Original Article

The effect of experimental hypothyroidism on nasal mucosa

Yeşim Başal[1], Ceren Günel[1], Nuket Özkavruk Eliyatkın[2], Gökhan Cesur[3], Aylin Eryılmaz[1]

[1]*Department of Otolaryngology, Medical Faculty of Adnan Menderes University, Aydın, Turkey*
[2]*Department of Pathology, Medical Faculty of Adnan Menderes University, Aydın, Turkey*
[3]*Department of Physiology, Medical Faculty of Adnan Menderes University, Aydın, Turkey*

ABSTRACT

Objectives: This study aims to investigate the histopathological changes in nasal mucosa and alterations in thyroid hormone receptors in an experimental postnatal hypothyroidism model.

Materials and Methods: Twenty-one Wistar albino newborn rats born from the pregnant rats were enrolled in this prospective randomized study and were divided into two groups: Group 1 (n=11, methimazole, MMI -induced hypothyroidism group) given MMI-water, and Group 2 (n=10, control group), normal tap water. When the offspring reached 90 days, they were decapitated and the nasal mucosa was removed. Thyroid hormone receptor (TR-α, TR-β) status was assessed by immunohistochemical staining.

Results: The difference between hypothyroidism and control groups regarding inflammation was statistically significant ($p<0.001$), as were the differences between hypothyroidism and control groups regarding edema and vascular proliferation ($p<0.001$, $p=0.001$). Staining was identified in sebaceous gland structures with immunohistochemical staining for thyroid hormone receptors. No statistically significant difference was found between the hypothyroidism and control groups regarding TR-α and TR-β.

Conclusion: Thyroid hormone receptors are present in nasal mucosa. Edema, inflammation, and vascular proliferation occur in nasal mucosa due to hypothyroidism.

Keywords: hypothyroidism, rhinitis, thyroid hormone receptor, TR-α, TR-β

Numerous factors can lead to anatomical and histological changes that cause rhinitis symptoms by disrupting normal nasal function.[1] Hormonal rhinitis is discussed within the non-allergic and non-infectious types of rhinitis.[2] Hypothyroidism, pregnancy, puberty, acromegaly, and oral contraceptive use are among the causes of hormonal rhinitis.[3]

In addition to clinical features affecting entire body systems in patients with hypothyroidism, clinical changes also occur in the ear-nose-throat region.[4] Few studies are present in the literature on this subject. In those studies, histological and physiological changes of nasal mucosa were determined in hypothyroidism.[5] Hypertrophy of mucous glands and increase in the basal substance of the connective tissue were identified in the nasal biopsies of patients with hypothyroidism and these were reported to be formed with the effect of increased thyroid stimulating hormone (TSH) release in hypothyroidism.[6]

The aim of our study was to investigate the histopathological changes occurring in nasal mucosa together with the status of thyroid hormone receptors in nasal mucosa in an experimental postnatal hypothyroidism model.

Received: November 04, 2016 **Accepted:** March 15, 2018

Correspondence: Yeşim Başal, MD. Adnan Menderes Üniversitesi Tıp Fakültesi Kulak Burun Boğaz Anabilim Dalı, 09010 Aydın, Turkey.
e-mail: yesimdurgun@gmail.com

Doi: http://dx.doi.org/10.5606/Tr-ENT.2018.91370

MATERIALS AND METHODS

Six-month-old pregnant Wistar albino rats weighing 250-300 grams were included in the study. Vaginal smear test was used for determination of pregnancy. The rats in which semen was detected in the vaginal smear test were considered pregnant. Twenty-one Wistar albino newborn rats born from the pregnant rats were enrolled in this prospective randomized study and were divided into two groups. Group 1 (Methimazole (MMI)-induced hypothyroid group, n=11) constituted the group with hypothyroidism, as MMI administration is typically used for inducing hypothyroidism in rats.[7,8] The mother rats had been fed with normal diet plus MMI-added water. Methimazole (SC-205747A, Santa Cruz Biotechnology, Inc., Dallas, TX) (0.025% wt/vole) was administered in their daily drinking water from birth up to 90 days. The rat offspring were fed with breast milk for 19-22 days, and drank MMI-added water daily up to 90 days, similar to their mothers. Group 2 (n=10) constituted the control group. The mothers of the rats in Group 2 were fed with normal diet and water that did not include MMI. The rat offspring were initially fed with breast milk and then with normal water daily up to 90 days, similar to their mothers. When the offspring were 90 days old, they were decapitated, and the nasal mucosa was removed.

