Araştırma Makalesi (Research Article)

Tonsil Hipertrofisinde Bağ Dokusu ve Lenfoid Dokunun Rolü

The Role of Connective and Lymphoid Tissues in Tonsil Hypertrophy

Mehmet Emre SIVRICE[1], Hasan YASAN[1], Gamze ERKILINC[2], Nermin KARAHAN[2], Vural AKIN[1]

[1] Department of Oto-Rhino-Laryngology and Head&Neck Surgery, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

[2] Department of Pathology, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

Amaç: Bu çalışmada tonsillektomi materyallerindeki mikroanatomik yapı farklılıklarını belirlemek için bağ dokusu ve lenfoid doku oranlarının incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Tonsillektomi materyalleri 3-13 yaş arası hastalardan toplandı ve üç grup oluşturuldu. Grup 1'de tonsil hacmi 3 cc'den fazla olup rekürren tonsillit öyküsü olan hastalar, grup 2'de tonsil hacmi 3 cc'den fazla olup rekürren tonsillit öyküsü olmayan hastalar ve grup 3'te tonsil hacmi 2 cc'den az olup rekürren tonsillit öyküsü olan hastalar yer aldı. Masson's Trichrome ile boyanan kesitler bağ ve lenfoid doku açısından değerlendirildi. Python programlama dili kullanılıp piksel sayımı yapılarak bağ dokusu alanlarının yaklaşık oranı hesaplandı.

Bulgular: Ortalama bağ dokusu yüzdeleri grup 1'de 5.45±2.50, grup 2'de 5.45±2.42 ve grup 3'te 10.90±3.41'di. Grup 3'te grup 1 ve grup 2'ye göre anlamlı olarak daha yüksek bağ dokusu oranları tespit edildi (p=0.001). Lenfoid doku, grup 1 ve grup 2'de grup 3'e göre anlamlı olarak daha yüksekti (p=0.001). Folikül sayıları üç grup arasında anlamlı olarak farklıydı (p=0.032).

Sonuç: Tonsil hipertrofisinin oluşum mekanizması henüz aydınlatılamamıştır. Biz tonsil hacmi arttıkça lenfoid dokunun da arttığı sonucuna ulaştık. Başka bir deyişle, hipertrofik tonsillerde tonsil hacminin artışına neden olan kısım lenfoid dokudur. Tekrarlayan inflamasyon ile palatin tonsilin mikroanatomik yapıları arasındaki ilişki, önceden tahmin edilen bilgilerden daha karmaşık görünmektedir.

Anahtar Kelimeler: Palatin tonsil, Hipertrofi, Bağ dokusu, Lenfoid doku

Abstract

Objective: This study aimed to investigate connective tissue and lymphoid tissue ratios in tonsillectomy specimens to determine differences between microanatomical structures.

Material and Methods: Tonsillectomy materials were collected from patients aged 3-13 years and three groups were created. Patients with larger tonsils than 3 cc and had recurrent tonsillitis history were included to the group 1, larger tonsils than 3 cc without recurrent tonsillitis history were included to the group 2 and tonsil sizes less than 2 cc with recurrent tonsillitis history were included to the group 3. Masson's Trichrome stained slides were evaluated to determine the connective and lymphoid tissues. We use Python programming language and count pixels to calculate the approximate ratio of the connective tissue area.

Results: The mean connective tissue percentages were 5.45±2.50 in group 1, 5.45±2.42 in group 2 and 10.90±3.41 in group 3. There was significantly higher connective tissue ratios in group 3 compared to group 1 and group 2 (p=0.001). Lymphoid tissue was significantly higher in group 1 and group 2 compared to group 3 (p=0.001). Follicle numbers were significantly different among 3 groups (p=0.032).

