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HEAD AND NECK

Histopathological changes of parotid and larynx in hypothyroid rats: experimental study

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Abstract

Objectives In this study, we aimed to investigate the laryngeal and parotid histopathological alterations in rats with experimentally induced postnatal hypothyroidism.

Materials and methods 200–300 g weighed Wistar albino rats were included in this study. The rats were randomly divided into four groups: group 1 is control and the other groups are experimental groups. Food and water were supplied ad libitum in group 1, no medication was administered. Propylthiouracil (PTU) was administered intraperitoneally for 15 days in group 2; for 30 days in group 3, for 45 days in group 4. The larynx and parotid glands of the rats were removed and intracardiac blood samples were collected for thyroid-stimulating hormone (TSH) analysis under anesthesia (ketamine hydrochloride, 100 mg/kg) 24 h after the last PTU injection. The same procedures were done for the control group at day 46. Histopathological evaluation was done for all the specimens.

Results While submucosal vascular dilatation was significantly higher in the experiment groups ($p < 0.05$), there was not a significant difference in lamina propria edema, inflammation, goblet cell loss, cilia loss between the groups in larynx specimens. In parotid gland specimens, serous asinus atrophy, stromal connective tissue increase were significantly higher in experiment groups ($p < 0.05$). In addition, there was a significant difference in nuclear morphology between control and experimental groups ($p < 0.05$).

Conclusion The results of the study showed that hypothyroidism may have effect on inflammatory procedure by causing vascular dilation in larynx and serous asinus atrophy nucleus changes, connective tissue increase in stroma in parotid gland.

Keywords Hypothyroidism · Rat · Larynx · Parotid · Xerostomia · Reinke's edema · Hoarseness

Introduction

The thyroid gland is an endocrine gland that is located in the anterior neck with two lobes and an isthmus connecting them to each other. The thyroid gland is responsible for the secretion of T3 and T4 hormones which exert a broad range of effects on almost all systems of the body including primarily growth and development, cardiovascular, neuromuscular, and gastrointestinal systems. Thyroid gland functions are controlled by thyrotropin releasing hormone (TRH) secreted by the hypothalamus and thyroid-stimulating hormone (TSH) secreted by the pituitary gland.

Hypothyroidism is associated with low plasma thyroid hormone levels and it may be acquired or congenital [1, 2]. Hypothyroidism may also result from hyperthyroidism treatment [1].

Previous studies showed that thyroid hormones may also affect functions of the larynx. Ritter et al. stated that hypothyroidism causes voice disorders including hoarseness and decreased fonation time [3]. Although the pathophysiological mechanism is not known exactly, it is considered that this condition results from the increased accumulation of fluid in the vocal folds together with the accumulation of polysaccharides and thickening of the vocal folds [4]. This

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condition is called as Reinke's edema clinically and it is a chronic edematous lesion in which both vocal folds are affected along their entire length [5].

According to literature, the most commonly seen finding in salivary glands in hypothyroidism is enlargement [6]. The changes in nucleus, asinus, ductal system, and glandular connective tissue were evaluated histopathologically. Serous acinar atrophy, nuclear morphological changes (small, irregular hyperchromatic nuclei), and an increase in the connective tissue between serous acini were observed on the parotid gland specimens [7]. In another study, it is concluded that, hypothyroidism may have effect on amylase secretion stimulated by 5-hydroxytryptamine (5-HT) from the parotid gland [8]. As a result of these mechanisms, salivation decreases, xerostomia develops and these cause a severe problem regarding oral and dental health [9].

The aim of this study was to investigate the effect of duration of hypothyroidism on larynx and parotid histopathology. The underlying background of this aim was to predict the answer to the question whether early initiation of hypothyroidism treatment may contribute to the prevention of laryngeal and parotid pathologies due to hypothyroidism. In this way, we can provide a contribution to elucidate pathophysiological base of common complaints in otolaryngology practice as xerostomia and voice disorders and find alternative ways to solve these problems.

Materials and methods

This study was performed on 32 female Wistar albino rats weighing 200–300 g after receiving approval of the Experimental Animal Ethics Committee dated 04.01.16 and numbered 009.2016.mar. The rats were maintained in an environment at a temperature of 22 ± 2 °C, 65–70% of humidity with a 12-h light/dark cycle. There was no food restriction and food and water were provided them ad libitum.