After keeping in 10% formalin for one night, all samples were processed in the automated tissue-tracking device and embedded in paraffin blocks. Sections prepared from the tissues in paraffin blocks were stained with hematoxylin eosin, and conventional morphological evaluation was made. The changes in nasal tissue, surface epithelium and vascular structures, subepithelial edema, inflammation and congestion were evaluated. The scoring was performed as follows: absence of submucosal edema and inflammation was scored as grade 0, light density was scored as grade 1, and moderate density as grade 2. Vascular proliferation and congestion were scored as present or absent.

Immunohistochemical staining evaluated thyroid hormone receptor (TR-α and TR-β) status. Slides with lysine were used for immunohistochemical processing. The process was performed manually, using the streptavidin - biotin peroxidase method. TR-α1 and TR-β1 (1/300; mouse monoclonal; sc-56873, Santa Cruz Biotechnology) were utilized as the primary antibodies. The stained slides were assessed using light microscopy and scoring was made in the presence and absence of staining in the nuclear and cytoplasmic areas. The approval of Medical Faculty of Adnan Menderes University Ethics Committee for Animal Experiments was obtained for the study.

Statistical analysis

All data were analyzed using IBM SPSS version 19.0 (IBM Corp., Armonk, NY, USA) software. Since the number of rats was less than 50, descriptive statistical results were reported as numbers. Mann-Whitney U test was used for determination of the relationship between variables. Fisher chi-square test was used for comparisons between the groups. The level of statistical significance was taken as $p<0.005$.

RESULTS

Eleven hypothyroid and 10 control group samples were analyzed. While two samples had inflammation in the control group, all of the samples in the hypothyroid group had inflammation. The difference between the hypothyroid and control groups regarding inflammation was statistically significant ($p<0.001$). None of the samples in the control group had edema whereas nine samples in the hypothyroid group had edema. The difference between the hypothyroid and control groups was statistically significant regarding edema ($p<0.001$). While vascular proliferation was absent in the entire control group, eight samples in the hypothyroid group had vascular proliferation. The difference between the hypothyroid and control groups was statistically significant regarding vascular proliferation ($p=0.001$). Increased inflammation and vascular proliferation in the group with hypothyroidism is shown in Figure 1a-c.

Immunohistochemical staining for thyroid hormone receptors revealed staining of sebaceous glandular structures whereas no staining was observed in the surface epithelium and subepithelial tissues. TR-α was detected in one control sample and five hypothyroid samples. TR-β was detected in none of the control samples and in three hypothyroid samples. No statistically

