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Apoptosis in chronic tonsillitis and tonsillar hypertrophy

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ABSTRACT

Objective: Chronic tonsillitis is the persistent inflammation of the tonsillar tissue that occurs due to recurrent, acute or subclinical infection. The recurrent and chronic inflammation of palatine tonsils sometimes results in hypertrophy. Apoptosis provides an important balance between lymphocytes in tonsillar lymphoid tissue. The aim of this study is to investigate the apoptosis in tonsillar diseases.
Methods: 43 patients with chronic tonsilitis and tonsillar hypertrophy underwent tonsillectomy. The specimens were examined immunohistochemically for apoptosis. Tonsils were assembled into groups according to their size. Specimens were compared for their apoptotic cell count.
Results: The apoptosis difference between the tonsil size groups is not statistically significant ($p > 0.05$). However, when the study group was divided into two at age 6, the difference was not statistically significant for patients at and below 6 years of age; but, the difference was statistically significant for patients above 6 years of age ($p < 0.05$). The comparison of apoptosis in microcompartments of tonsil tissue (intrafollicular, interfollicular, subepithelial and intraepithelial) between tonsil size stages and between chronic tonsillitis and tonsillar hypertrophy groups revealed no statistical significance ($p > 0.05$). There was a statistically significant positive correlation between intrafollicular and interfollicular, interfollicular and intraepithelial & subepithelial and intraepithelial areas ($p < 0.05$).
Conclusions: In the light of these findings, it was concluded that apoptosis played a role in the tonsillar hypertrophy and atrophy. Apoptosis functioned to balance lymphocyte proliferation in tonsil tissue. The association of apoptosis with tonsillar hypertrophy seemed to be age-dependent.

1. Introduction

Immunologic reactions within tonsils may lead to hypertrophy and chronic infection. Chronic tonsillitis is a persistent inflammation resulting from recurrent acute tonsillitis or subclinical infections. Chronic tonsillitis is a clinical diagnosis relying upon history of tonsillitis and sore throat relapsing 3–4 times a year and not responding to sufficient antibiotic therapy [1]. Hypertrophy in tonsils develops as a result of parenchymal hyperplasia or fibrinoid degeneration leading to obstruction of crypts. However, chronic infection may also lead to atrophy.

The etiology of hypertrophy in tonsillar lymphoid tissue is not exactly known; however, diet, genetics and humoral change may play a role [2]. Furthermore, the causes of tonsillar hypertrophy and the effect on immune cell composition of recurrent tonsillitis is not entirely clear, yet [3].

Apoptosis is a morphological involution caused by a cellular suicide program which is associated with programed cell death. Fas antigen mediated apoptosis and death of immunocytes in lymphoid tissues are induced through apoptotic mechanisms. The number of apoptotic cells in tonsil is higher in adults than in children. Apoptosis may be related to morphological and immunologic involutions of the tonsil [4]. Germinal center of lymphoid tissues plays an important role both in cell proliferation and cell death. Apoptosis is the result of programmed, not pathological cell death, and it plays an important role in the maintenance, immunity and development of life [5]. Apoptosis is scarce in immature type of germinal center, but abundant in mature type. Apoptosis has an important function in the germinal center of tonsil [6]. The number of lymphocytes in tonsil and their

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role in immune response is dependent on their proliferation and migration status. Apoptosis provides the balance among lymphocytes. Thence, stimulated, autoreactive T lymphocytes with low specificity are removed from the environment [7,8]. Apoptosis plays a role in continuation of immune response by providing lymphocyte homeostasis in normal tonsil tissue, as well as pathologic conditions like hypertrophy and chronic infection. Apoptosis functions to balance mitosis in order to protect tissue remodeling and cellular homeostasis during development. That is why it plays an important role in control of tissue hyperplasia by equilibrating lymphocytes [7]. There must be a balance between apoptosis and proliferation in normal lymphoid tissue in order to keep total lymphocyte count constant [9]. Apoptosis regulates life span of inflammatory cells. Therefore, increase or decrease in apoptosis determines the course of inflammatory process. Decreased apoptosis leads to chronic inflammation and increased severity of disease. Kucera et al. [10] showed that proliferation and cell death effected mostly B cells in chronic inflammation. Apoptosis in immune system and in lymphocytes has been extensively studied; however, there is only one study in the literature about apoptosis in tonsillar disorders [7].