The animals were divided into four groups one as control and three as an experiment and each of them including eight rats. While propylthiouracil (PTU) (Sigma Chemical Company, Poole, UK) was administered intraperitoneally with a dose of 10 mg/kg body-weight in the experiment groups for 15, 30, and 45 days; physiological saline was administered in the control group [control group (Group 1), 15-day hypothyroid group (Group 2), 30-day hypothyroid group (Group 3), and the 45-day hypothyroid group (Group 4)].

24 h after the last PTU injection, intracardiac blood samples were taken for the measurement of TSH and the larynx and parotid glands of each experiment group were removed under anesthesia (using 100 mg/kg body-weight ketamine hydrochloride [Ketalar®, Pfizer İlaçları Ltd Şirketi, Istanbul, Turkey]) (Group 2 at day 15; Group 3 at day 30; Group 3 at day 45; Group 1 (control group) at day 45) (Fig. 1). The rats were sacrificed by decapitation. The specimens were kept in 10% formalin (pH 7.4) for one night and then subjected to the procedure in the automated tissue tracking device.

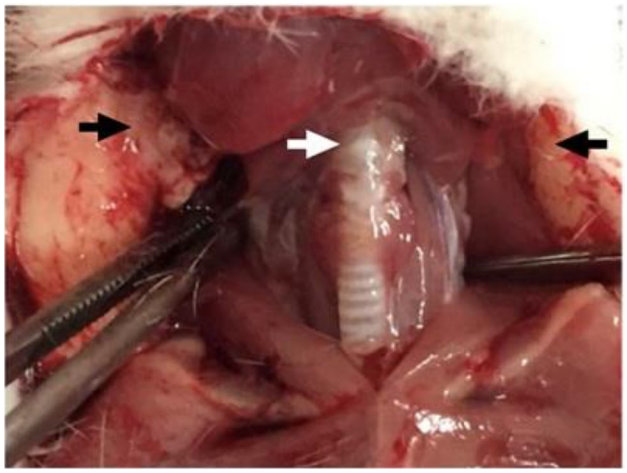

Fig. 1 Rat larynx (white arrow) and parotid glands (black arrows)

One rat in the control group was found to be dead in the cage during the experiment. The remaining seven rats were kept under normal laboratory circumstances for 45 days and, thereafter, their parotid gland and larynx specimens were removed at day 46 by performing the same procedures in other groups.

Biochemical investigation

Blood samples taken from rats were placed in serum gel tubes and centrifuged at 2000 rpm for 20 min. Blood samples obtained were placed in Eppendorf tubes and stored at temperature − 20 °C until the date of the study. Rat serum TSH analysis was performed using a commercial test kit (SunRed Biotechnology, Cat.Nr: 201-11-0181-48T) and sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method.

Histopathological investigation

After the specimens kept in 10% formalin, dissections were embedded into paraffin blocks. Laryngeal specimens were sliced into 5 µm thick sections and parotideal specimens were sliced into 10 µm thick sections. All slices were stained with hematoxylin–eosin and examined in light microscopy with 20×, 40× magnification. Then the differences between groups were compared.

To eliminate any bias, all preparations were coded randomly and assessed by one pathologist. The pathologist graded the laryngeal and parotideal specimens to assess them quantitatively. Edema, vascular dilatation, and inflammation were used as parameters to evaluate specimens

Table 1 Comparison of TSH values and weight between groups

Variable	Control ($n=7$)	15-day hypothyroid ($n=8$)	30-day hypothyroid ($n=8$)	45-day hypothyroid ($n=8$)	p
Weight (g)	233.14 ± 38.8	231.75 ± 23.25	246.63 ± 30.04	246.38 ± 33.97	0.678*
TSH (IU/mL)	0.84 (0.78–0.99)	1.39 (1.29–1.67)	1.82 (1.45–2.33)	2.80 (1.85–5.77)	**$p<0.001$****

Bold values denote statistical significance at the $p < 0.05$ level. p-value less than 0.05 was statistically significant