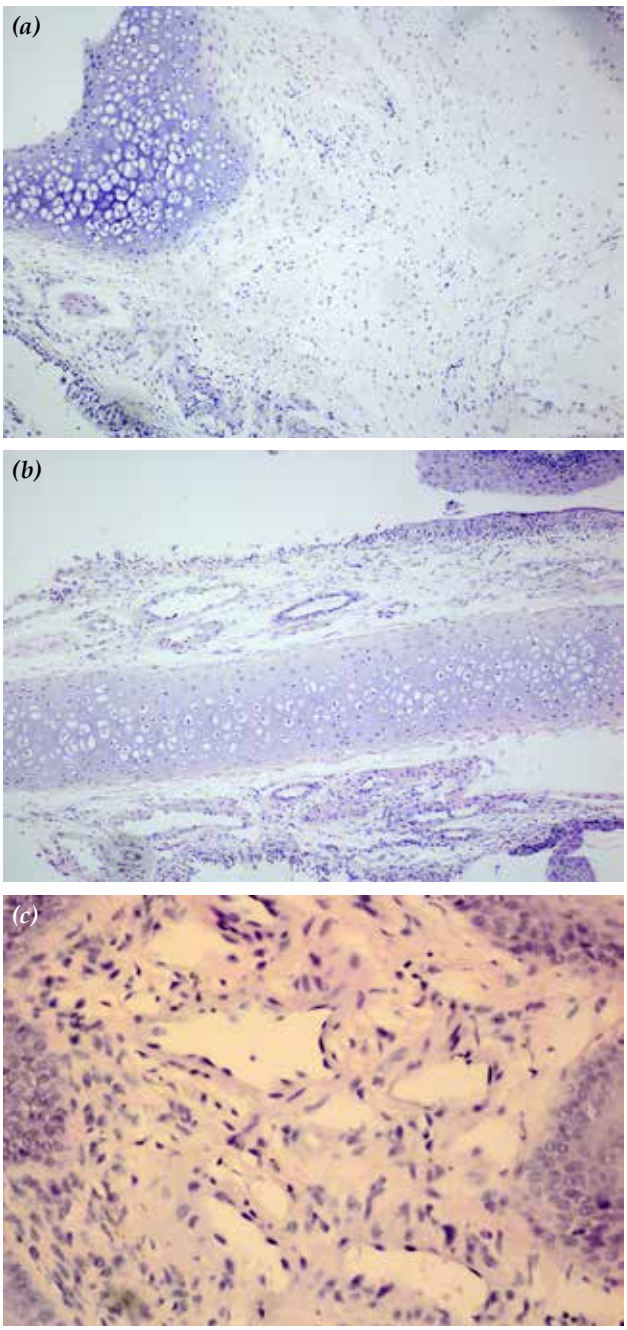

Figure 1. ***(a)*** *Inflammation observed in the edematous stroma in the group with hypothyroidism (H-E×100).* ***(b, c)*** *Moderate-degree inflammation accompanied by vascular proliferation observed in the group with hypothyroidism (1b; H-E×100 and 1c; H-E×200).*

significant differences were found between the control and hypothyroid group samples regarding staining results. Staining with TR-α1 in the group with hypothyroidism is shown in Figure 2a, b.

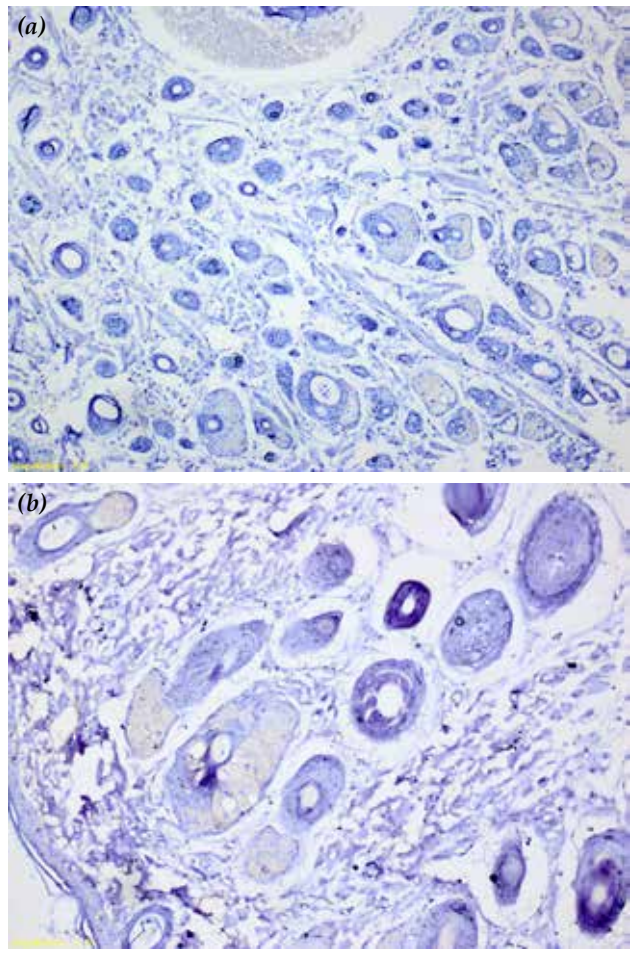

Figure 2. ***(a, b)*** *Staining with TR-α1 in sebaceous glandular structures is observed in the group with hypothyroidism (2a; TR-α1 ×100 and 2b; TR-α1 ×200).*

