Nobel Med 2024; 20(3): 173-178

THE ROLE OF FIBROBLAST GROWTH FACTOR RECEPTOR-1 IN TONSILLAR HYPERTROPHY

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ABSTRACT

Objective: *Even though pathologies of palatine tonsils are common, the mechanisms leading to these pathologies are not clearly understood. The main objective of this study is to investigate the effect of fibroblast growth factor receptor-1 (FGFR1) on tonsillar hypertrophy.*

Material and Method: *Children who underwent total tonsillectomy were included in the study. Subjective tonsil size, tonsil weight and volume were measured for all patients. The tonsil samples were than homogenized and their FGFR1 concentrations were measured with enzyme-linked immunoassay (ELISA) method. The correlations between tonsil measurements and FGFR1 concentrations were analyzed.*

Results: *59 patients were included in the study. Tonsil size and weight was found to be positively correlated to the patient age. No significant correlation was found between subjective tonsil size and objective tonsil measurements. The FGFR1 concentration of the tonsil tissue showed significant negative correlation with the tonsil volume (r:-0.22, p: 0.04) and weight (r:-0.28, p: 0.01). Subjective tonsil size showed no correlation with the FGFR1 concentration.*

Conclusion: *There is a negative correlation between tonsil size and tonsillar FGFR1 concentration. This finding might indicate that FGFR1 plays a role in the mechanisms which determine the tonsil size.*

Keywords: *Palatine tonsil, adenoids, hypertrophy, fibroblast growth factors, inflammation.*

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DELIVERING DATE: 19 / 11 / 2023 • **ACCEPTED DATE:** 20 / 02 / 2024

TONSİLLER HİPERTROFİDE FİBROBLAST BÜYÜME FAKTÖRÜ RESEPTÖRÜ 1'İN ROLÜ

ÖZET

Amaç: Palatin tonsillerin hastalıkları toplumda sık görülmekle birlikte, bunların oluşumuna sebep olan mekanizmalar net olarak bilinmemektedir. Bu çalışmanın amacı, tonsil hipertrofisinde fibroblast büyüme faktörü reseptörü 1'in (FGFR1) etkisini incelemektir.

Materyal ve Metot: Total tonsillektomi yapılan çocuk hastalar çalışmaya dahil edilmiştir. Tüm hastalarda tonsillerin subjektif büyüklüğü, tonsil ağırlığı ve hacmi ölçülmüştür. Ardından tonsil örnekleri homojenize edilmiş ve içeriğindeki FGFR1 konsantrasyonu enzyme-linked immunoassay (ELISA) yöntemi ile ölçülmüştür. Tonsil ölçümleri ve FGFR1 konsantrasyonları arasındaki korelasyonlar araştırılmıştır.

Bulgular: Çalışmaya 59 hasta dahil edilmiştir. Tonsil büyüklüğü ve ağırlığının hasta yaşı ile pozitif korelasyon gösterdiği görülmüştür. Subjektif tonsil büyüklüğü ve objektif tonsil ölçümleri arasında anlamlı korelasyon gösterilememiştir. FGFR1 konsantrasyonu ile tonsil volümü (r:-0,22, *p*:0,04) ve tonsil ağırlığı (r:-0,28, *p*:0,01) arasında anlamlı negatif korelasyon saptanmıştır. Subjektif tonsil büyüklüğü ve FGFR1 konsantrasyonu arasında anlamlı ilişki saptanmamıştır.

Sonuç: Tonsil büyüklüğü ve tonsil FGFR1 konsantrasyonu arasında negatif korelasyon mevcuttur. Bu bulgu, FGFR1'in tonsil büyüklüğünü belirleyen mekanizmalarda rol oynayabileceğini göstermektedir.

Anahtar kelimeler: Palatin tonsil, adenoid, hipertrofi, fibroblast büyüme faktörleri, inflamasyon.

