

ORIGINAL RESEARCH

Histopathological characteristics of larynx in hypothyroidism in an experimental rabbit model

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Abstract

Objective: Hypothyroidism has a significant effect on the patients' voices. This study evaluated the histopathological characteristics of larynx following hypothyroidism in an experimental rabbit model.

Methods: Eleven male Dutch rabbits were included. Methimazole-induced hypothyroidism was done for nine rabbits. The remaining two rabbits were assigned as controls. Six weeks after starting methimazole, a histological examination was performed with parameters of inflammation, ulceration, hemorrhage, and thickness of epithelium, Reinke's space, vocal ligament, thyroarytenoid muscle, collagen deposition, and periodic acid-Schiff (PAS)-positive materials, as well as confirmation of hypothyroidism by T4 measurement.

Results: Histologic examination showed a significant thickening of epithelium, Reinke's space, vocal ligament, and collagen PAS-positive materials deposition in hypothyroid rabbits ($p < .05$). There was significant reduction in thyroarytenoid muscle thickness ($p < .05$). Inflammation, ulceration, and bleeding were not significantly different between hypothyroid and control rabbits' laryngeal specimens.

Conclusion: Hypothyroidism causes significant changes in the laryngeal tissues. Thickening of epithelium, Reinke's space, vocal ligament, collagen, PAS-positive materials, and reduced thickness of thyroarytenoid muscle are the major findings of this study.

KEYWORDS

animal model, histology, hypothyroidism, larynx, voice

1 | INTRODUCTION

Hypothyroidism is a common endocrine condition that affects the larynx and other organs of the body. Hypothyroidism has been linked to

changes in vocal perception and acoustic characteristics. Intensity perturbation, frequency perturbation, noise-to-harmonics ratio, grade, roughness, breathiness, asthenia, and strain scale and voice handicap index are parameters, which can be changed in the hypothyroid patients.¹⁻³

The thyroid gland synthesizes and releases the major thyroid hormones of thyroxine (T4) and triiodothyronine (T3) that mediate

Level of evidence: NA.

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Variable	Group		p value ^a
	Case mean ± SD	Control mean ± SD	
Epithelium	134.53 ± 24.02	101 ± 1.41	.034
Reinke's space	84.88 ± 15.23	39.25 ± 0.35	.033
Vocal ligament	335.33 ± 103.52	159.50 ± 6.36	.034
Thyroarytenoid muscle	1078.79 ± 528.61	1930 ± 77.78	.034
Collagen deposition	145.11 ± 22.02	32.62 ± 10.78	.034
PAS-positive materials	47.87 ± 8.86	11.08 ± 1.52	.034

^aMann-Whitney test.

many metabolic and developmental activities in the body. Inside the cell, T4 is converted to the T3 (most biologically active form of thyroid hormone) via deiodinases activity.⁴ T3 exerts its main action by binding to nuclear thyroid hormone receptors (THR). The hormone-receptor complex binds to the promoter regions of target genes to induce a regulatory ligand-dependent transcription activity (genomic action).⁵

Thyroid hormones operate on receptors in the plasma membrane, cytoplasm, and mitochondria (nongenomic action). Cell proliferation, angiogenesis, microfilament organization, and thermogenesis are all affected and regulated by this activity. Nongenomic and genomic functions, on the other hand, influence and overlap each other to control cellular metabolism and development.⁶⁻⁸

Altman et al. showed the presence of alpha and beta THR in human larynx especially in the fibrous connective tissue of the lamina propria of the vocal cords.⁹

Despite hypothyroidism's impact on the larynx, the pathophysiology of voice problems in hypothyroidism remains a controversial issue. We evaluated the histopathological characteristic of larynx in methimazole-induced hypothyroid rabbits to better clarify the mechanism of voice changes in hypothyroidism.

2 | MATERIALS AND METHODS

Eleven male Dutch rabbits, aged 5–6 months and weighing about 2 kg, were used in this study. The rabbits were kept in a controlled environment in an animal laboratory, including temperature, humidity, a 12-hour light/dark cycle, and full access to food and drink. The study protocols were approved by the local Ethics Committee of SUMS (IR.SUMS.MED.REC.1400.167) and according to the regulation of the animal care of Shiraz University.

