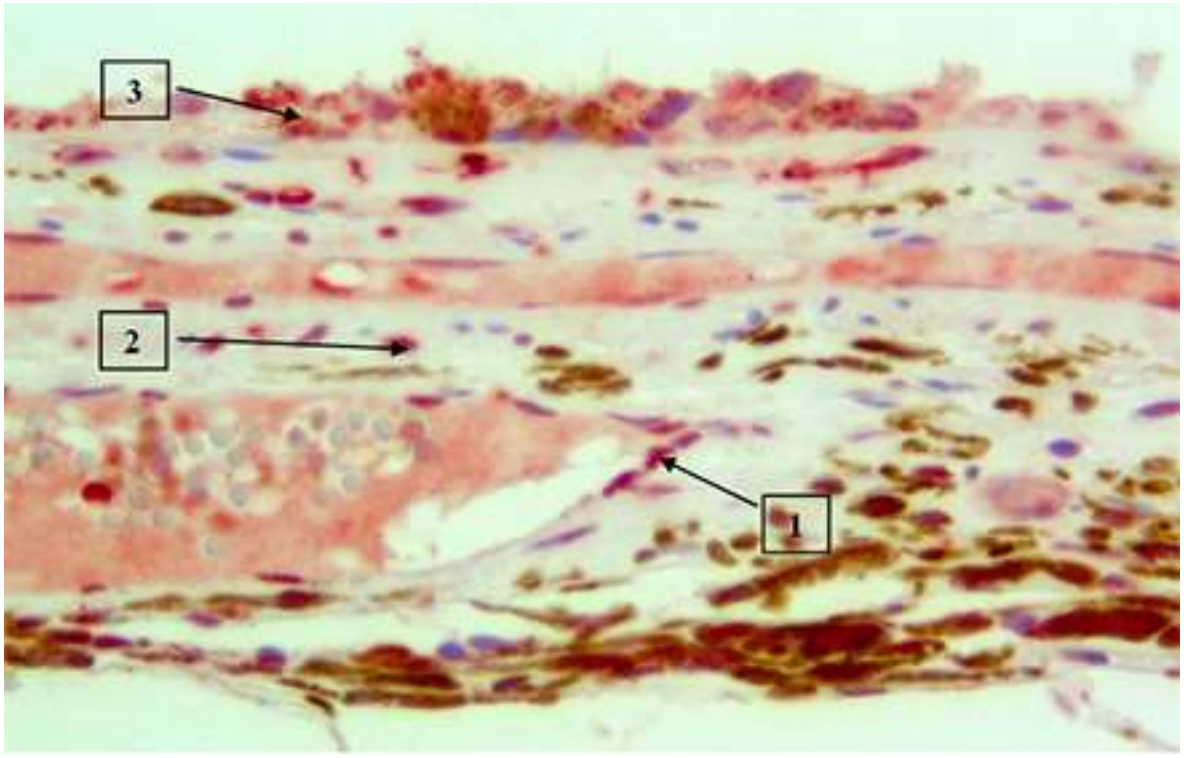

Fig. 3. IHC staining of chroidea with anti-THR monoclonal antibodies (1 – endotheliocytes nuclei, 2 – stromal cells nuclei, 3 – retinal pigment epithelium).

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