INTRODUCTION

Diseases of the palatine tonsils are commonly encountered in ear, nose, throat (ENT) practice. Tonsillectomy is widely used to treat these diseases and is among the most common surgical procedures performed in pediatric patients. In the US>530,000 tonsillectomies are performed in children annually.[1] The two most common forms of tonsillar disease are tonsillar hypertrophy (leading to sleep-disordered breathing), and recurrent or chronic tonsillitis. Even though these diseases are prevalent, their etiologies are poorly understood.[2]

Fibroblast growth factor receptor-1 (FGFR1) is a protein of the transmembrane tyrosine kinase receptor family.[3] FGFR1 binds fibroblast growth factors and mediates such essential processes as cell survival, apoptosis, proliferation, and angiogenesis.[4] Earlier studies reported the presence of FGFR1 in tonsillar tissue.[5] Additionally, FGFR1 was shown to play a role in inflammatory pathologies of the liver and kidneys.[6,7] A previous study conducted at our clinic on the effect of FGFR1 on nasal concha hypertrophy observed a difference (not significant) in the tissue Fibroblast Growth Factor level in hypertrophic and normal conchae, which formed the basis of the present study.[8] The present study aimed to determine the effect of FGFR1 on tonsillar hypertrophy.

MATERIAL AND METHOD

Study design and patients

The study included patients aged 2-12 years that underwent total tonsillectomy with the dissection technique between March 2019 and December 2020 at Hacettepe University, School of Medicine, Department of Otolaryngology, Ankara, Turkey, for treatment of recurrent tonsillitis, chronic tonsillitis, or tonsillar hypertrophy. Patients that underwent surgery for other indications or underwent partial tonsillectomy were excluded, as were those with evident tonsillar asymmetry. In addition, patients with systemic diseases, anatomical abnormalities of the head and neck region, genetic syndromes, and metabolic disorders were excluded. In all, 59 patients (30 male and 29 female) that met the inclusion criteria were included in the study. Recorded patient data included age, gender, clinical symptoms, tonsillar volume and weight, and the FGFR1 concentration in tonsillar tissue. The study protocol was approved by the Hacettepe University Ethics Committee (project ID: 17389), and written informed consent was received from all the patients' parents.

Evaluation of Tonsil Size and Surgical Intervention

All patients underwent complete ear-nose-throat examination preoperatively while awake and intraoperatively under general anesthesia. Tonsil size was evaluated after placing a Boyle-Davis mouth gag and an appropriately sized tongue retractor blade. Tonsils were photographed with the mouth gag in place, and the distance between the medial edges of the tonsils and between the anterior tonsillar pillars was measured at the level where the tonsils were situated most closely. The ratio of these distances was recorded as percentiles, which were used to group the patients according to the Brodsky tonsil grading system.[9]

All surgeries were performed using the dissection technique and tonsils were excised completely. After excision, the volume and weight of both tonsils were measured. The weight of the tissue was measured using a scale with 0.01 g sensitivity. Tonsil volume was measured via the water displacement method in a cylinder with 0.2 mL sensitivity. Patients with >10% discordance between the 2 tonsils were not included in the study. The surgical specimen with the least surgical trauma was used to measure the FGFR1 concentration.

Immunohistochemical Staining

Immunohistochemical staining was used to evaluate the presence and distribution of FGFR1 in tonsillar tissue. Slices 4-µm thick were cut from the specimens, and then slides were prepared for immunohistochemical analysis. The reliability of the antibody solution used was tested on kidney tissue specimens. To determine the optimal antibody concentration antibody solution concentrations ranging from 1:25 to 1:800 were used. The 1:25 antibody solution concentration yielded the best staining results; therefore, it was used to prepare the final specimens.

Evaluation of FGFR1 Levels in Specimens

As tonsils are asymmetrical and non-homogenous organs, the first step in the preparation of the specimens for evaluation of the FGFR1 concentration was to obtain standardized tissue samples that contained all components of the organ. To that end, the obtained tonsils were cut into 2 equal pieces containing both the mucosal and capsular sides of the tonsil. Next, a 4-mm-thick sample was cut from the upper pole of 1 of the halves. With that method, a sample that equally represented the mucosal and capsular surfaces of the tonsil was obtained. After the samples were obtained, they were mechanically crushed and homogenized. The total protein concentrations of the samples were measured using the bicinchoninic acid (BCA) assay method. Next, all samples were diluted to the protein concentration of 500 µg/mL, and then the ELISA method was used to measure the FGFR1 concentration in these diluted samples.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows v.26 (IBM Corp., Armonk, NY). After the normality of the distribution of data was analyzed, Independent samples t-test was used for intergroup analyses. The correlation between the FGFR1 concentration and other parameters was analyzed using Pearson's correlation test. For all analyses the level of statistical significance was set at $p<0.05$.