Hypothyroidism was induced by methimazole (15 mg/kg/day) via the gastric gavage for nine rabbits. To designate a control group for future comparison, the remaining two rabbits were gavaged with distilled water. Based on earlier animal investigations, the kind of medication and dose for hypothyroidism induction were identified.^{10,11}

After 6 weeks, laryngeal harvesting was done in methimazole-treated and controlled rabbits following Phenobarbital (90 mg/kg) intravenous injection. For thyroid function assessment, 1 cc of blood was taken from a marginal ear vein with a 25 gauge needle in the

TABLE 1 Thickness of histologic layers (μm)

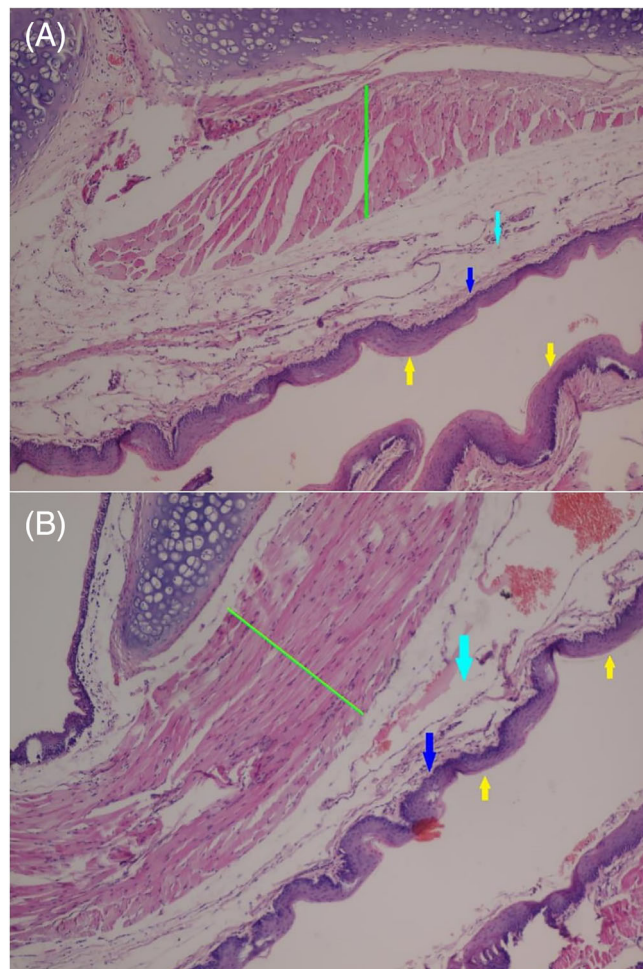


FIGURE 1 In hypothyroid group (A) in comparison to control (B) the thickness of epithelium (yellow Arrow), Reinke's space (dark blue arrow) and vocal ligament (light blue arrow) are increased, where the muscle thickness (green line) is decreased, H&E Stain (×100).

morning and after 8 h of fasting immediately before harvesting. In our research, total T4 levels less than 1 μg per deciliter were deemed to prove hypothyroidism since the diagnosis of hypothyroidism is based on T4 in many species such as rats.¹²

Laryngeal specimens were kept in 10% formalin and sent for histopathologic examination. Specimens were sectioned into 5-μm thickness and stained with hematoxylin–eosin, Masson trichrome and