*p value belongs to one-way ANOVA test

**p value belongs to the Kruskal–Wallis test. Paired comparison for TSH: control-15: $p<0.001$, control-30: $p<0.001$, control-45: $p<0.001$, 15–30: $p<0.001$, 15–45: $p<0.001$, 30–45: $p=0.005$

Table 2 Distribution of TSH levels between groups

	TSH (IU/mL) Average ± SD (median)
Group 1	0.86 ± 0.07 (0.8)
Group 2	1.45 ± 0.15 (1.4)
Group 3	1.88 ± 0.28 (1.8)
Group 4	2.97 ± 1.21 (2.8)
p	**0.001***

Bold values denote statistical significance at the $p < 0.05$ level. p-value less than 0.05 was statistically significant

Kruskal–Wallis test *$p<0.05$

histopathologically. In the system constituted to evaluate edema and vascular dilatation in light microscopy, grades were expressed as follows: grade 0 (absent), grade 1 (mild), grade 2 (medium), and grade 3 (marked). Polymorphonuclear leukocytes (PMNL) and lymphocytes were counted to evaluate the severity of inflammation. Grades were determined as follows: Grade 0 (no inflammation; 1–20 lymphocyte infiltration, PMNL was absent), Grade 1 (mild inflammation; 21–50 lymphocyte infiltration, 1–2 PMNL infiltration), Grade 2 (severe inflammation; 51–80 lymphocyte infiltration, 3–10 PMNL infiltration).

Results

All results are summarized in Tables 1, 2, 3, 4, 5, 6, 7 and Figs. 2, 3, 4.

Lamina propria edema, submucosal vascular dilatation, mucosal inflammation, goblet cell loss, and cilia loss were used as parameters to compare the histopathological

Table 3 The relationship between laryngeal pathological parameters and groups

Larynx	Control n (%)	15-day H n (%)	30-day H n (%)	45-day H n (%)	p	Paired comparison p
Lamina propria edema						
Absent	7 (100%)	7 (87.5%)	7 (87.5%)	6 (75%)	0.886	
Present	0 (0%)	1 (12.5%)	1 (12.5%)	2 (25%)		
Vascular dilatation						
Mild	3 (42.9%)	0 (0%)	0 (0%)	0 (0%)	**0.025**	**Paired comparison p value** 15–30: 15–45: 30–45: **> 0.05**
Medium	4 (57.1%)	6 (75%)	7 (87.5%)	8 (100%)		
Severe	0 (0%)	2 (25%)	1 (12.5%)	0 (0%)		
Inflammation						
Absent	3 (42.9%)	5 (62.5%)	6 (75%)	5 (62.5%)	0.695	
Present	4 (57.1%)	3 (37.5%)	2 (25%)	3 (37.5%)		
Goblet cell loss						
Present	3 (42.9%)	3 (37.5%)	5 (62.5%)	5 (62.5%)	0.711	
Absent	4 (57.1%)	5 (62.5%)	3 (37.5%)	3 (37.5%)		
Cilia loss						
Present	3 (42.9%)	3 (37.5%)	3 (37.5%)	5 (62.5%)	0.777	
Absent	4 (57.1%)	5 (62.5%)	5 (62.5%)	3 (37.5%)		

Bold values denote statistical significance at the $p < 0.05$ level. p-value less than 0.05 was statistically significant

H Hypothyroidy

*p values belong to Fisher–Freeman–Halton and Fisher's exact tests. Results of paired comparison for vascular dilatation; Control-15: $p=0.058$, Control-30: $p=0.128$, Control-45: $p=0.077$, 15–30 $p=1$, 15–45: $p=0.467$, 30–45: $p=1$

