International Journal of Pediatric Otorhinolaryngology 79 (2015) 191–195

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Apoptosis in chronic tonsillitis and tonsillar hypertrophy

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ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form 6 December 2014
Accepted 8 December 2014
Available online 15 December 2014

Keywords:
Palatine tonsil
Tonsillitis
Apoptosis
In situ nick-end labeling

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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http://dx.doi.org/10.1016/j.ijporl.2014.12.005
0165-5876/

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

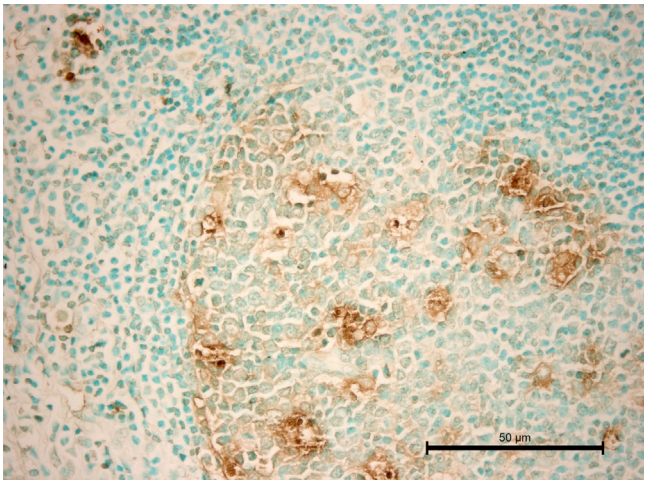

Fig. 1. Apoptosis in tonsil stage 1, TUNEL, ×400.