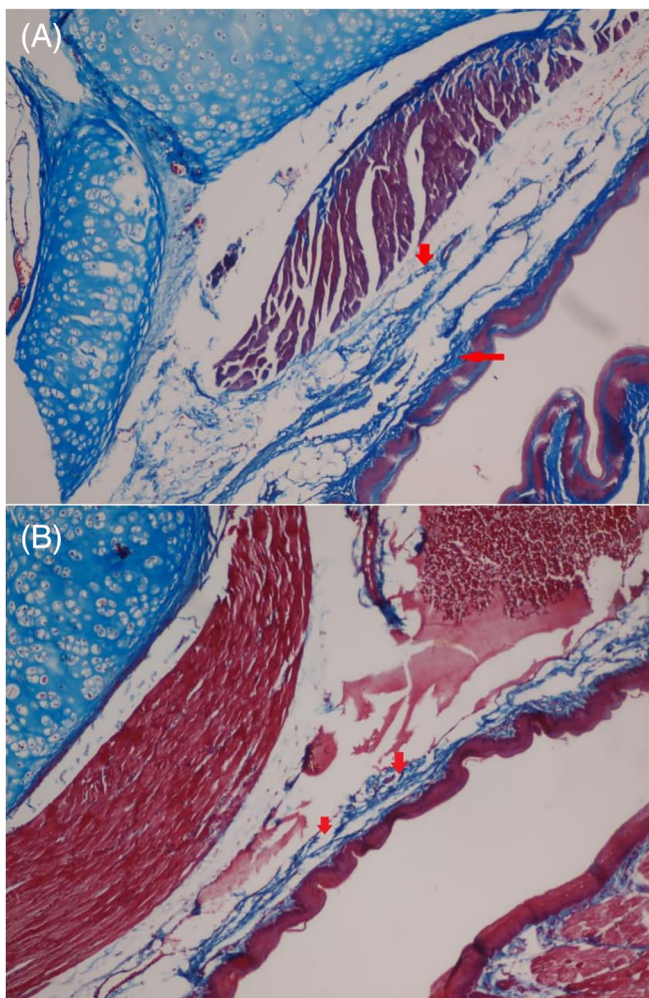


FIGURE 2 Deposition of collagen is more prominent in hypothyroid group (A) than control (B). Masson trichrome stain (×100)

combination of Alcian-Blue/periodic acid-Schiff (PAS) for light microscopy examination. In both the case and control groups, both sides of the vocal cords were examined. The histologic characteristics that we investigated and compared between hypothyroid and control rabbits were inflammation, ulceration, bleeding, and thickness measurement of epithelium, Reinke's space, vocal ligament, thyroarytenoid muscle, collagen deposition, and PAS-positive materials such as mucopolysaccharides.

2.1 | Statistical analysis

Data were analyzed using SPSS software package version 19.0 (Chicago, Illinois). Continuous variables were presented by mean ± SD and those related to qualitative data were shown by frequency and percentage. The difference between two qualitative variables was assessed using the Chi-Square and Fisher's Exact tests. The quantitative and binary qualitative variables were compared using the Mann-Whitney test. We used Spearman Correlation to investigate the relationship between two quantitative variables. *p* value less than .05 was considered to be statistically significant.

