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The Role of Cytokines in the Pathophysiology of Recurrent Tonsillitis

Rekürren Tonsillit Patofizyolojisinde Sitokinlerin Rolü

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ABSTRACT Objectives: Recurrent tonsillitis and tonsillar hypertrophy are two diseases develop in the same tissue but most probably with different pathophysiological mechanisms. The role of many chemokines in tonsillitis is still being investigated. In this study, IL-1 α and β, IL-6, IL-8, IL-15, and TNF α expressions in chronic-inflamed and hypertrophic tonsil tissue were studied. **Material and Methods:** The study was conducted with Baskent University Department of Otolaryngology and Medical Genetics. A total of 45 specimens from patients with recurrent tonsillitis (17) and tonsillar hypertrophy (28) who underwent tonsillectomy were included in the study. Comparison between expression levels of cytokines and tissue groups was performed by Real Time Polymerase Chain Reaction (RT-PCR) technique. **Results:** We found a statistically significant difference only in the expression of IL-8. The mean expression level of IL-8 was higher in the hypertrophy group than in the other group. **Conclusion:** In our study, we didn't find a significant difference in the expression levels of IL-1 α and β, IL-6, IL-15, and TNF α. Only IL-8 expression was higher in tonsillar hypertrophy group. This result is thought to be related to the increased number of germinal centers seen in the hypertrophy group. It is thought that new studies investigating these cytokines and other cytokines will guide us to find the difference between hypertrophy and recurrent/chronic inflammation.

Keywords: Hypertrophic tonsil; recurrent tonsillitis; cytokines; interleukins

ÖZET Amaç: Rekürren tonsillit ve tonsillar hipertrofi aynı dokuda muhtemel farklı patofizyolojik mekanizmalar ile gelişen farklı iki hastalıktır. Tonsillit gelişiminde kemokinlerin rolü hala araştırılmaktadır. Bu çalışmada kronik inflamasyonlu ve hipertrofik tonsil dokusunda IL-1 α ve β, IL-6, IL-8, IL-15, ve TNF α ekspresyon durumunu araştırdık. **Gereç ve Yöntemler:** Bu çalışma Başkent Üniversitesi Kulak Burun Boğaz ve Tıbbi Genetik Anabilim Dalları tarafından yürütüldü. Rekürren tonsillit (17) ve tonsillar hipertrofi (28) nedeni ile tonsillektomi uygulanan hastalardan elde edilen toplam 45 doku kullanılarak RT-PCR yöntemi ile doku grupları arasındaki sitokin düzeyleri karşılaştırıldı. **Bulgular:** İncelenen sitokinler arasında sadece IL-8 ekspresyon düzeyi açısından istatistiksel fark tespit edildi. IL-8 ekspresyon düzeyi hipertrofik tonsil grubunda daha yüksek saptandı. **Sonuç:** Bu çalışmada of IL-1 α ve β, IL-6, IL-15, ve TNF α ekspresyonları açısından anlamlı bir farklılık tespit etmedik. Sadece IL-8 ekspresyon düzeyinin tonsillar hipertrofi grubunda yüksek çıkmasını, hipertrofik tonsillerde germinal merkezlerin fazla olmasıyla ilişkilendirdik. Sitokinlerin tonsillit ve hipertrofi patofizyolojisindeki rollerinin araştırılması için yeni çalışmalar gereklidir.

Anahtar Kelimeler: Hipertrofik tonsil; rekürren tonsillit; sitokinler; interlökinler

Palatine tonsils, which are one of the first defense points of the body against pathogenic microorganisms, are the organs of the secondary lymphoid system. The palatine tonsils are located in Waldeyer's lymphatic system and are primarily responsible for defense against pathogens.[1] The lymphatic ring consists of the pharyngeal, lingual, and torus tubarius tonsil, and lymphatic tissue distributed along the posterior oropharyngeal wall.[2]

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Tonsillar disease is one of the most common disorders in otorhinolaryngology. There are different types of tonsillar disease as recurrent tonsillitis, chronic tonsillitis, and tonsillar hypertrophy. Treatment options are generally antibiotic using or tonsillectomy.