Table 4 The relationship between parotideal pathological parameters and groups

Parotid	Control *n* (%)	15-day H *n* (%)	30-day H *n* (%)	45-day H *n* (%)	*P*	Paired comparison *p*
Serous acinar atrophy						
Present	0 (0%)	1 (12.5%)	5 (62.5%)	8 (100%)	***p* < 0.001**	Control-15: 1 Control-30: **0.026** Control-45: ***p* < 0.001** 15–30: 0.119 15–45: **0.001** 30–45: **0.200**
Absent	7 (100%)	7 (87.5%)	3 (37.5%)	0 (0%)		
Nuclear morphology						
Large, round, euchromatic	7 (100%)	6 (75%)	5 (62.5%)	5 (62.5%)		
Mixed	0 (0%)	2 (25%)	3 (37.5%)	1 (12.5%)	217	–
Small, irregular, hyperchromatic	0 (0%)	0 (0%)	0 (0%)	2 (25%)		
Increase in the connective tissue						
Present	3 (42.9%)	8 (100%)	8 (100%)	8 (100%)	1	Control-15: **0.026** Control-30: **0.026** Control-45: **0.026** 15–30: > **0.05** 15–45: > **0.05** 30–45: > **0.05**
Absent	4 (57.1%)	0 (0%)	0 (0%)	0 (0%)		

Bold values denote statistical significance at the $p < 0.05$ level. *p*-value less than 0.05 was statistically significant

H Hypothyroidy

Table 5 ROC curve analysis results

Area the under ROC curve (AUC)	1
95% confidence interval	0.88–1
p value	$p < 0.001$
Cut-off	> 0.99
Sensitivity–specificity	% 100–100

Bold values denote statistical significance at the $p < 0.05$ level. *p*-value less than 0.05 was statistically significant

changes in larynx specimens between groups (Fig. 3a–c). On the other hand, serous acinar atrophy, changes in nucleus morphology, and increase in stromal connective tissue were used for the same purpose in parotid specimens (Fig. 4a–c). After cut-off value of TSH was determined, histopathological results of all groups were also compared according to TSH levels.

No differences were found between groups in terms of weight ($p = 0.678$) (Table 1). When TSH levels of rats in the control and experiment groups were investigated, there was a significant difference between groups ($p < 0.001$) (Table 2). This difference results from an increase in TSH levels of experiment groups compared to the control group

Table 6 The relationship between pathological laryngeal parameters and TSH groups

Larynx	TSH < 0.99 *n* (%)	TSH > 0.99 *n* (%)	*p*
Lamina propria edema			
Absent	6 (22.2%)	21 (77.8%)	0.561
Present	0 (0%)	4 (100%)	
Vascular dilatation			
Mild	3 (100%)	0 (0%)	**0.009**
Medium	3 (12%)	22 (88%)	
Severe	0 (0%)	3 (100%)	
Inflammation			
Absent	2 (10.5%)	17(89.5%)	0.174
Present	4 (33.3%)	8 (66.7%)	
Goblet cell loss			
Present	3 (18.8%)	12 (80%)	1
Absent	3 (20%)	13 (81.3%)	
Cilia loss			
Present	3 (21.4%)	11 (78.6%)	1
Absent	3 (17.6%)	14 (82.4%)	

Bold values denote statistical significance at the $p < 0.05$ level. *p*-value less than 0.05 was statistically significant

H Hypothyroidy

**p* values belong to Fisher–Freeman–Halton and Fisher's exact tests

Table 7 The relationship between pathological parotideal parameters and TSH groups

Parotid	TSH < 0.99 *n* (%)	TSH > 0.99 *n* (%)	*P*
Serous acinar atrophy			
Present	0 (0%)	14 (100%)	**0.021**
Absent	6 (35.3%)	11 (64.7%)	
Nuclear morphology			
Large, round, euchromatic	6 (26.1%)	17 (73.9%)	0.401
Mixed	0 (0%)	6 (100%)	
Small, irregular, hyperchromatic	0 (0%)	2 (100%)	
Increase in the connective tissue			
Present	2 (7.4%)	25 (92.6%)	***p* < 0.001**
Absent	4 (100%)	0 (0%)	

Bold values denote statistical significance at the $p < 0.05$ level. *p*-value less than 0.05 was statistically significant