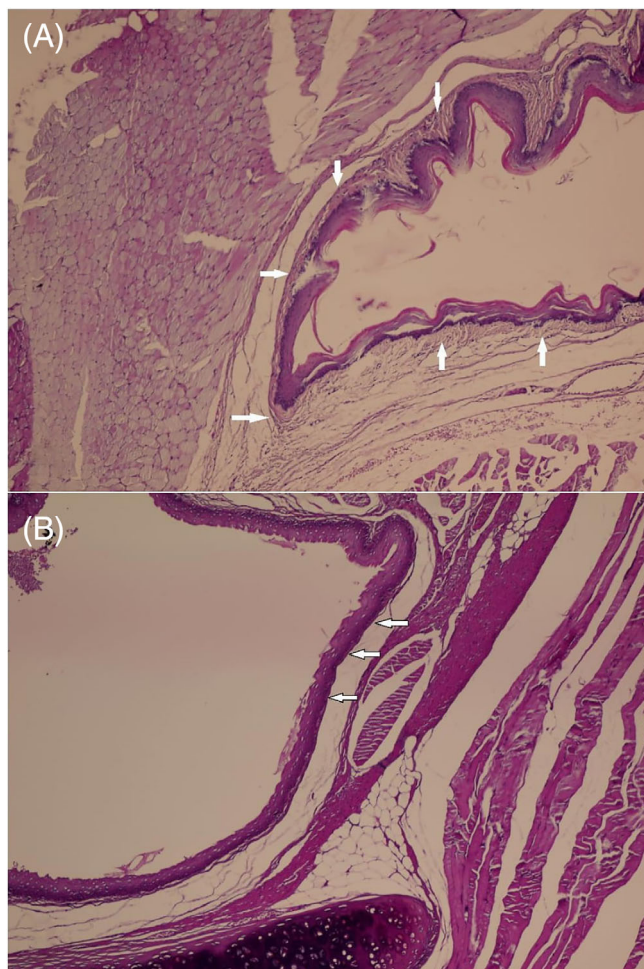


FIGURE 3 Subepithelial deposition of PAS-positive materials (white arrow) in hypothyroid group (A), PAS stain ×100

TABLE 2 Qualitative histologic parameters compared to controls

Variable		Group		<i>p</i> value ^a
		Case N (%)	Control N (%)	
Inflammation	Yes	4 (44.4)	0	.491
	No	5 (55.6)	2 (100)	
Hemorrhage	Yes	1 (11.1)	0	1
	No	8 (88.9)	2 (100)	
Ulceration	Yes	1 (11.1)	0	1
	No	8 (88.9)	2 (100)	

^aFisher's exact test.

3 | RESULTS

The effects of hypothyroidism on epithelial, Reinke's space, vocal ligament, thyroarytenoid muscle, collagen deposition and PAS-positive materials thicknesses are shown in Table 1. In compared to controls, significant increases in thickness of epithelium, Reinke's space, vocal ligament, and reductions in thyroarytenoid muscle thickness were

TABLE 3 Spearman Rho correlation of histological parameters

		Collagen deposition	Epithelium	Reinke	Ligament	Lamina propria	PAS positive-material	Muscle
Collagen deposition	Correlation coefficient	1.000	-0.250	0.184	0.267	0.350	0.583	-0.133
	Sig. (two-tailed)		0.516	0.635	0.488	0.356	0.099	0.732
	N	9	9	9	9	9	9	9
Epithelium	Correlation Coefficient	-0.250	1.000	-0.259	-0.583	-0.417	-0.250	-0.783 ^a
	Sig. (two-tailed)	0.516		0.500	0.099	0.265	0.516	0.013
	N	9	9	9	9	9	9	9
Reinke	Correlation coefficient	0.184	-0.259	1.000	0.117	0.234	0.728 ^a	-0.067
	Sig. (two-tailed)	0.635	0.500		0.764	0.544	0.026	0.864
	N	9	9	9	9	9	9	9
Ligament	Correlation coefficient	0.267	-0.583	0.117	1.000	0.950 ^{**}	0.483	0.500
	Sig. (two-tailed)	0.488	0.099	0.764		0.000	0.187	0.170
	N	9	9	9	9	9	9	9
Lamina propria	Correlation coefficient	0.350	-0.417	0.234	0.950 ^{**}	1.000	0.600	0.367
	Sig. (two-tailed)	0.356	0.265	0.544	0.000		0.088	0.332
	N	9	9	9	9	9	9	9
PAS positive-materials	Correlation coefficient	0.583	-0.250	0.728 ^a	0.483	0.600	1.000	-0.133
	Sig. (two-tailed)	0.099	0.516	0.026	0.187	0.088		0.732
	N	9	9	9	9	9	9	9
Muscle	Correlation coefficient	-0.133	-0.783 ^a	-0.067	0.500	0.367	-0.133	1.000
	Sig. (two-tailed)	0.732	0.013	0.864	0.170	0.332	0.732	
	N	9	9	9	9	9	9	9

^aCorrelation is significant at the .05 level (two-tailed).

Colored values significes 0.026.

Colored values significes 0.013.

discovered (Figure 1). Collagen deposition and PAS-positive materials thicknesses were increased in hypothyroid rabbits compared to controls (Figures 2 and 3).

In Table 2, the effects of hypothyroidism on inflammation, ulceration and hemorrhage of laryngeal tissues are presented. Histopathologic criteria of inflammation was based on more than 10 inflammatory cells per microscopic field ($\times 10$). We found not any significant inflammation, ulceration and hemorrhage in hypothyroid rabbits compared to controls.