This study was performed to evaluate the apoptosis in chronic tonsillitis and tonsillar hypertrophy.

2. Material and methods

This study was approved by the institutional ethics committee (Date: number 11/27). This research was supported totally by our institution's Scientific Research Projects Coordination Unit (Project number: 012 D06 101 008). The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Informed consent was obtained from the parents of all patients prior to their inclusion in the study.

This study was performed on 43 patients with the clinical diagnosis of chronic tonsillitis and tonsillar hypertrophy. Their ages ranged between 2 and 16 years with a mean of 6. 23 were males and 20 were females. The patients having systemic disorders and other otolaryngologic problems were not included in the study. Tonsil size was determined according to Friedman staging system as stage 1, 2, 3 and 4 [11]. Tonsillectomy was performed under general anesthesia using dissection method. Those patients with symptoms and/or signs of recurrent acute tonsillitis, recurrent sore throat, foul mouth smelling and tonsil size of stage 1 and 2 were considered chronic tonsillitis group ($n = 22$). Those patients with symptoms and/or signs of snoring, open mouth breathing in addition to those symptoms and/or signs above and tonsil size of stage 3 and 4 were called tonsil hypertrophy group (n = 21). The tonsil tissues were sent in formaldehyde tubes to the Department of Histology and Embryology for investigation of apoptosis. Apoptosis was evaluated in different compartments of tonsil tissue.

All patients were operated on an outpatient basis. None had any postoperative complication.

Tissue samples in 10% formalin solution were passed through series of various concentrations of ethyl alcohol and xylene. They were then parafinized and paraffin blocks were prepared. 4–5 μm thick sections were obtained. Apoptosis was determined according to "TdT-dUTP nick-end-labeling" (TUNEL) method using ApopTag® Plus Peroxidase kit (In Situ Apoptosis Detection Kit, Chemicon (Millipore), Billerica, MA, USA) in various steps as suggested in terms of use in the kit.

Apoptosis was evaluated in most densely stained areas using light microscopy under x400 magnification (Leica DMR, Wetzlar, Germany). Apoptotic cells were counted according to Kerr criteria in 1972 [12]: Brown staining, morphologically oval to round shaped nuclear condensation and fragmentation with narrow to dense cytoplasm. Three different areas in each microcompartment were evaluated in each specimen and their mean was taken (Figs. 1–4).

For statistical analysis of results SPSS 15.0 for Windows was used. For analysis of difference between groups, Kruskal-Wallis analysis of variance, Student's *t*-test for independent samples and Mann–Whitney *U* test was used. For correlation analysis Spearman's rho correlation test was utilized. Significance was taken as $p < 0.05$.

3. Results

Mean apoptosis cell counts of tonsil stages were shown in Table 1. The differences were not statistically significant ($p > 0.05$).

Stage 1 and 2 were combined into chronic tonsillitis group and stage 3 and 4 into tonsil hypertrophy group. Their apoptosis counts were shown in Table 2. The differences were not statistically significant ($p > 0.05$).

The comparison of apoptosis in microcompartments of tonsil tissue (intrafollicular, interfollicular, subepithelial and intraepithelial) between tonsil size stages were demonstrated in Table 3. The differences were not statistically significant ($p > 0.05$).

The comparison of apoptosis in microcompartments of tonsil tissue (intrafollicular, interfollicular, subepithelial and intraepithelial) between chronic tonsillitis and tonsillar hypertrophy groups were demonstrated in Table 4. The differences were not statistically significant ($p > 0.05$).

The correlation analysis of apoptosis among microcompartments of tonsillar tissue was shown in Table 5. There was a statistically significant positive correlation between intrafollicular and interfollicular, interfollicular and intraepithelial and subepithelial and intraepithelial areas ($p < 0.05$).

The age six was used as a cut-off to compare apoptosis in tonsillar tissue. The comparison of apoptosis in tonsillar tissue between chronic tonsillitis and tonsillar hypertrophy groups in patients at and below 6 years of age and above 6 were shown in Tables 6 and 7. The difference was not statistically significant for patients at and below 6 years of age; however, the difference was statistically significant for patients above 6 years of age.