Conclusion: The mechanism of the tonsillar hypertrophy has not been elucidated, yet. We found that; as long as the tonsil volume increases, lymphoid tissue ratio increases to. In other words, the lymphoid region is the main part responsible for the enlargement of the tonsil in hypertrophic tonsils. Relationship between recurrent inflammation and microanatomical structures of the palatine tonsil seem to be more complicated than previously supposed knowledge.

Keywords: Palatine tonsil, Hypertrophy, Connective tissue, Lymphoid tissue

Yazışma Adresi: Mehmet Emre SİVRİCE, Süleyman Demirel Üniversitesi Tıp Fakültesi KBB ABD, Isparta, Türkiye ,

Telefon: +905447724319, Mail: emresivrice@gmail.com

ORCID No (Sırasıyla): 0000-0002-2396-6794, 0000-0002-5470-6784, 0000-0003-4704-7415, 0000-0003-0883-4037, 0000-0002-0050-4837

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INTRODUCTION

Tonsils are secondary lymphoid organs, which are located at the beginning of the respiratory tracts. They belong to Waldeyer's ring and their primary work is antibody secretion against antigens (1).

Adenoidectomy, tonsillectomy and adenotonsillectomy are three of the most common Ear Nose Throat (ENT) operations in the pediatric population (2). The most common indications for tonsillectomy in the pediatric population are obstructive sleep apnea (OSA) and recurrent tonsillitis. Tonsil hypertrophy and adenoid hypertrophy are the main causes of OSA in children. Although tonsil hypertrophy and recurrent tonsillitis are the most common indications for tonsillectomy, the pathogenic mechanism underlying the tonsillar hypertrophy is still unclear. Recurrent tonsillitis may present either as a small tonsil or tonsillar hypertrophy. On the other hand tonsillar hypertrophy may occur without recurrent infection. Also the cause of hypertrophy in asymmetric tonsils and indications for surgery remains controversial (3).

Kalcioglu et al. concluded that histopathological examination of routine tonsillectomy specimens is not necessary but histologic structural evaluation may be useful for determination of tonsillar hypertrophy etiology (4). To our knowledge the data on the histologic evaluation of tonsillar connective tissue is lacking and there are no studies focusing on tonsillar connective tissue. In this study we used a programming language to read the images and compare connective tissue ratios in tonsil hypertrophy to find an answer to this questions; which part of tonsils (connective or lymphoid) enlarge in hypertrophic tonsils and what are striking features of microanatomical structures of tonsils either hypertrophied or not?

MATERIAL and METHODS

Patient Selection and Study Design

This study was conducted between January 2018-December 2018 at our department of Otolaryngology Head and Neck Surgery. Hospital database were reviewed for tonsillectomy patients between the ages of 3 and 13. Patients who met the following criteria were included: Children without chronic diseases such as diabetes mellitus, hepatic or renal disease, haematological disease, autoimmune disease or known allergic diseases. Patients who did not meet the inclusion criteria and had tonsil volume between 2-3 cc were excluded from the study. Traditional tonsillectomy by use of cold knife dissection and snare was performed by one (same) surgeon. The study was carried out in accordance with international ethical standards of the Helsinki Declaration. The ethics committee of the institution approved the study protocol (date: 05.02.2019, number: 58). Informed consent was obtained from the parents of all participants.

The real tonsil volumes were measured using Archimedes principle. Removed tonsils were placed into a syringe with full of water in the operating room. Water displaced by tonsils was measured using 50 mL syringes to determine the real tonsil volume (5).

Three groups were created according to real tonsil size and the medical history of tonsillar infections. The patients who had larger tonsils than 3 cc and had recurrent tonsillitis (at least four times a year for more than two years) were included to the group 1. The patients who had larger tonsils than 3 cc without recurrent tonsillitis were included to the group 2. The patients who had tonsil sizes less than 2 cc and had recurrent tonsillitis (at least four times a year for more than two years) were included to the group 3. We had 11 patients in each group.