H Hypothyroidy

**p* values belong to Fisher–Freeman–Halton and Fisher's exact tests

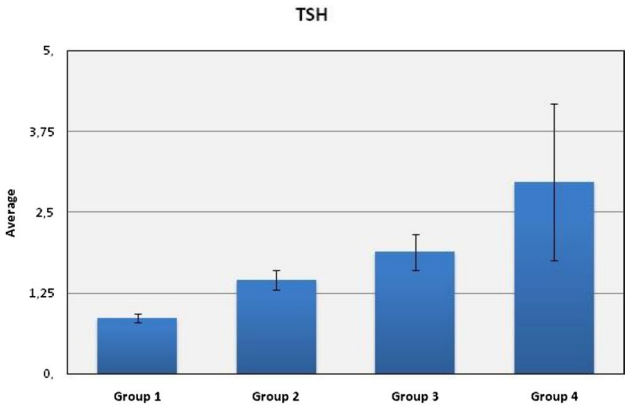

Fig. 2 TSH results distribution between groups

as the number of hypothyroid day increases. In consequence of paired comparison performed for TSH levels, there was a significant difference between groups at the significance level of $\alpha^* = \alpha/k = 0.05/6 = 0.008$. TSH levels of rats in the control group were found to be less than 15, 30, and 45-day hypothyroid groups ($p < 0.05$); TSH levels of rats in the 15-day hypothyroid group were found to be less than 30 and 45-day hypothyroid groups ($p < 0.05$); TSH levels of rats in the 30-day hypothyroid group were found to be less than 45-day hypothyroid group ($p < 0.05$) (Table 2) (Fig. 2).

When the groups were compared according to pathological parameters (lamina propria edema, inflammation, goblet cell loss, and cilia loss); there was no significant difference ($p > 0.05$). In contrast, in terms of vascular dilatation, there was a significant difference between the control and experiment groups ($p = 0.025$) (Table 3).

When the histopathological findings of parotid specimens were investigated according to groups, serous acinar atrophy was found significantly different between the control and 30-day hypothyroid group, the control and 45-day hypothyroid group, 15-day and 45-day hypothyroid groups. In addition, it was important to see that as the number of hypothyroid days increases, serous acinar atrophy increases.

Another finding showed that there was no significant difference between groups according to nuclear morphology ($p = 0.217$).

When the groups were compared in terms of connective tissue increase, although there was a significant difference between hypothyroid groups and the control group ($p < 0.05$), no significant difference was determined among hypothyroid groups (Table 4).

Determination of the cut-off value for TSH level

The results of the analysis performed to determine the cut-off value for TSH level of the hypothyroid and control groups in the study are shown in Table 5.

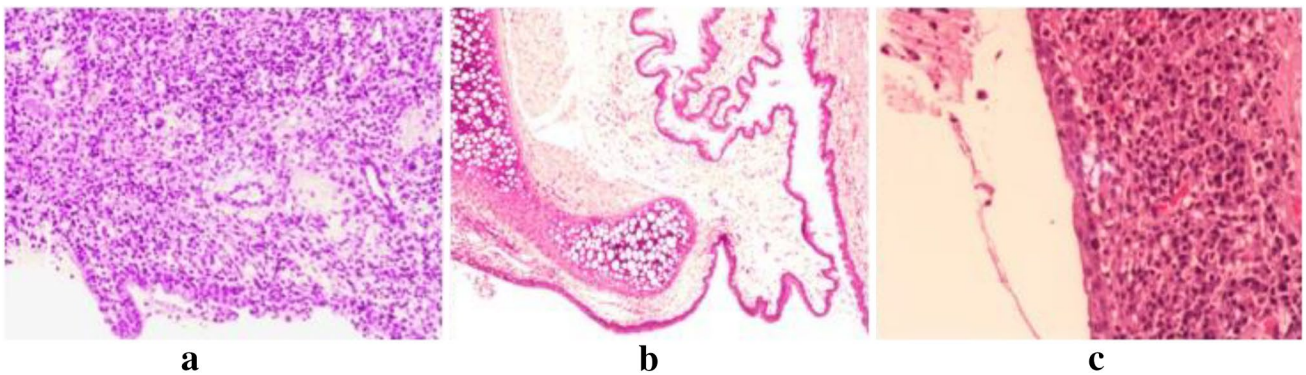

Fig. 3 Histopathological changes in larynx. **a** Inflammation in laryngeal mucosa, **b** Edema in laryngeal mucosa, **c** Cilia loss in laryngeal mucosa