Table 3 shows the relationships between histological characteristics in hypothyroid rabbits using the Spearman correlation method. PAS-positive materials deposition was shown to have a substantial and positive correlation with increased Reinke's space thickening ($p = .026$). There was an inverse and significant correlation between thickness of epithelium and thyroarytenoid muscle ($p = .013$). There was not any statistically significant correlation between collagen deposition and thicknesses of epithelium, Reinke's space, vocal ligament, and muscle.

4 | DISCUSSION

Many changes in the function and histology of organs in the body are caused by hypothyroidism. The larynx has functional alterations as a result of hypothyroidism, but structural changes are unknown. This study revealed that hypothyroidism significantly increases the thickness of the epithelium, Reinke's space, vocal ligament, collagen deposition, PAS-positive materials and also decreases the thickness of the thyroarytenoid muscle.

Connective tissue is one of the targets of thyroid hormones. Thyroid hormones have been shown in many studies to regulate fibroblast activity and collagen metabolism. The quantity of collagen in the tissues rises with hypothyroidism, according to experimental investigations.^{13,14} Increasing the amount of collagen in the tissues leads to an increase in stiffness that affects on organ function.¹⁵ Increased accumulation of fluid and polysaccharides in lamina propria and especially Reinke's space is another cause of connective tissue thickening in

hypothyroidism.¹⁶ Our results are consistent with prior research on increased collagen and PAS-positive materials thickness in Reinke's area, and they may explain why certain hypothyroid patients have long-term voice problems (Figures 2 and 3). According to statistical evidence, the role of PAS-positive materials is more than collagen deposition in Reinke's space thickening (Table 3).

Epithelial thickening is another finding of this study. Accumulation of myxomatous materials, hyaluronan, inflammatory factors, and fluid have been considered as factors that cause this condition.^{17,18}

The presence of muscle weakness in hypothyroidism has been proven although the exact mechanism of its development is not well recognized. It seems that the reduction in mitochondrial biogenesis and proteins and impaired lipid catabolism contribute to myopathy following hypothyroidism.^{19,20} In our work, a significant decrease in thyroarytenoid muscle thickness is highly in favor of altered muscle metabolism in hypothyroid rabbits. In hypothyroidism, decreased thyroarytenoid mass causes muscular weakness and dysphonia, as well as vocal fatigue. Another finding of this research showed an inverse relationship between thyroarytenoid muscle thickening and epithelial thickness (Table 3). This study's findings did not reveal a significant correlation between hypothyroidism and inflammation in laryngeal tissues of rabbits. Some studies indicated the sterile inflammation in thyroid hormones deficiency, which seems to be closer to the results of our study. Sterile inflammation is a condition, which occurs in the absence of cell infiltration. Inflammatory biomarkers, such as C-reactive protein, tumor necrosis factor alpha, and interleukins are increased in the serum. This type of inflammation is initiated via the multiprotein intracellular complexes called inflammasome. The activation of inflammasome leads to release of pro-inflammatory markers such as interleukins 1 α , 1 β , and 18.²¹⁻²³

This experimental study is based on histopathological findings of the larynx in hypothyroidism. Histopathological changes of the larynx in hypothyroidism can be considered a basis for better understanding the pathophysiology of functional disorders. This is one of the strengths of this study, which is based on the basic sciences for better understanding of clinical disorders. A limitation of this research is the small number of the control group. Nevertheless, in light of the evaluation of both sides of the vocal cords, we believe it is unlikely to be a serious issue and that this alleviates the concerns of a small control group to some degree.

Future studies may be based on the laryngeal tissue changes over a longer period of time in hypothyroidism.

5 | CONCLUSION

Hypothyroidism is a metabolic disorder that affects many organs including the larynx. Our experimental study showed that hypothyroidism increases the thickness of epithelium, Reinke's space, vocal ligament, collagen deposition, PAS-positive materials in vocal folds, and decreases the thickness of the thyroarytenoid muscle. This research may lead to a greater comprehension of the functional

alterations associated with hypothyroidism and, therefore, a more effective therapy for dysphonic patients.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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