Auris Nasus Larynx 42 (2015) 449–452

Contents lists available at ScienceDirect

Auris Nasus Larynx

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

# Possible role of apoptosis in pathogenesis of adenoid hypertrophy and chronic adenoiditis: Prospective case–control study

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ARTICLE INFO

*Article history:*
Received 3 February 2015
Accepted 27 April 2015
Available online 21 May 2015

*Keywords:*
Adenoids
Nasal obstruction
Apoptosis
In situ nick-end labeling

ABSTRACT

*Objective:* Apoptosis is a programmed cell death; it provides an important balance between lymphocytes in adenoid tissue. The aim of this study is to investigate the role of apoptosis in chronic adenoiditis and adenoid hypertrophy. This is the first study in the literature about apoptosis in adenoid hypertrophy and chronic adenoiditis.

*Methods:* Prospective case–control study in a tertiary referral university hospital was conducted. 46 patients who had chronic adenoiditis and adenoid hypertrophy underwent adenoidectomy. Adenoids were evaluated for apoptosis and assembled into groups according to their size. Apoptotic cells were counted in three different microscopic fields and their average was taken for every microcompartment. As a result of immunohistochemical staining, specimens were compared for their apoptotic cell rate.

*Results:* The difference in apoptosis of chronic adenoiditis and adenoid hypertrophy groups is statistically significant ($p < 0.05$). The age 6 was used as a cut-off to compare apoptosis in adenoid tissue. 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. The comparison of apoptosis in microcompartments of adenoid tissue (intrafollicular, interfollicular, subepithelial and intraepithelial) between chronic adenoiditis and adenoid hypertrophy groups revealed significant differences for intrafollicular and intraepithelial areas, and insignificant differences for interfollicular and subepithelial areas.

*Conclusion:* Although apoptosis could not totally explain the pathogenesis of chronic adenoiditis and adenoid hypertrophy, it appeared to play an important role in it. Apoptosis functions to limit adenoid hypertrophy. Adenoid apoptosis appears to be age-dependent.



## 1. Introduction

Adenoids are parts of the immune system. Immunologic reactions within adenoids may lead to hypertrophy and chronic infection. Chronic adenoiditis is the persistent inflammation of the adenoid tissue that occurs due to recurrent, acute or subclinical infection. The recurrent and chronic inflammation of adenoids sometimes results in hypertrophy [1]. Hypertrophy in adenoids develops as a result of parenchymal hyperplasia or fibrinoid degeneration leading to obstruction of crypts. However, chronic infection may also lead to atrophy.

Chronic adenoiditis is a clinical condition characterized by persistent purulent rhinorrhea, foul smelling breath, postnasal drainage, cough and infected adenoids with chronic congestion and crusts on it. Adenoid may be hypertrophic or normal in size. In case of adenoid hypertrophy, nasal obstruction causes difficulty in breathing; as a result snoring and mouth breathing are observed [2,3]. The etiology of this hypertrophy in lymphoid tissue is not exactly known; however, diet, genetics and humoral change may play a role [4]. Furthermore, the etiology of adenoid hypertrophy and the effect on immune cell composition of recurrent adenoiditis is not entirely clear, yet.

Apoptosis in immune system and in lymphocytes has been extensively studied; however, there is no study in the literature about apoptosis in adenoid hypertrophy and chronic adenoiditis.

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http://dx.doi.org/10.1016/j.anl.2015.04.012
0385-8146/

This is the first study in the literature about apoptosis in adenoid tissue. This study was performed to evaluate the possible role of apoptosis in the pathogenesis of chronic adenoiditis and adenoid hypertrophy.

## 2. Materials and methods

This study was approved by the institutional ethics committee of our university (number 11/29). This research was supported totally by our institution's Scientific Research Projects Coordination Unit (Project number: 012 D06 101 007). The study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. Informed consent was obtained from the parents of all patients.

This study was performed on 46 patients with the clinical diagnosis of chronic adenoiditis and adenoid hypertrophy. Their ages ranged between 2 and 14 years with a mean of 6. 22 were males and 24 were females. The patients having systemic disorders and other otolaryngologic problems were not included in the study. Patients were evaluated only at least 2 weeks after active infection had been controlled with antibiotics. None of the patients had a recent history (less than a month) of intranasal steroid use. Adenoids were examined with a flexible pediatric endoscope with a diameter of 2.5 mm (Karl Storz, Tuttlingen, Germany) through the nose. All borders of choana and adenoid tissue were visualized and the ratio of adenoid size to choana diameter was expressed as a percentage [5]. Clear clinical and morphological distinction between chronic adenoiditis and adenoid hypertrophy does not exist; there is a significant overlap between these two entities. All the patients were assumed to have some degree of chronic adenoiditis; however, those with and without hypertrophy were differentiated. Those patients with symptoms and/or signs of purulent rhinorrhea, foul smelling breath, postnasal discharge and those also having 50% or less adenoid-to-choana ratio were called chronic adenoiditis without hypertrophy group ($n = 21$). Those patients with symptoms and/or signs of snoring, open mouth breathing and hyponasal speech in addition to those symptoms and/or signs above and those also having more than 50% adenoid-to-choana ratio were named adenoid hypertrophy group ($n = 25$).