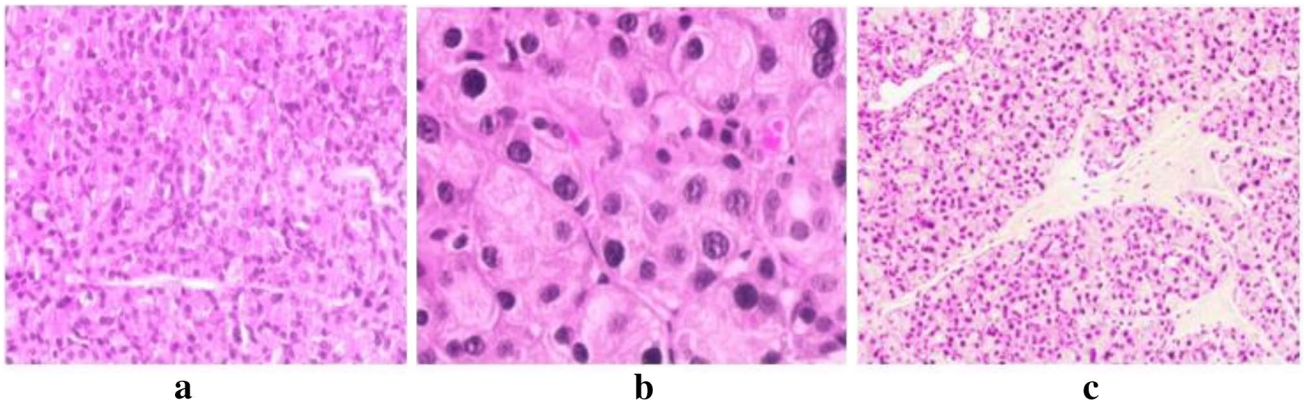

Fig. 4 Histopathological changes in parotid gland. **a** Serous asinus atrophy, **b** Small, irregular, hyper chromatic nucleus in serous asinus, **c** Connective tissue increase in parotid gland

Area under the ROC curve was found to be 1 in consequence of ROC curve analysis performed to determine the cut-off value for TSH levels of the allgroups. Area under the ROC Curve for the cut-off value determined to be > 0.99 was significant ($p < 0.001$). The distinctiveness of the cut-off value determined in this condition was higher (Table 5).

When the groups constituted by considering the cut-off values determined for TSH level were compared regarding pathological laryngeal parameters, the rate of vascular dilatation in the group with TSH level > 0.99 was determined to be significantly higher than the other group ($p < 0.05$) (Table 6). In the investigation performed regarding lamina propria edema, inflammation, goblet cell loss, and cilia loss, there was no significant difference between groups ($p > 0.05$) (Table 6).

When the relationship between pathological parotideal parameters and groups divided according to the cut-off values determined for TSH level was investigated; the presence of serous acinar atrophy and increase in the connective tissue were observed to be significantly higher in the group with TSH level > 0.99. When the groups were compared regarding nuclear morphology, no significant difference was found between groups ($p > 0.05$) (Table 7).

Statistical evaluations

The conformity of the data used in the study to a normal distribution was tested using the Shapiro–Wilk test. The one-way ANOVA test was used for the comparison of three or more independent groups with a normal distribution. Significant results obtained after ANOVA test were compared with post hoc test. Mann Whitney *U* test was used for the comparison of two independent groups without normal distribution. The Fisher–Freeman–Halton and Fisher's exact tests were used for the investigation of relationships of categorical variables. Descriptive statistical methods of the data with normal distribution were expressed as a mean ± standard deviation; descriptive statistical methods of the data without normal distribution were expressed as median (min–max) and descriptive statistical methods of categorical variables were expressed as *n* (%). ROC analysis was used to determine the cut-off value of the parameter and it was expressed as sensitivity and specificity values. All statistical analyses were performed using IBM SPSS Statistics version 22.0 with 95% confidence interval, at a significance level of $\alpha = 0.05$ and with a significance level of $\alpha^* = \alpha/k = 0.05/6 = 0.008$ (k: number of groups which would be compared) for post hoc comparisons and then reported.