DISCUSSION

Hypothyroidism has been known to affect the ear, nose, and throat region.[9] The most frequent symptoms of rhinitis related to hypothyroidism are nasal obstruction and rhinorrhea, which are caused by an increase in submucosal connective tissue and hypertrophy of mucous glands.[9,10] The pathophysiological mechanism of hormonal rhinitis associated with hypothyroidism has not been clearly identified.[11] In the hypothyroidism model of Proud and Lange, cilia loss, submucosal infiltration of inflammatory cells and hypertrophy were determined in nasal mucosa.[12] Few studies on this subject are present in the medical literature.

Thyroid hormone T_3 shows its effect by binding to thyroid hormone receptors, which are found in

the nucleus. These nuclear receptors are important mediators in the physiological activities of thyroid hormones.[13] Two genes are present for thyroid hormone receptors in humans; the alpha and beta genes. Thyroid beta-receptors are found more likely in brain, heart, liver, kidney, pituitary gland and hypothalamic tissue.[13]

The presence of TR-α1 and TR-β1 receptors was identified in cochlear hair cells.[14] The TR-α1 and TR-β1 receptors were also shown to be present in the human larynx in males and females, but the same study was not able to demonstrate the presence of receptors in laryngeal mucosa and muscles.[15] The existence of thyroid hormone receptors during embryological developmental was previously determined in the nasal cavities of rats.[16]

TR-α1 and TR-β1 receptors were shown to be increased in the epididymis of rats with hypothyroidism.[17] Thyroid hormone receptors were shown to be increased in the pituitary glands of rats with hypothyroidism when compared to euthyroid rats.[18] Also, an increase in thyroid hormone receptors was determined in the brains of rats in which hypothyroidism was created.[19] An increase of edema findings was determined together with TR-α1 and TR-β1 in the larynges of rats in which hypothyroidism was created when compared to the normal group.[20]

In our study, inflammation, edema, and vascular proliferation were found to be more intense in the group with hypothyroidism when compared to the control group. Hypothyroidism was found to lead to increasing inflammation, edema, and vascular proliferation; these findings were consistent with the literature.

We identified thyroid hormone receptors in sebaceous glandular structures in our study. Although differences were found between the groups regarding thyroid hormone receptors, these differences were not statistically significant. We were not able to encounter any other study in the medical literature that showed thyroid hormone receptors in the sebaceous glandular structures of the nasal mucosa.

Thyroid hormone receptors were shown to have an anti-inflammatory effect in one study.[21] Thyroid hormone receptors with anti-inflammatory effect may increase as the hypothyroidism-related nasal inflammation increases. The absence of a statistically significant difference between groups regarding receptors may be related to the duration of exposure to hypothyroidism and the degree of inflammation in our study. A longer duration of hypothyroidism may be considered to increase the significance of the difference regarding the thyroid hormone receptors and new studies may be planned.