Pathologic Evaluation

Weight and size of the specimens were recorded. Outer and section surfaces of the specimens were examined macroscopically for evaluating occupying lesions. The specimens were cut in to 4-5 mm sections. One or two samples that represented entire specimen were taken in the absence of detectable lesion. Routine tissue follow up was performed. Paraffin embedded samples were cut in to 5 µm sections stained with Masson's Trichrome stain (ChemBio Laboratory Research, Istanbul, Turkey).

Staining procedure was performed according to directions of manufacturer company. The collagen (connective tissue) is in blue colour, the cytoplasm is in red colour and the nuclei are in black colour when staining is performed with Masson's Trichrome.

Masson's Trichrome stained slides were evaluated to determine the ratio of connective tissue and lymphoid tissues. Pictures including entire section surface were taken (30-80 photos for each case). All pictures were combined into one picture using Image Composite Editor Program (version 2.0.3.0. (64 bit)) (Microsoft, Albuquerque, New Mexico, USA).

For all combined pictures Python programming language and the open source computer vision library (OpenCV) were used to read the images and convert into Hue, Saturation, and Brightness Value (HSV) colour space. We found an average colour range in HSV colour space to detect the pixels which represented the area affected. We count those pixels to calculate the ratio of the area to the total area. Figure 1-2-3 are samples for each group (group 1-2-3 respectively). A section of the figures shows Masson's Trichrome staining and B section shows the HSV colour space. The white areas in section B represent connective tissue.

Statistical Methods

The statistical analyses of the study were performed by SPSS 20.0 (IBM Inc, Chicago, IL, USA) software. Descriptive statistics were presented as mean±SD (median, min, max) for numerical variables, and frequency (percentage) for categori-

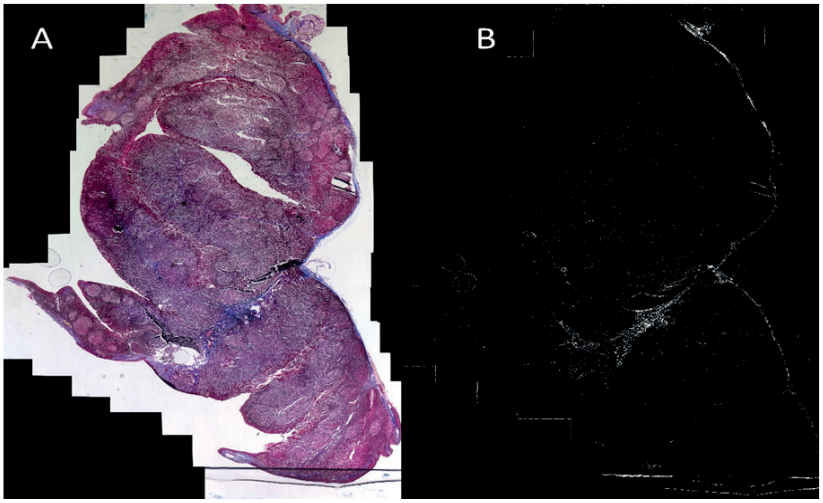

Figure 1. Group 1-Tonsil hypertrophy with increased follicle numbers (A section-Masson's Trichrome staining, B section-HSV colour space 'white areas represents connective tissue')

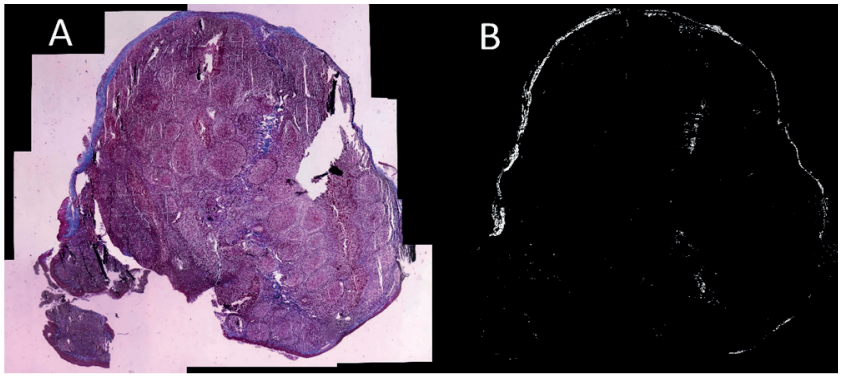

Figure 2. Group 2- Tonsil hypertrophy without recurrent tonsillitis (A section-Masson's Trichrome staining, B section- HSV colour space 'white areas represents connective tissue')