Discussion

In the experiments performed on animal models, the thyroid gland has been made hypofunctional with PTU administration [10–12]. Functional status of the thyroid gland has been shown with histological changes or changes in serum fT3, fT4, and TSH [13]. PTU belongs to a class of drugs called the thionamides and these agents inhibit thyroid hormone synthesis by interfering with thyroid peroxidase which catalyzes iodination of tyrosine residues in thyroglobulin [14]. It is accepted as the drug serves as a substrate for thyroid peroxidase and so it inhibits iodination of tyrosine groups in thyroglobulin [11].

Upon demonstration of the development of hypothyroidism/goiter in euthyroid rats with the administration of thionamide in 1947, PTU has been approved by the FDA (Food and Drug Administration) for use in treatment [12, 15]. In our experimental model, we induced hypothyroidism by administration of intraperitoneally PTU and we confirmed the hypofunctional status of the thyroid gland with serum TSH levels (Table 2).

Granley et al. undermined the role of hypothyroidism in the etiology of hoarseness by demonstrating favorable voice characteristic changes due to thyroid hormone replacement in patients with hypothyroidism. Pathophysiological mechanisms causing these changes are controversial. In addition, definitive findings are not yet available [16]. Deposition of myxomatous material in the vocal folds and edema in the cricothyroid muscle are one of the accused causes in the etiology [17]. The increased amount of mucopolysaccharide in the lamina propria layer of the vocal fold causes edema in Reinke's space by leading to accumulation of fluid. Other factors accused in the pathophysiology of Reinke's edema are submucosal vascular dilatation, increased numbers of goblet cells and inflammation [18]. Also with the involvement of muscle weakness to all these, hoarseness and vocal fatigue may occur [6].

In our study, when the groups were compared according to the data obtained from histopathological investigation of larynx specimens, vascular dilatation was significantly higher in the experimental hypothyroid groups than the control group. However, there was no significant difference between hypothyroid groups regarding the duration of hypothyroidism. On the other hand, no significant difference was determined between hypothyroid groups and the control group regarding lamina propria edema, inflammation, goblet cell hyperplasia, and cilia loss (Table 3).

Also, when the groups were compared regarding TSH level organized by the cut-off values, while vascular dilatation was found to be higher in the group with TSH > 0.99 ($p < 0.05$), no difference was determined regarding other parameters (lamina propria edema, inflammation, goblet cell hyperplasia, and cilia loss) (Table 5). This result was similar with hypothyroid groups and control group comparation. In contrast to some studies in the literature [19, 20], in our study no significant difference was determined between groups regarding edema. When the groups were compared with regards to the cut-off value for TSH level (< 0.99, > 0.99), similar results were also obtained (Table 5). Likewise, White et al. did not find significant difference between patients with Reinke's edema and healthy control group according to the presence of hypothyroidism [20]. There was also no difference between groups in terms of cilia loss and inflammation according to our study results.

Another factor accused of the development of Reinke's edema is increased numbers of goblet cells [21]. In our study, no significant difference was determined between hypothyroid groups and the control group regarding the rates of goblet cell loss (Tables 3, 6). However, it should be kept in mind that the duration of experimental hypothyroidism is a maximum of 45 days in this study. In the experimental hypothyroidism study performed by Eryilmaz et al. [18] by adding of methimazole to the drinking water of rats for 90 days, edema and goblet cell number on surface epithelium of the vocal folds were evaluated in the histopathological investigation. According to the results obtained from their study, while a positive correlation was determined between hypothyroidism and edema, no significant difference was observed regarding goblet cell number similar to our study [18, 22]. When it is taken into consideration that 90-day of hypothyroidism period in the study performed by Eryilmaz et al. is twice the maximum period of 45 days in our study, it can be thought that the different result obtained regarding edema is correlated with the duration of hypothyroidism.

In the literature, it was stated that hypothyroidism could cause histopathological changes and affect salivation function of the parotid gland [23]. In another study, it was revealed that the levels of thyroid hormones should be within normal limits for the preservation of the histological structure of the parotid gland and continuation of salivation physiologically [24].

Cellular changes in the parotid gland may develop due to the systemic side effects of hypothyroidism. Secretory function of the glandular tissue is only possible with cells in a regularly functioning metabolism. Serous acinar cells are specifically affected in hypothyroidism [23]. One of the most important mechanisms causing hyposalivation is serous acinar atrophy responsible for the synthesis of saliva.