Palatine tonsil's immune reactions contains specific adaptive and nonspecific innate immune responses. T cells are found in the palatine tonsils with high proportion. These lymphoid organs can produce cell-mediated and humoral immune responses.[3] Beside T cells, also interferon expression in tonsils found to be related allergy, age, and viral infections.[4]

Cytokines are molecules that have an important role for organization of the immune system and inflammatory events. In particular, they ensure the control and regulation of reactions against foreign antigens, they also play an important role in local and systemic inflammatory response by regulating intercellular relationships. An important part of the cytokines secreted from the immune system is interleukins and their main task is to stimulate the immune system cells.[5]

The role of various chemokines related to inflammatory events in tonsillar tissue has been demonstrated in studies by different methods.[1,6-10] Some authors found with little or no difference related to various immunity factors in the tonsillar tissue between hypertrophy and tonsillitis except tissue morphology. Some authors believe that hypertrophy of palatine tonsils and tonsillitis significantly differs in nonspecific immunity and inflammation.[11]

This study compared the expression of interleukin (IL)-1 α and β, IL-6, IL-8, IL-15 and tumor necrosis factor alpha (TNF-α) cytokines between recurrent tonsillitis and hypertrophic tonsil tissues.

MATERIAL AND METHODS

The study was conducted with Baskent University Department of Otolaryngology and Medical Genetics. This study is a prospective, nonrandomized, controlled clinical trial. Informed consent was obtained from the participants and their families. Local ethics committee approval was obtained. This study was approved by the Baskent University Institutional Review Board (date: 29 April, 2015, no: KA15/137) and supported by Baskent University Research Fund. This study was carried out in accordance with the Principles of the Declaration of Helsinki.

STUDY POPULATION

The study was conducted in 2 separate patient groups (Group 1: Recurrent tonsillitis group, Group 2: Tonsillar hypertrophy group). Group 1 was included 17 patients under the age of 18 who underwent tonsillectomy due to recurrent tonsillar infection, regardless of gender differences (0-18 years, due to child age group). Group 2, tonsillar hypertrophy group, was included 28 patients under the age of 18 who underwent tonsillectomy surgery due to sleep apnea/snoring without recurrent infections history, regardless of gender differences (0-18 years, due to child age group).

Exclusion criteria were known immunodeficiency, malignancy, history of cystic fibrosis, ciliary dysfunction, congenital syndrome, and people who had used antibiotics in the last 15 days.

IL-1β, IL-1β, IL-6, IL-8, IL-15, TNF-α GENE EXPRESSION

The tissues obtained after the surgical operation was delivered with the transport medium on the same day. Total cellular RNA was isolated using TriZOL reagent (Roche Diagnostics GmbH, Mannheim, Germany). Isolated RNA samples were translated into cDNA using the Transcriptor High-Fidelity cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). Expression levels of IL-1α (Assay ID: 100544), IL-1β (Assay ID: 100950), IL-6 (Assay ID: 144013), IL-8 (Assay ID: 103136), IL-15 (Assay ID: 141328), TNF-α (Assay ID: 103295), GAPDH (Assay ID: 141139) were determined by applying the real-time polymerase chain reaction. Expression levels were investigated using 2-ΔΔCt technique. Comparisons were made between tissue groups.

STATISTICAL ANALYSIS

IBM SPSS Statistics 22 software was used for statistical assessments. Comparisons between groups were made using the Chi-Square test, student-t test, Mann-Whitney U test, Kruskal-Wallis tests. $p<0.05$ value was considered significant.

RESULTS

Forty-five tissues were involved in the study. Of these tissues, 32 belong to male individuals and 13 belong to female individuals. Mean age was 7.60±2.58. Of the 28 samples were included in the hypertrophy tonsil group, 23 were males and 5 were females. The average age of this group was 7.18±2.37. The recurrent tonsillitis group included 17 samples, 9 of them were male, and 8 of them were female. The average age of this group was 8.29±2.82 (Table 1).

Only at statistically important difference was found in the expression of IL-8. IL-8 expression in the hypertrophic tonsil group was found to be more significant than in chronic tonsillitis group ($^{*}p<0.05$) (Figure 1).