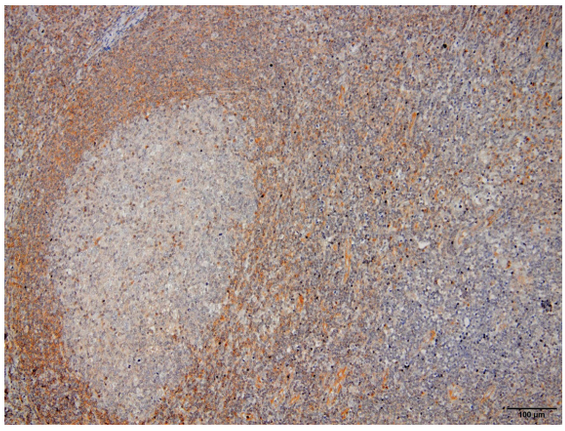

Figure 1. Immunohistochemical staining of the tonsil tissue with fibroblast growth factor receptor-1 (FGFR1) antibody. Germinal centers stain negative with the FGFR1 antibody, while the cells around the germinal centers show positive staining.

Table 1. Correlation analysis of tonsil measurements and age

		Age
Mean tonsil volume (ml)	r	0.516*
	p	<0.001
Mean tonsil weight (gr)	r	0.475*
	p	<0.001
Airway narrowing caused by tonsils (%)	r	-0.102
	p	0.440
Mean tonsil FGFR1 concentration (ng/ml)	r	-0.192
	p	0.156

FGFR1: Fibroblast growth factor receptor-1, Pearson correlation analysis, ***:** statistically significant at $p<0.01$

RESULTS

The study included 59 patients (30 male and 29 female). According to the Brodsky system, 8 (13.6%) patients had grade 1, 24 (40.6%) had grade 2, 18 (30.5%) had grade 3, and 9 (15.3%) had grade 4 tonsils. Mean tonsil volume was 2.78±0.95 mL and mean tonsil weight was 2.91±1.03 g. The mean FGFR1 concentration in tonsillar tissue was 7.66±1.93 ng $mL.^{-1}$ Based on immunohistochemical staining, the distribution of FGFR1 in tonsil tissue was determined. As shown in Figure, there was no staining in the germinal centers, but there were cells that demonstrated positive staining outside the germinal centers. Endothelial cells also exhibited positive staining for FGFR1.

Correlation analysis of tonsil measurements and patient age was performed. There was a significant positive correlation between tonsil volume and age (r=0.516, $p<0.001$), and between tonsil weight and age (r=0.475, $p<0.001$). Even though relative tonsil size and the FGFR1 concentration in tonsils were negatively correlated with age, these findings were not significant ($p=0.44$ and $=0.15$, respectively) (Table 1).

Table 2. Correlation analysis between tonsil volume, tonsil weight, airway narrowing and tonsillar fibroblast growth factor receptor-1

		Mean tonsil volume (ml)	Mean tonsil weight (gr)	Airway narrowing (%)	FGFR1 concentration (ng/ml)
Mean tonsil volume (ml)	r	1	0.930**	0.058	-0.222*
	p		0.000	0.590	0.041
Mean tonsil weight (gr)	r		1	0.070	-0.276*
	p			0.512	0.011
Airway narrowing (%)	r			1	0.170
	p				0.121
FGFR1 concentration (ng/ml)	r				1
	p				

FGFR: Fibroblast growth factor receptor-1, concentration
Pearson correlation analysis, ***:** statistically significant at $p<0.05$, ****:** statistically significant at $p<0.01$