4. Discussion

Tonsillectomy is among the most frequently performed operations in ENT practice. Among the most frequent indications for tonsillectomy are tonsillar hypertrophy and chronic tonsillitis

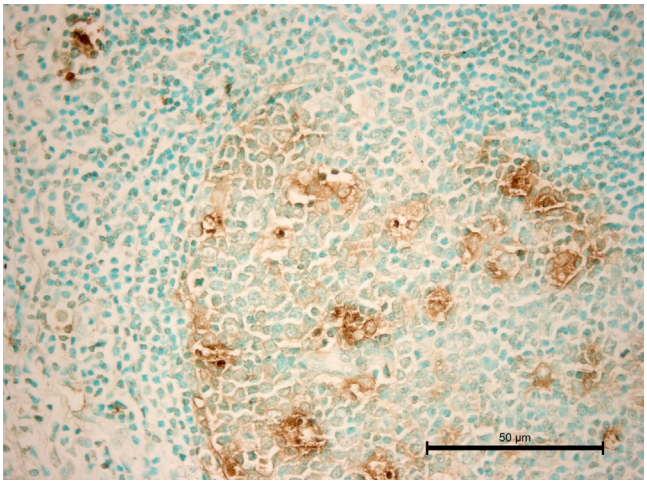

Fig. 1. Apoptosis in tonsil stage 1, TUNEL, ×400.

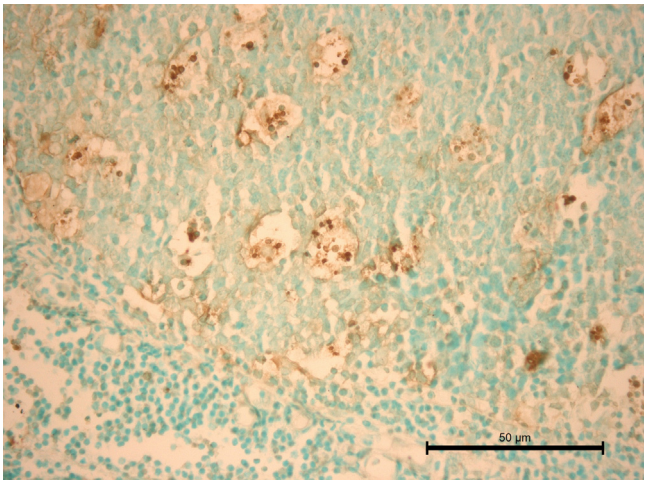

Fig. 2. Apoptosis in tonsil stage 2, TUNEL, ×400.

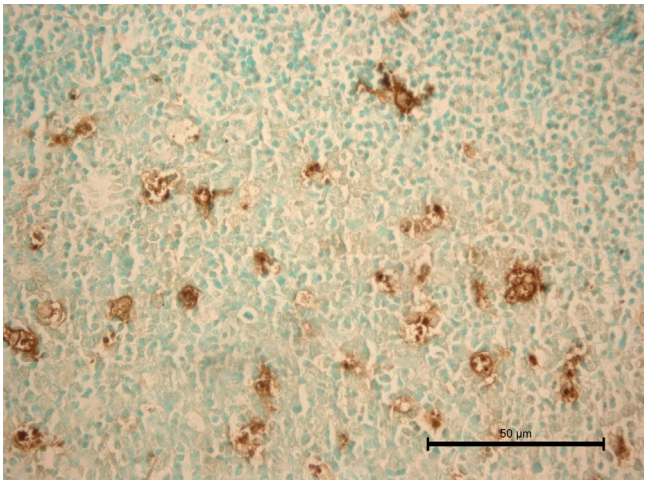

Fig. 3. Apoptosis in tonsil stage 3, TUNEL, ×400.

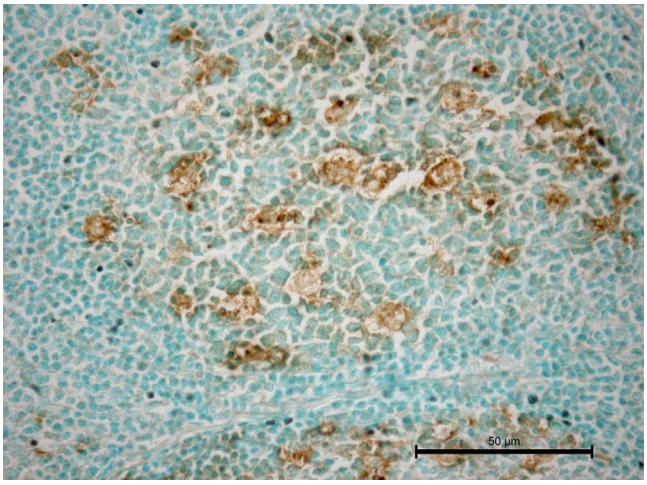

Fig. 4. Apoptosis in tonsil stage 4, TUNEL, ×400.