In the previous studies, it was shown that the nuclei became smaller, tended to gain an irregular appearance and become hypochromatic after hypothyroidism [11, 12]. It is known that the nucleus is large in euchromatic status and RNA synthesis is active in the nucleus in euchromatic status. This activity is also a marker of metabolic activity of the cell [25].

In the comparison performed in our study regarding the ratio of parotid nuclear morphologies between the control group and hypothyroid groups, while there was a numerical difference in the groups, this difference was determined to be not statistically significant (Table 4). In the evaluation performed regarding the cut-off value of TSH level, while all nuclei were large, round, euchromatic in the group with TSH level < 0.99 ($n = 6$; 6/6), it was observed that there were small, irregular, hyperchromatic nuclei ($n = 25$; large, round, chromatic = 17, mixed = 6, small, irregular, hyperchromatic = 2) in the group with TSH level > 0.99 ($p > 0.05$). Although this result was not statistically significant, observation of large, round, chromatic morphology in all rats ($n = 6$) of the group with TSH < 0.99 and small, irregular, hyperchromatic and mixed morphology in the rats ($n = 8$) of the group with TSH > 0.99 was remarkable. These results obtained are suggestive of statistically significant results can be obtained with studies performed in larger groups with longer duration of hypothyroidism.

In the studies in the literature, enlarged salivary glands in patients with hypothyroidism were considered to be a common finding [24, 26]. In the study performed by Hayat

NQ et al. rendering the rats hypothyroid by feeding them with methimazole 0.02% for 3 weeks, the authors determined marked atrophy in serous acini in the parotid gland [26]. Similarly also in our study, a significant difference was determined between hypothyroid groups and the control group regarding the presence of serous acinar atrophy in the parotid gland ($p < 0.05$). When the effect of the duration of hypothyroidism on serous acini in the parotid gland was evaluated, while there was no difference between the control group and 15-day hypothyroid group and 15-day and 30-day hypothyroid groups; a significant difference was determined between the control group and 30-day hypothyroid group, the control group and 45-day hypothyroid group, 15-day and 45-day hypothyroid groups. This result supports that as the duration of hypothyroidism increases atrophy in serous acinar structure increases ($p < 0.05$) (Table 4). Similarly also in the evaluation performed according to the cut-off value for TSH level, the rate of serous acinar atrophy was found to be significantly higher in the group with higher TSH level (Table 6).

In another study performed on submandibular glands of rats, the cause of hypertrophy in the gland was demonstrated to be due to an increase in the connective tissue despite serous acinar atrophy [24].

Also in this study, a difference was determined in the comparison performed between the control group and hypothyroid groups regarding the presence of an increase in the connective tissue in the parotid gland ($p < 0.05$) (Table 4). However, no significant difference was determined in the comparison performed regarding the duration of hypothyroidism. Similarly also in the evaluation performed according to the cut-off value for TSH level, the rate of increase in the connective tissue in the parotid gland was found to be significantly higher in the group with higher TSH level (Table 6).

Conclusion

Thyroid hormone has an important role in the regulation of metabolic events in the body. In thyroid hormone insufficiencies; morphological changes in different organ and structures in the body and functional changes associated with this condition may occur. In this experimental study, histopathological changes of larynx and parotid that might compromise a basis for the difference in functions of these organs due to hypothyroidism in rats were investigated. The evaluations performed in this study demonstrated that vascular dilatation which was among early findings of inflammation in the larynx was increased significantly with hypothyroidism. Another findings were serous acinar atrophy and increase in the connective tissue in the parotid gland. It was determined that no marked difference occurred between the periods of 15–30–45 days of hypothyroidism regarding histopathological changes in the larynx. In contrast, in parotid gland, serous acinar atrophy increased as the duration of hypothyroidism increased. The humidity of mucosa lining the oropharynx, hypopharynx and larynx are important for both swallowing and phonation. Histopathological changes in the parotid gland and early period inflammation findings determined in the larynx at the histopathological level of this study are supporting histopathological ground related to swallowing and voice disorders caused by hypothyroidism. New studies with a longer period of hypothyroidism may demonstrate results with more marked differences at the tissue level.

Declarations

Conflict of interest The authors declare that they have no conflict of interests.

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