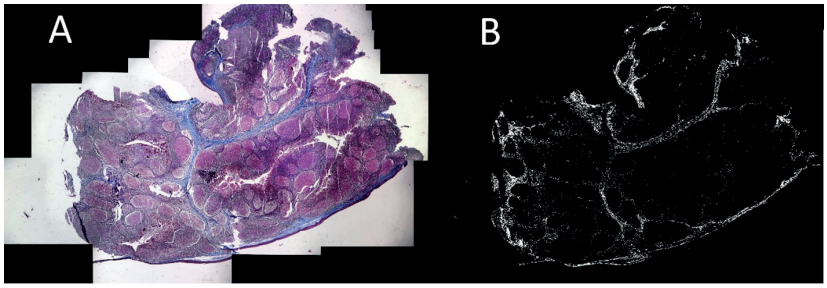

Figure 3. Group 3- Non-hypertrophic tonsils without recurrent tonsillitis and with increased connective tissue (A section-Masson's Trichrome staining, B section-HSV color space 'white areas represents connective tissue')

cal variables. Kruskal-Wallis test was used for comparison of study groups since the distribution of the numerical variables is not normal. Post-hoc test for Kruskal-Wallis was used to make the pairwise comparisons. The relations were calculated by Spearman's Rho correlation analysis in each groups. P<0.05 value was considered as statistically significant results for 5% type-I error.

RESULTS

The mean age of patients at the time of tonsillectomy were 6.8±3.06 in group 1, 4.81±1.40 in group 2 and 5.72±2.45 in group 3. No statistically significant difference was found among the 3 groups in terms of age and gender (p=0.301 and p=0.88 respectively). **Table 1** summarises demographic features.

The mean connective tissue percentages were 5.45±2.50 in group 1, 5.45±2.42 in group 2 and 10.90±3.41 in group 3 **(Figure 4).** There was significantly higher connective tissue ratio in group 3 compared to group 1 and group 2 (p=0.001, group 1 vs group 3 p=0.05-group 2 vs group 3 p=0.05).

Table 1. Kruskal-Wallis test results between groups

	Group I	Group II	Group III	p
Age	**6.81±3.06**	**4.81±1.40**	**5.72±2.45**	**0.301**
Gender				
Female	7 (63.6)	7 (63.6)	6 (54.5)	0.88
Male	4 (36.4)	4 (36.4)	5 (45.5)	

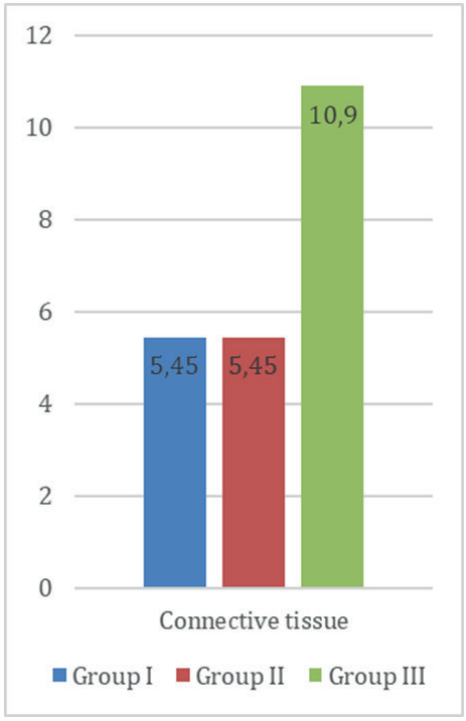

Figure 4. Connective tissue results of the groups (p=0.001)