In conclusion, edema, inflammation, and vascular proliferation occur in the nasal mucosa due to hypothyroidism. Thyroid hormone receptors are present in the sebaceous glands of the nasal mucosa. Our study suggests that thyroid hormone receptors may play a role in the mechanism of action of hypothyroidism on nasal mucosa and shows that new studies related to thyroid hormone receptors are needed.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Gluckman JL, Stegmoyert R. Nonallergic rhinitis. In: Paparella MM, Shumrick DA, Gluckman JL, Meyerhoff WL, editors. Otolaryngology. Vol 3. 3rd ed. Philadelphia: W.B. Saunders; 1991. p. 1889-95.
2. Günel C, Başak HS, Güney E. The relationship between hypothyroidism and rhinitis Tr-ENT 2010;20:163-8.
3. Scadding GK. Non-allergic rhinitis: diagnosis and management. Curr Opin Allergy Clin Immunol 2001;1:15-20.
4. El-Shafie KT. Clinical presentation of hypothyroidism. J Family Community Med 2003;10:55-8.
5. Black RR, Maxon HR. Benign diseases of the thyroid gland. In: Richard Z, editor. Otolaryngology. 3rd ed. Philadelphia: W.B. Saunders; 1991. p. 2483-97.
6. Weisskopf A. Connective tissue: a synthesis of modern thought and its impact on the understanding of nasal disease. Laryngoscope 1960;70:1029-59.
7. Kocaturk T, Ergin K, Cesur G, Evlicoglu GE, Cakmak H. The effect of methimazole-induced postnatal hypothyroidism on the retinal maturation and on the Sirtuin 2 level. Cutan Ocul Toxicol 2016;35:36-40.
8. Sack J, Weller A, Rigler O, Rozin A. A simple model for studying the correction of in utero hypothyroidism in the rat. Pediatr Res 1995;37(4 Pt 1):497-501.
9. Ritter FN. The effects of hypothyroidism upon the ear,

nose and throat. A clinical and experimental study. Laryngoscope 1967;77:1427-79.
10. Dykewicz MS, Fineman S, Skoner DP, Nicklas R, Lee R, Blessing-Moore J, et al. Diagnosis and management of rhinitis: complete guidelines of the Joint Task Force on Practice Parameters in Allergy, Asthma and Immunology. American Academy of Allergy, Asthma, and Immunology. Ann Allergy Asthma Immunol 1998;81(5 Pt 2):478-518.
11. Salib RJ, Harries PG, Nair SB, Howarth PH. Mechanisms and mediators of nasal symptoms in non-allergic rhinitis. Clin Exp Allergy 2008;38:393-404.
12. Proud GO, Lange RD. The effect of thyroidectomy on the nasal mucosa of experimental animals. Trans Am Laryngol Rhinol Otol Soc 1957;59:46-55.
13. Yen PM. Genomic and nongenomic actions of thyroid hormones. In: Braverman LE, Utiger RD, editors. The thyroid: Fundamental and clinical text. Philadelphia: Lippincott Williams and Wilkins; 2005. p. 135.
14. Dettling J, Franz C, Zimmermann U, Lee SC, Bress A, Brandt N, et al. Autonomous functions of murine thyroid hormone receptor TRα and TRβ in cochlear hair cells. Mol Cell Endocrinol 2014;382:26-37.
15. Altman KW, Haines GK 3rd, Vakkalanka SK, Keni SP, Kopp PA, Radosevich JA. Identification of thyroid hormone receptors in the human larynx. Laryngoscope 2003;113:1931-4.
16. Nagasawa T, Suzuki S, Takeda T, DeGroot LJ. Thyroid hormone receptor beta 1 expression in developing mouse limbs and face. Endocrinology 1997;138:1276-81.
17. De Paul AL, Mukdsi JH, Pellizas CG, Montesinos M, Gutiérrez S, Susperreguy S, et al. Thyroid hormone receptor alpha 1-beta 1 expression in epididymal epithelium from euthyroid and hypothyroid rats. Histochem Cell Biol 2008;129:631-42.
18. Li M, Boyages SC. Expression of beta2-thyroid hormone receptor in euthyroid and hypothyroid rat pituitary gland: an in situ hybridization and immunocytochemical study. Brain Res 1997;773:125-31.
19. Chattopadhyay N, Kher R, Virmani J, Godbole MM. Differential expression of alpha- and beta-thyroid hormone receptor genes in the developing rat brain under hypothyroidism. Biol Neonate 1995;67:64-71.
20. Eryilmaz A, Gunel C, Eliyatkin N, Cesur G, Ture M, Basal Y. Laryngeal effect of experimental postnatal hypothyroidism: do thyroid hormone receptors change? Tr-ENT 2016;26:164-8.
21. Billon C, Canaple L, Fleury S, Deloire A, Beylot M, Dombrowicz D, et al. TRα protects against atherosclerosis in male mice: identification of a novel anti-inflammatory property for TRα in mice. Endocrinology 2014;155:2735-45.