[13]. Pathologic features of chronic tonsillitis and tonsillar hypertrophy are not distinct from each other; both disorders are histopathologically entitled as reactive lymphoid hyperplasia. Immunologic parameters, genetic predisposition and local lymphocyte dysfunction appear to play a role in the etiology of

Table 1
The comparison of apoptotic cell counts according to tonsil stages (Kruskal Wallis analysis of variance).

Stage	Mean apoptotic cell count ± SD	Statistics
Stage 1 ($n=6$)	9.5 ± 5.54	$\chi^2=3.96$, $p=0.266$
Stage 2 ($n=16$)	12.88 ± 7.29	
Stage 3 ($n=13$)	13.31 ± 5.69	
Stage 4 ($n=8$)	12 ± 2.14	

SD: Standard deviation.

Table 2
The comparison of apoptotic cell counts according to chronic tonsillitis and tonsil hypertrophy groups (Mann–Whitney *U* test).

Study groups	Mean apoptotic cell count ± SD	Statistics
Chronic tonsillitis ($n=22$)	11.95 ± 6.90	$Z=1.355$, $p=0.175$
Tonsil hypertrophy ($n=21$)	12.81 ± 4.63	

SD: Standard deviation.

recurrent tonsillitis and tonsillar hypertrophy [14]. Several studies showed that these disorders developed as a result of hypofunction in local and systemic immunity [15,16]. Bieluch et al. [17] indicated that there was no histopathologic difference between chronic tonsillitis and recurrent tonsillitis and they accepted lymphocyte infiltration in crypt epithelium and crypt epithelial defect as a marker of chronic tonsillitis.

Tonsillar hypertrophy developed as a result of chronic and recurrent infection of palatine tonsils [14]. In hypertrophic tonsils, the increase in T and B cell count showed a positive correlation with bacterial count and tonsil size [17]. However, the cause of tonsillar hypertrophy has not been elucidated, yet. Epidemiologic studies demonstrated that tobacco smoking, allergies and recurrent respiratory infections might be associated with transient or permanent hypertrophy of lymphoid tissue [18].

The increase in size and number of germinal centers was found more in patients with tonsillar hypertrophy compared to those without hypertrophy. However, the presence of stage-1-sized palatine tonsils in patients with chronic tonsillitis indicated that chronic tonsillitis might also be associated with atrophic palatine tonsils. Zhang et al. [19] demonstrated that tonsillar hypertrophy was associated with increased lymphoid follicle size, but not the number of follicles. Alataş and Baba [20] determined that tonsillar hypertrophy was related to increased tonsil weight, increased follicle diameter, area and number.

Apoptosis is an indicator of proliferative activity. In a tissue with high cellular proliferation, apoptosis has to be increased, too. The increase in lymphocytes secondary to recurrent infections is limited by the increase in apoptosis in tonsils. As expected from antigenic stimulus-increased lymphocyte proliferation-increased cell death cycle, significantly increased cellular apoptosis in hypertrophic tonsils functions to limit or control cellular hyperplasia. However, we found that this increased apoptosis was age dependent and that it was true for patients above 6 years of age only. However, there was no statistically significant difference in apoptosis of microcompartments.

In this study there was no statistically significant difference in apoptosis of chronic tonsillitis and tonsillar hypertrophy. This lack of difference can be interpreted that immunoregulatory mechanisms involving lymphocyte proliferation and cell death is not well preserved in tonsils, because increased apoptosis is expected in case of tonsillar hypertrophy. We determined that the increased tonsillar apoptosis was associated with age. The lack of significance

Table 3
Comparison of apoptosis in microcompartments of tonsil tissue (Kruskal Wallis analysis of variance).

Stage	Mean apoptotic cell count ± SD			
	Intrafollicular	Interfollicular	Subepithelial	Intraepithelial
Stage 1 (n = 6)	5.67 ± 1.87	1.67 ± 0.56	1.33 ± 0.33	0.83 ± 0.31
Stage 2 (n = 16)	8.06 ± 1.12	2.06 ± 0.34	1.50 ± 0.28	1.25 ± 0.42
Stage 3 (n = 13)	7.23 ± 1.00	2.15 ± 0.32	2.15 ± 0.32	1.77 ± 0.43
Stage 4 (n = 8)	6.75 ± 0.59	2.25 ± 0.31	1.38 ± 0.32	1.63 ± 0.49
Statistics	$\chi^2 = 2.875$, $p = 0.411$	$\chi^2 = 1.624$, $p = 0.654$	$\chi^2 = 3.703$, $p = 0.295$	$\chi^2 = 2.902$, $p = 0.407$

SD: Standard deviation.