Lymphoid tissue percentages are shown in **Figure 5.** Lymphoid tissue (percentage of tonsillar area excluding connective tissue and surface epithelium) was significantly higher in group 1 and group 2 compared to group 3 (p=0.001, group 1 vs group 3 p=0.05-group 2 vs group 3 p=0.05). AGA IgG/IgM, EMA, and tTG IgG/IgM were tested in all patients. Follicle numbers of the groups are shown in **Figure 6.** Follicle number differences was significant among 3 groups (p=0.032 and group 1 vs group 3 p=0.03).

According to Spearman's correlation test, only in the third group a significant relationship was observed between

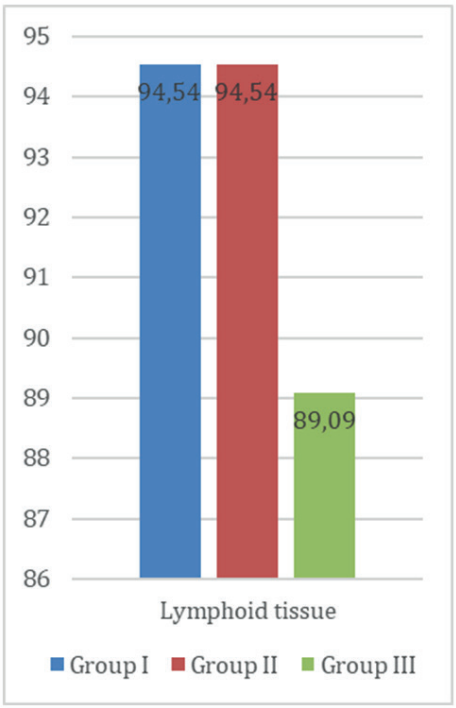

Figure 5. Lymphoid tissue percentages of the groups (p=0.001)

connective tissue and tonsil volume (Rho=0.613, p=0.045). Correlation was not observed among other variables.

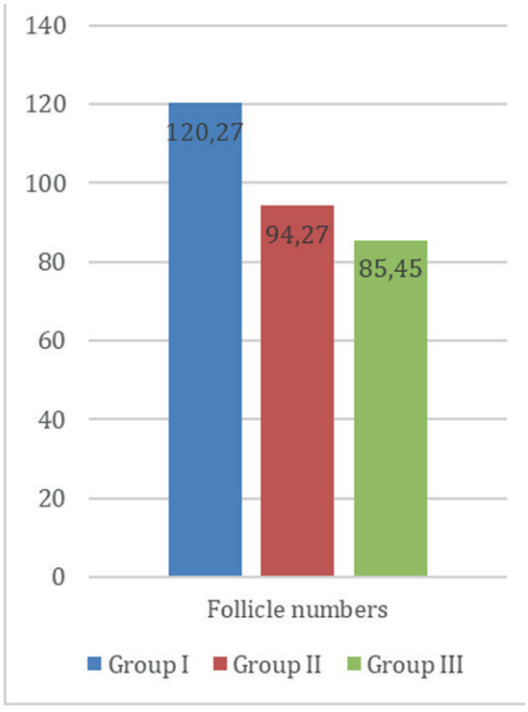

Figure 6. Follicle numbers of the groups (p=0.032)

DISCUSSION

Many factors have been supposed to be responsible for the etiology of tonsil hypertrophy but it remains unclear. The current study focused on histologic evaluation of tonsil hypertrophy with Masson Trichrome stain by using a programing language software. The main finding was that patients with tonsil size less than 2 cc and had recurrent tonsillitis showed higher connective tissue scores but lymphoid tissue is the major part in hypertrophic tonsils.