The patients were grouped according to Brodsky tonsil grade, and these groups were compared in terms of tonsil volume and weight, but there weren't any significant differences between the groups (p=0.420 and p=0.356, respectively). Correlations between tonsil volume, weight, relative tonsil size, and the FGFR1 concentration were analyzed. As expected, there was a strong significant correlation between tonsil size and weight (r=0.930, $p<0.001$). Additionally, there was a significant negative correlation between the FGFR1 concentration, and tonsil volume (r=–0.222, p=0.041) and tonsil weight (r=–0.276, p=0.011). There wasn't a significant correlation between relative tonsil size and other variables. These findings are summarized in Table 2.

DISCUSSION

Problems caused by tonsillar hypertrophy constitute a significant proportion of otolaryngology practice. Despite how common it is, its pathogenesis is not clearly understood. Treatment of tonsillar hypertrophy is possible via surgical removal of the tonsils. Improving our understanding of the mechanisms underlying tonsillar hypertrophy might lead to novel management strategies, especially in patients with significant comorbidities that complicate surgical intervention. Although multiple molecules and mechanisms probably play a role in tonsillar hypertrophy, the present study aimed to determine the role of FGFR1.

Previous studies reported on the presence and distribution of FGFR1 in tonsillar tissue. Uhlén *et al.* observed that staining with FGFR1 antibodies was positive in non-germinal center cells and surface epithelium, but was negative in germinal centers.[5] In addition, positive staining in high-endothelial venules was reported by Hughes.[10] The present findings confirm these earlier findings and show that FGFR1 primarily affects non-germinal center areas of the tonsils.

Even though it was not a primary aim, the present study analyzed the data obtained according to patient age. Correlation analysis showed that there is a strong significant positive correlation between tonsil volume and weight, and patient age; tonsil volume and weight increased with age in patients aged 2-12 years. Nonetheless, it should be noted that the study sample might not be representative of the general population because it only included patients that met the criteria for tonsillectomy. Nevertheless, the present findings are in agreement with earlier findings. Aydın and Üner studied tonsillar size in 274 children aged 0-16 years using ultrasonography and noted that tonsil volume increases rapidly between age 1 and 4 years, with an acceleration in volume increase until age 16 years.[11] Hong *et al.* evaluated tonsil size in 161 children using ultrasonography and reported a stable increase in tonsil size with age, which is in agreement with the findings reported by Öztürk in 2017 in their series of 680 patients.[12,13] In the present study relative tonsil size and patient age were not significantly correlated. Papaionnau *et al.* reported that in the general population relative tonsil size increases up to age 7-8 years, and then starts to decrease, but that decrease is not observed in patients with snoring; as the present study included patients with tonsillar pathologies, it's findings are in agreement with those of Papaionnau *et al.*[14]

When the present study's patients were grouped according to Brodsky tonsil grade there weren't any significant differences in tonsil size or weight between the groups. Based on this finding, we think that relative tonsil size does not necessarily indicate actual tonsil size. This finding contradicts many earlier studies, especially adult studies that reported that the preoperative tonsil grade correlates with actual tonsil size. Lu *et al.*, Cahali *et al.*, and Jara *et al.* investigated this relationship in adult patients, and observed a significant correlation between relative and actual tonsil size.[15-17] Yasan *et al.* noted a significant correlation in the 292 children included in their study.[18] Probably the most detailed study on this subject was conducted by Wang *et al.*, who studied this relationship in adult and pediatric patients.[19] They also measured embedded tonsil volume. They reported that even though there is a significant correlation between relative and actual tonsil size in both adults and children, there can be significant discordance in children, as some significantly deviate from the mean values of their groups. The present findings are similar to those reported by Wang *et al.*, and the lack of significant correlation in the present study might have been due to its smaller patient population.[19] As surgeons, we know from experience that some patients with seemingly small tonsils may actually have

very large tonsils, most of which lie embedded in the tonsillar bed, or vice versa. As such, we think more studies that take embedded tonsil volume into account are required.