Table 4
The comparison of apoptosis in microcompartments of tonsil tissue between chronic tonsillitis and tonsillar hypertrophy groups (Mann-Whitney *U* test).

Study groups	Mean apoptotic cell count ± SD			
	Intrafollicular	Interfollicular	Subepithelial	Intraepithelial
Chronic tonsillitis (n = 22)	7.41 ± 4.52	1.95 ± 1.33	1.45 ± 1.06	1.14 ± 1.49
Tonsil hypertrophy (n = 21)	7.05 ± 2.97	2.19 ± 1.03	1.86 ± 1.11	1.71 ± 1.45
Statistics	$Z = 0.086$, $p = 0.932$	$Z = 1.017$, $p = 0.309$	$Z = 1.197$, $p = 0.231$	$Z = 1.661$, $p = 0.097$

Table 5
The correlation between apoptosis cell counts of microcompartments of tonsillar tissue. (Spearman's rho correlation analysis).

Microcompartment	Correlation coefficient	
Intrafollicular and interfollicular	$r = 0.453$	($p = 0.002$)
Interfollicular and subepithelial	$r = 0.273$	($p = 0.076$)
Interfollicular and intraepithelial	$r = 0.296$	($p = 0.05$)
Subepithelial and intraepithelial	$r = 0.576$	($p < 0.001$)

Table 6
The comparison of apoptosis in tonsillar tissue between chronic tonsillitis and tonsillar hypertrophy groups in patients at and below 6 years of age (Mann–Whitney *U* test).

Study groups	Mean apoptotic cell count ± SD	Statistics
Chronic tonsillitis (n = 11)	14.27 ± 8.41	$Z = 0.302$, $p = 0.762$
Tonsillar hypertrophy (n = 14)	12.50 ± 4.95	

Table 7
The comparison of apoptosis in tonsillar tissue between chronic tonsillitis and tonsillar hypertrophy groups in patients above 6 years of age (Mann–Whitney *U*-test).

Study groups	Mean apoptotic cell count ± SD	Statistics
Chronic tonsillitis (n = 11)	9.64 ± 4.17	$Z = 2.195$, $p = 0.028$
Tonsillar hypertrophy (n = 7)	13.43 ± 4.19	

disappeared when we controlled apoptosis with age. The significantly increased apoptosis in tonsillar hypertrophy compared to chronic tonsillitis was valid for patients above 6 years of age; this significance diminished in tonsils of patients at and below 6 years of age. The authors felt that increased apoptosis with increasing age might account for the noted involution of lymphoid tissue as patients get older.

It has been asserted that bacteriology of tonsillar hypertrophy might be different from that of chronic tonsillitis [17]. Kielmovitch et al. [21] determined *Streptococcus pyogenes* as predominant microorganism in tonsillar hypertrophy and *Staphylococcus aureus* as predominant microorganism in chronic tonsillitis. Kuhn et al. [22] found *S. aureus* ve *Haemophilus influenza* more frequently in tonsillar hypertrophy than chronic tonsillitis irrespective of the presence of recurrent infections.

Recurrent stimuli by pathogenic agents lead to activation of monocytes and macrophages. The secreted cytokines cause proliferation of entothelial cell and fibroblasts besides stimulating immunity. In time, immunologically active tissue is replaced by fibrotic tissue. Thus, inflammatory process in tonsils does not always lead to hypertrophy, but sometimes ends with scleroatrophic tonsil [23]. From the results of this study we infered that chronically infected, but small-sized tonsils had low lymphoid tissue and high fibrotic tissue and that thus they should have low apoptosis. Furthermore, large-sized tonsils should have high lymphoid tissue, low fibrotic tissue and thus high apoptosis. However, in this study the ratio between proliferation and apoptosis was not specifically studied. This ratio would be useful to determine whether the tonsil would be enlarging or contracting.