Koch et al. blamed chronic and recurrent infections of palatine tonsils for tonsillar hypertrophy (6). Bieluch et al. found that increased T and B cell numbers had a positive correlation with bacterial growth and tonsil size (7). Alatas and Baba indicated that tonsillar hypertrophy was related with increased follicle diameter, area and number but Zhang et al. indicated that tonsillar hypertrophy was related with increased lymphoid follicle size, but not the number of follicles (8,9). In our study we found follicle numbers was significantly higher in group 1 compared to group 2 and 3 (p=0.032). We observed that tonsillar hypertrophy was related with increased follicle numbers in patients that has hypertrophied tonsils with recurrent tonsillitis. But we did not observed this increased numbers of follicles in patients with idiopathic tonsillar hypertrophy. Unlike the other two studies we divided tonsillar hypertrophy into two groups and found this result. We believe that the underlying mechanism of the difference between these two groups needs further investigation.

Yılmaz et al. determined that inflammation in tonsil tissue does not always cause hypertrophy and sometimes inflammation can result with replacement of the lymphatic tissue to fibrotic tissue (10). In our study we found connective tissue ratio is significantly higher in group 3. And according to Spearman's correlation test, only in the third group a significant relationship was observed between connective tissue and tonsil volume (Rho=0.613, p=0.045). Our results are compatible with this study. But the underlying mechanism between hypertrophy and fibrosis still unclear.

Allergy is also blamed as an important risk factors for adenoid and tonsillar hypertrophy in children. Endo and Bellioni have shown in different studies that allergic reactions of the nasal mucosa are also present in pharyngeal tonsillar tissues (11,12). Zielinski described that lymphatic follicles are various and bigger in children with allergic rhinitis (13). Sadeghi-Shabestari et al. found 70.3% of children with adenotonsillar hypertrophy had positive skin prick test. But only 10% of children in control group had positive skin prick test. So they concluded controlling the allergy may play a role in reducing adenotonsillectomy operations (14). In our study we included patients who were not previously diagnosed with allergic diseases so we cannot make a comment on the role of allergy in tonsillar hypertrophy.

Yasan et al. determined that objective tonsil volume is correlated with subjective tonsil size in patients with recurrent acute tonsillitis (15). But current tonsil measure classifications, such as Friedman classification assess tonsil size medial to tonsillar columns relative to oropharyngeal orifice diameter in coronal plane; tonsil size deep to the tonsillar columns and tonsil size in sagittal plane are not assessed (16). This staging may be a reason for false comparison of the connective tissue area. So in this study we calculated the real tonsil volumes according to the Archimedes principle to overcome this problem (5).

In this study we compared connective tissue ratios between 3 groups. Connective tissue ratio is significantly higher in group 3. Lymphoid tissue was significantly higher in group 1 and group 2 compared to group 3. Follicle numbers was significantly higher in group 1 compared to group 2 and 3. According to our results, we confirmed that palatine tonsil hypertrophy is caused by increased lymphoid tissue. Connective tissue volume is also low against to the total tonsil volume. But in order to reach this conclusion, it was necessary to carry out such a study, which focused on connective tissue. To our knowledge this is the first study which used Masson's Trichrome staining in tonsillectomy materials and connective tissue areas are calculated for the first time by our method. Our results also demonstrated another fact. Recurrent tonsil infections cause hypertrophy in some patients but not in all patients.

The main weaknesses of the present study was the limited number of cases. Another weakness of our study is the exclusion of patients with history of allergic diseases. We believe these groups should also be studied for allergic patients.

As a conclusion, Etiology of the tonsillar hypertrophy has not been elucidated, yet. Tonsillary connective tissue volume is higher in patients with recurrent tonsillitis without hypertrophy than the others. These finding may be related with correlation of long term inflammation and increased fibrosis. In hypertrophic tonsils, the major part responsible for the enlargement of the tonsil size is the lymphoid region. So the attention should be focused on lymphoid tissue hyperplasia in order to prevent tonsillar enlargement in long term course. Relationship between the inflammation and microanatomical structures of the palatine tonsil should be focused to understand the etiology.