The primary aim of the present study was to determine the role of the FGFR1 concentration in tonsillar hypertrophy. The findings show that there is a significant negative correlation between the FGFR1 concentration, and tonsil volume and tonsil weight; smaller tonsils (i.e., in cases of chronic or recurrent tonsillitis with or without tonsillar hypertrophy) have a higher FGFR1 concentration in their cells than their large, hypertrophic counterparts. Although this finding does not prove causality, it shows that there is a possible relationship between the FGFR1 concentration and tonsil size.

To the best of our knowledge the present study is the first to evaluate the relationship between the FGFR1 concentration and benign tonsillar pathology; therefore, the findings cannot be compared and validated based on the literature. As such, the present findings were compared to those of studies that evaluated the FGFR1 concentration in patients with inflammatory pathologies of other organs. Wang *et al*. aimed to identify the mechanism by which the activation of FGFR1 causes progression of mouse prostate cancer, reporting that FGFR1 activation increases inflammation in the tumor microenvironment via promotion of nuclear factor-κB (NF-κB) and that inflammation is the key to tumor progression.[20] They also observed a decrease in inflammation in response to FGFR1 suppression. Lou *et al*. investigated the relationship between hepatic fibrosis and FGFR1, noting that activation of FGFR1 via lipopolysaccharide molecules released in response to tissue injury promoted tissue fibrosis.[6] Additionally, they reported that NF-κB mediated inflammation, tissue fibrosis, and the mitosis rate, and that proinflammatory cytokine levels decreased when FGFR1 activation was artificially suppressed. The researchers suggested that FGFR1 promoted hepatic fibrosis and that its suppression could be a possible target in hepatic fibrosis patients. Rossini *et al*. studied the effect of FGFR1 in patients with inflammatory renal pathologies.[7] They observed increased FGF1 and FGFR1 concentrations in macrophages and lymphocytes that infiltrated the kidneys in patients with inflammatory renal diseases. Moreover, they reported increased FGFR1 staining in the renal tubules of pathologic kidneys. The researchers also highlighted the close relationship between myofibroblasts, which mediate the fibrosis process, and FGFR1-positive cells, noting that FGFR1 activation might play a role in inflammation and fibrosis in patients with inflammatory renal diseases.

According to the above-mentioned studies, activation of FGFR1 can be related to NF-κB-mediated inflammation and tissue fibrosis. To improve our knowledge hypertrophic tonsils and chronically infected tonsils without tonsillar hypertrophy need to be compared. Gao *et al*. reported that the levels of inflammatory markers, including NF-κB, are significantly higher in patients with chronic tonsillitis than in those with tonsillar hypertrophy.[21] Some studies have shown significantly more fibrosis and increased collagen content in chronic tonsillitis patients.[22-24] As both fibrosis and inflammation are more commonly seen in patients with chronically inflamed tonsils without hypertrophy, and the present study observed a higher FGFR1 concentration in smaller tonsils, these findings are supported by the literature, although indirectly. Nonetheless, the design of the present study was not suitable for evaluating the totality of FGFR1's action in the tonsils and more detailed research is required.

The present study has some limitations. First, this study only focused on the FGFR1 concentration, which limits our ability to comment on the action mechanisms of the molecule. Additionally, to further prove its significance, an ideal study would have evaluated the effects of FGFR1 inhibition in tonsils (not applicable to the present study design), but could be attempted in animal studies. Moreover, the concentrations of total FGFR1 and activated FGFR1 can differ, and the separate measurement of the activated FGFR1 concentration might yield more salient findings.

CONCLUSION

Both the volume and weight of the tonsils tend to increase with age in children aged 2-12 years. The FGFR1 concentration is higher in patients with smaller tonsils; therefore, tonsils with recurrent or chronic inflammation without hypertrophy have a higher FGFR1 concentration than their hypertrophic counterparts. This elevated FGFR1 concentration might be related to increases in inflammatory molecule content and fibrosis in chronic/recurrent tonsillitis patients.

Acknowledgements

This research was fully funded by the Scientific Research Project Coordination Office of the Hacettepe University (Project ID: 17389).

*The authors declare that there are no conflicts of interest.

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