TABLE 1: Comparative of age and gender between hypertrophic tonsils and recurrent tonsillitis tissue groups.

Group	Male	Female	Age	Total
Hypertrophic tonsil	23	5	7.18±2.37	28
Recurrent tonsillitis	9	8	8.29±2.82	17
Total	32	13	7.60±2.58	45

DISCUSSION

The differences between chronic or recurrent tonsillitis and hypertrophic tonsils are still unknown exactly. We would like to analyze these 2 conditions in terms of some cytokines. ILs are the most important part of cytokines. We studied IL-1, IL-6, IL-8, IL-15, and TNF. We only found that IL-8 expression is significantly higher in hypertrophic tonsil tissues.

IL-1 has got 2 different protein parts as IL-1β and IL-1α. It is produced by all cells, but it is made in macrophages, keratinocytes, endothelial cells, smooth muscle cells, dendritic cells, fibroblasts and neutrophils. Although IL-1 can be made continuously in some cells, more IL-1 is made after stimulation with substances such as microorganisms, lipopolysaccharide, muramil dipeptide. Agents that stimulate T lymphocytes can also stimulate macrophages, causing IL-1 to form. IL-1 has a more protective effect on cells. IL-1 increases the capacity of antigen presenting cells, proliferation of B lymphocytes, immunoglobulin synthesis and the number of immunoglobulin (Ig) receptors on the cell surface. IL-1 causes local neutrophil infiltration, delayed-type cellular sensitivity, fibroplasia and angiogenesis.[5,12]

IL-6 expression is mainly synthesized by bone marrow stromal cells, mesenchymal cells, fibroblasts, T and B lymphocytes, monocytes, keratinocytes, as-

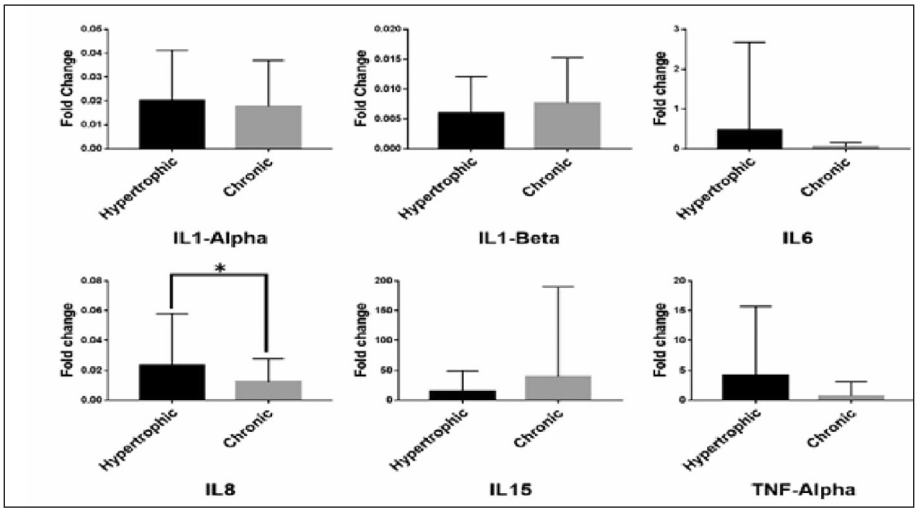

FIGURE 1: Comparative of the expressions of cytokines were between hypertrophic tonsil and recurrent tonsillitis tissue groups. IL: Interleukin; TNF-α: Tumor necrosis factor alpha.

trocytes, and endothelial cells. It is one of the essential factors for B lymphocytes to make antibodies and increase the conversion of stimulated lymphocytes into plasma cells that produce IgG, IgM, IgA.[13]

IL-8 expression is carried out by fibroblasts, peripheral blood mononuclear cells, endothelial cells and keratinocytes. When IL-1 and TNF are stimulated, IL-8 synthesis begins. In the immune response, it is thought to be the most important mediator causing neutrophil chemotaxis to the inflammation site.[14]