Indications for adenoidectomy were large obstructing adenoids (more than 50% adenoid-to-choana ratio) and chronic adenoiditis (pus drainage from adenoid and exudate on adenoid) unresponsive to oral antibiotics.

All the patients underwent adenoidectomy under general anesthesia using a curettage technique and the adenoid tissues were sent in formaldehyde tubes to the Department of Histology and Embryology for investigation of apoptosis. Histologists (EB, SFM) were uninformed about clinical characteristics of patients and were therefore blinded. Apoptosis was evaluated in different compartments of adenoid tissue.

All the patients were operated on as outpatients. 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-labelling" (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 [6]: brown staining, morphologically oval to round shaped nuclear condensation and fragmentation with narrow to dense cytoplasm (Figs. 1 and 2). Three different areas in each microcompartment were evaluated in each specimen and their mean was taken.

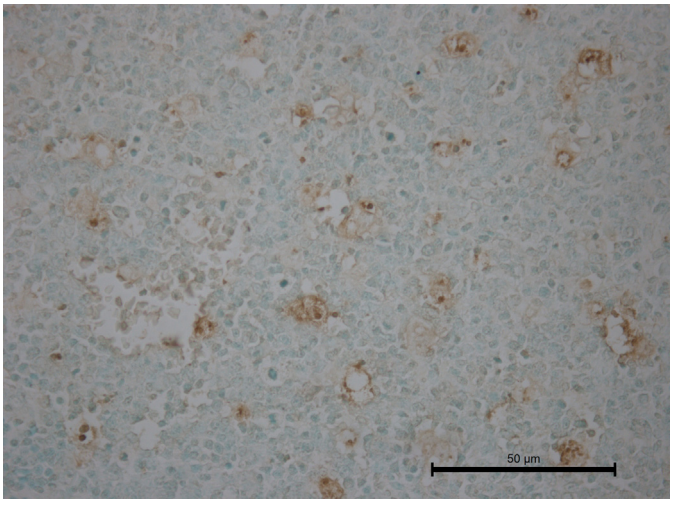


**Fig. 1.** Apoptosis in chronic adenoiditis, TUNEL, 400×. (For interpretation of the references to color in the text, the reader is referred to the web version of the article.)

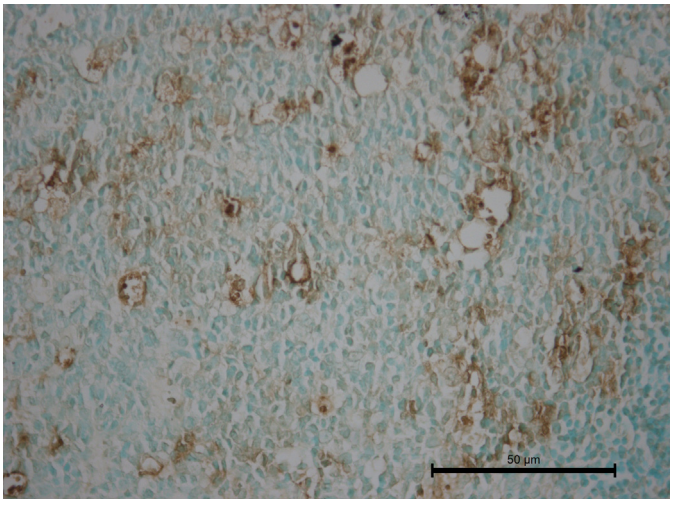


**Fig. 2.** Apoptosis in adenoid hypertrophy, TUNEL, 400×. (For interpretation of the references to color in the text, the reader is referred to the web version of the article.)

For statistical analysis of results SPSS 15.0 for Windows was used. For the analysis of difference between groups, 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

Information about age and sex of study groups are summarized in Table 1.

Adenoid tissue apoptosis values are shown in Table 2. The apoptosis difference between chronic adenoiditis and adenoid hypertrophy groups was statistically significant ($p < 0.05$).

The comparison of apoptosis in microcompartments of adenoid tissue (intrafollicular, interfollicular, subepithelial and intraepithelial) between chronic adenoiditis and adenoid hypertrophy groups is demonstrated in Table 3. The differences were statistically significant for intrafollicular and intraepithelial areas,