Research Contribution Rate Statement Summary

Idea: M.E.S.-H.Y. Design:M.E.S.-H.Y. / Data Collection:M.E.S.-H.Y.-V.A. / Analysis:G.E.-N.K. / Writing: M.E.S.-H.Y.-G.E.-V.A.

Conflict of Interest and Financial Status

Our study has not been financed by an institution and institution. In this study, there is no conflict of interest among the authors on any subject.

REFERENCES

1. Ogra PL. Mucosal immune response in the ear, nose and throat. Pediatr Infect Dis J. 2000;19(5):4-8.
2. Parker NP, Walner DL. Trends in the indications for pediatric tonsillectomy or adenotonsillectomy. Int J Pediatr Otorhinolaryngol. 2011;75(2):282-285.
3. Hanege FM, Acar GO, Tekin M, Ozkanlı S, Saygı HI. What is the cause of hypertrophia in asymmetric tonsils?. B-ENT. 2016;12(3):175-179.
4. Kalcioglu MT, Gurses I, Erdem T. Is the pathological examination of routine tonsillectomy and adenoidectomy specimens necessary? A retrospective study of 559 adenoidectomy and 1132 tonsillectomy specimens and a literature review. B-ENT. 2010;6(2):91-95.
5. Sağıroğlu A, Acer N, Okuducu H, Ertekin T, Erkan M, Durmaz E, et al. Palatine tonsil volume estimation using different methods after tonsillectomy. Anat Sci Int. 2017;92(4):500-508.
6. Koch RJ, Brodsky L. Qualitative and quantitative immunoglobulin production by specific bacteria in chronic tonsillar disease. Laryngoscope. 1995;105(1):42-48.
7. Bieluch VM, Martin ET, Chasin WD, Tally FP. Recurrent tonsillitis: histologic and bacteriologic evaluation. Ann Otol Rhinol Laryngol. 1989;98(5):332-335.
8. Alatas N, Baba F. Proliferating active cells, lymphocyte subsets, and dendritic cells in recurrent tonsillitis: their effect on hypertrophy. Arch Otolaryngol Head Neck Surg. 2008;134(5):477-483.
9. Zhang PC, Pang YT, Loh KS, Wang DY. Comparison of histology between recurrent tonsillitis and tonsillar hypertrophy. Clin Otolaryngol Allied Sci. 2003;28(3):235-239.
10. Yılmaz T, Kocan EG, Besler HT, Yılmaz G, Gursel B. The role of oxidants and antioxidants in otitis media with effusion in children. Otolaryngol Head Neck Surg. 2004;131(6):797-803.
11. Bellioni P. Histological changes in chronic allergic tonsillitis. Clin Otolaryngoiatr. 1968;20(2):116-124.
12. Endo LH, Vassalo J, Leitão SR. Allergic tonsillitis: histopathological study. Adv Otolaryngol. 1992;47:41-45.
13. Zieliński A, Dobek-Smiech J, Fortak W, Brzezińska H, Zakrzewska A. Clinical and histological studies of pharyngeal tonsil in children. Otolaryngol Pol. 1985;39(1):47.
14. Sadeghi-Shabestari M, Moghaddam YJ, Ghaharri H. Is there any correlation between allergy and adenotonsillar tissue hypertrophy. Int J Pediatr Otorhinolaryngol. 2011;75(4):589-591.
15. Yasan H, Aynali G, Erdogan O, Yariktas M. Does subjective tonsillar grading reflect the real volume of palatine tonsils. Int J Pediatr Otorhinolaryngol. 2011;75(5):618-619.
16. Friedman M, Tanyeri H, La Rosa M, Landsberg R, Vaidyanathan K, Pieri S, et al. Clinical predictors of obstructive sleep apnea. Laryngoscope. 1999;109(12):1901-1907.