The biological properties of IL-15 are similar to IL-2. While causing T lymphocyte proliferation, it also increases antibody-dependent cellular cytotoxicity and natural killer (NK) cytotoxicity. It regulates the production of NK-derived cytokines such as interferon-alpha (IFN-α), granulocyte-macrophage colony-stimulating factor, TNF, and differentiation of anti-IgM-stimulated B lymphocytes. It increases the proliferation and antibody formation of B lymphocytes.[15]

Previous studies have shown that TNF-α, IL-1β, IL-1α, IL-8, IL-6, IL-15 are expressed in different tissues and affect cell functioning. The role of various cytokines related to inflammatory events in tonsillar tissue has been demonstrated in studies by different methods.[1,6-10]

Reis et al. found in their study that people with tonsil hypertrophy had a higher concentration of germinal centers and lymphatic follicles.[2] They also showed that lymph follicles and germinal centers were smaller in people with hypertrophy. The presence of the germinal center was known to play an active role in lymphocyte production in the lymphoid follicle.

We know from previous studies that antigenic stimulation of intraepithelial T lymphocytes in the tonsil-crypt epithelium produces proinflammatory cytokines of Th1 type as IFN-, IL-2, TNF-, and Th2 type cytokines as IL-4-5-6-13.[16-19] In tonsillitis production of cytokines starts with Th1 type cytokines and later on secretion of Th2 cytokines. The existence of Th1-type cytokines as IFN-γ and TNF-α is found to be higher in recurrent tonsillitis group when it is compared with hypertrophic tonsil group by Todorović et al.[1]

Bredun et al. also found that the content of proinflammatory- IL-1β is significantly higher in chronic tonsillitis.[11] We did not find any differences related expression IL-1, also TNF-α, IL-15, and IL-6.

It has been shown in previous studies that IL-8 is a chemokine. It has a key role in adhesion of monocytes and neutrophils in the intrafollicular area of tonsil tissue. The cause of leukocyte migration to the inflammatory region in the immune response suggests that it is vital for body defense.[20] In tonsillar hypertrophy especially in obstructive sleep apnea syndrome IL-8 was found to be increased in some studies.[7,20,21] We also found that IL-8 expression was higher in hypertrophic tonsil tissue. It was thought that the increased IL-8 expression in the hypertrophic tonsil group would be associated with the increased number of lymphoid follicles in the germinal center in hypertrophic tonsil groups.

Even though some studies found differences in many cytokines' levels at tonsillar diseases, it cannot really possible to say the reason related the difference between hypertrophy and recurrent/chronic tonsillitis. Both condition may alter inflammatory markers. A limitation of this study is that we did not investigate bacterial/viral colonization of the tonsils in these patients. Also, we did not study in other age groups.

CONCLUSION

There is no difference between hypertrophic tonsil and chronic tonsillitis tissues due to levels of expression of IL-1β, IL-1α, IL-15, IL-6, and TNF-α; IL-8 expression is significantly higher in hypertrophic tonsils. It is still not yet possible to create a consensus on the difference between hypertrophic tonsil and recurrent/chronic tonsillitis. Cytokine profiles of tonsil tissues in different groups can be investigated in a larger series to clarify the mechanisms of inflammation.

Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Seda Türkoğlu Babakurban, Selin Akad Dinçer, Yunus Kasım Terzi, Feride İffet Şahin;* ***Design:*** *Feride İffet Şahin;* ***Control/Supervision:*** *Seda Türkoğlu Babakurban, Feride İffet Şahin, Yunus Kasım Terzi;* ***Data Collection and/or Processing:*** *Seda Türkoğlu Babakurban;* ***Analysis and/or Interpretation:*** *Seda Türkoğlu Babakurban, Selin Akad Dinçer, Yunus Kasım Terzi, Feride İffet Şahin;* ***Literature Review:*** *Seda Türkoğlu Babakurban, Selin Akad Dinçer;* ***Writing the Article:*** *Seda Türkoğlu Babakurban, Selin Akad Dinçer;* ***Critical Review:*** *Seda Türkoğlu Babakurban, Yunus Kasım Terzi, Feride İffet Şahin;* ***References and Fundings:*** *Seda Türkoğlu Babakurban, Selin Akad Dinçer, Yunus Kasım Terzi, Feride İffet Şahin;* ***Materials:*** *Seda Türkoğlu Babakurban.*

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