The discrepancies in tonsil size and apoptosis may originate from current tonsil size classifications. Current tonsil size classifications, such as Friedman classification [11], evaluate tonsil size medial to tonsillar pillars relative to oropharyngeal orifice diameter in coronal plane; tonsil size deep to the tonsillar pillars and tonsil size in sagittal plane are not evaluated. Thus, a tonsil deep in the tonsillar fossa may be actually big, but downstaged; or a tonsil superficial in tonsillar fossa may be upstaged. Lack of significance in difference of apoptosis of tonsil size may be due to this false staging. This may be overcome by measuring volume of tonsils using radiologic studies or by measuring volume and weight of tonsils after excision.

There was a statistically significant positive correlation between intrafollicular and interfollicular, interfollicular and intraepithelial & subepithelial and intraepithelial microcompartments of tonsils. This significant positive correlation is related to the proximity of these microcompartments to each other in tonsil tissue. The antigen contacts first the epithelium and it is presented first to the intraepithelial lymphocytes, then to the subepithelial lymphocytes; interfollicular and intrafollicular lymphocytes follow in a row. After stimulus the lymphocytes proliferate against the antigen; hence, apoptosis also increases. Dono et al. [24] indicate that subepithelial B cells belong to a subset of non-recirculating B cells and that they are virtually absent in the peripheral blood. They fail to produce polyspecific antibodies and fail to undergo spontaneous apoptosis. Their most striking function is their ability to produce IgM antibodies to T cell-independent type-2 antigens. They participate in the chain of events required to promote the proliferation of allogeneic T cells in tonsil.

Apoptosis of eosinophils is decreased in patients with allergic asthma and allergic rhinitis in order to limit inflammation. Besides eosinophils the lifespan of T lymphocytes is prolonged in allergic

inflammation. Thus, allergy may affect apoptosis in tonsils. Sadeghi et al. [25] asserted that allergy and sensitivity to allergens was a risk factor for tonsillar hypertrophy in children. Furthermore, there are studies which indicate that tonsillar hypertrophy is more frequent in children with allergies [26,27].

The percentage of basophils is increased in histology of hypertrophic tonsils. The activation of endogeneous endonucleases is closely associated with apoptosis in many cells. Lopez et al. [7] determined significant difference in endonuclease activities of chronic tonsillitis and tonsillar hypertrophy. IL-4, secreted by basophils, regulates apoptotic endonucleases and causes proliferation of tonsillar tissues. IL-4 prevents induction of apoptosis of T, B and NK cells. In recurrent tonsillitis endonucleases are activated and lymphoid apoptosis develops. However, in tonsillar hypertrophy basophils trigger chronic inflammatory process and inhibit apoptosis [7]. Furthermore, Agren et al. [28] determined increased IL-4 in tonsillar hypertrophy compared to recurrent tonsillitis. They also found increased basophil count in tonsillar hypertrophy compared to recurrent tonsillit + tonsillar hypertrophy group. Thus, they inferred that decreased apoptosis in tonsillar hypertrophy might be due to increased basophil count. They identified lowest basophil count in recurrent tonsillitis group. In our study we determined high apoptosis in tonsil stages 2, 3 and 4 and lowest apoptosis in stage 1.

Changes in DNA methylation alter many biologic processes including cell cycle and apoptosis by modifying gene expression. Lopez-Gonzalez et al. [29] identified increased hypomethylation in patients with tonsillar hypertrophy compared to those with recurrent tonsillitis. Hypomethylation is thought to play a role in tonsillar hypertrophy by altering expression of genes related to cell cycle or apoptosis; or by increasing IL-4 level, secreted by basophils. The three aforementioned studies [7,28,29] indicate that apoptosis is decreased in tonsillar hypertrophy. However, our results show the contrary, that apoptosis is increased in tonsillar hypertrophy in order to balance cellular hyperplasia. The different result may originate from the fact that the patients in aforementioned studies have obstructing tonsils, but no recurrent infection and our patients have both. The presence of recurrent infection appears to stimulate apoptosis of lymphoid tissue.

5. Conclusions

Apoptosis functions to balance lymphocyte proliferation in tonsil tissue. To ensure lymphoid homeostasis, cell death (apoptosis) has to increase in tonsillar hypertrophy, where there is cell proliferation. Apoptosis appears to be playing an important role in tonsillar hypertrophy or atrophy, because tonsillar hypertrophy develop secondary to lymphoid hyperplasia. The association of apoptosis with tonsillar hypertrophy seem to be age-dependent.

Conflicts of interest

None.

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