Apoptosis in Vocal Fold Polyps

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Summary: Objective. To examine the degree of apoptosis and its role in the pathogenesis of polyps of the vocal folds.

Material and Method. A review of the pathology department's archives for patients diagnosed with vocal fold polyps between 2010 and 2016 has been conducted. As a control group, gross and microscopically intact vocal fold from laryngectomy specimens was collected. A total of 61 vocal fold polyps from 51 patients and 41 unremarkable vocal folds from the control group were identified. Microscopically, the parameters studied were as follows: apoptosis, mitosis, inflammation, and exocytosis. Apoptotic index (number of apoptotic cells) was determined by the number of apoptotic cells per millimeter square in the epithelium. Apoptotic cells were readily identified by deeply shrunken eosinophilic cells detached from the surrounding environment with pyknotic-degenerated nuclei.

Results. In polyps, the apoptotic index was statistically higher than the control group ($\rho = 0.000$). In addition, the increased apoptotic index in polyps showed a statistically proportional increase in mitotic index, inflammation, and exocytosis, which were significantly higher compared to control group.

Conclusion. As a key for several therapeutic modalities, manipulation of apoptosis can be a future route for approaching vocal fold polyps by deciphering the complex signal pathways that allow the specified apoptotic cell to be targeted without damaging its surrounding counterpart.

Key Words: Polyps–Vocal folds–Apoptosis–Mitosis–Epithelium.

INTRODUCTION

Vocal fold polyps are among the most common exudative lesions of the vocal folds arising from the superficial layer of the lamina propria. These lesions are usually unilateral located at the free edge of the vocal fold with either a pale translucent or hemorrhagic appearance. The base can be sessile or pedunculated leading to aperiodic vibration of the vocal folds which in turn leads to a change in voice quality.[1] This latter is perceived as dysphonia secondary to mass imbalance in the vocal folds often associated with compensatory hyperactivity of the supraglottic laryngeal structures. Acoustically there is a drop in the fundamental frequency and an increase in the perturbation parameters and noise to harmonic ratio.[2] The most accepted therapeutic modality is phonomicrosurgery for removal of the lesions coupled with voice therapy and rehabilitation that result in improvement in the self-reported, perceptual, acoustic, and aerodynamic measures.[3]

The change in voice quality in patients with polyps both perceptually and acoustically has been attributed to structural and histological changes in the submucosal plane. Remacle et al has previously reported histological changes in patients with benign vocal fold lesions among which are vocal fold polyps. Pathological examination of the removed specimens revealed the presence of vascular proliferation and fibrosis.[4,5] These results were further corroborated by numerous authors, confirming the preponderance of fibrosis, neovascularization, and bleeding in patients with basal membrane thickening and epithelial hyperplasia nodules exhibited.[6,7]

The etiology behind the microscopic and structural changes as well as the alterations in the immune expression of many of the extracellular matrix constituents in patients with polyps has been attributed mainly to exogenous and endogenous factors such as smoking, reflux, and phonotraumatic behavior with little discussion at all on the role of apoptosis in the pathogenesis of these lesions. Apoptosis plays a very important protective role in the remodeling of tissues and their proliferation and carries a pivotal role in various processes, including cell death, development and aging, and defense mechanism during immune reactions and in response to disease or noxious agents.[8,9] To that end, apoptosis is a key indicator of the proliferative activity of any cell group and provides the balance or homeostasis in any tissue proliferation activity. In addition, a process of selective apoptosis might be involved in the development of the lamina propria within the human vocal fold which bears important consequences on the human's ability to phonate and engage in complex singing and speaking.[10]

Given the important role of apoptosis in tissue remodeling, the authors of this manuscript hypothesize that dysregulation of apoptosis may be a contributing factor to the onset or perpetuation of diseases of the lamina propria. No previous report has investigated the apoptotic activity in the vocal fold epithelial lining in patients with vocal fold polyps. The hypothesis is that there is elevated index of apoptosis in patients with vocal fold polyps compared to a control group with normal vocal folds. The purpose of this investigation is to examine the degree of apoptosis and its role in the pathogenesis of polyps of the vocal folds.

MATERIALS AND METHODS

After having obtained an approval from the institution research board, a review of the pathology department's archives for patients diagnosed with vocal fold polyps between 2010 and 2016 has been conducted. As a control group, gross and

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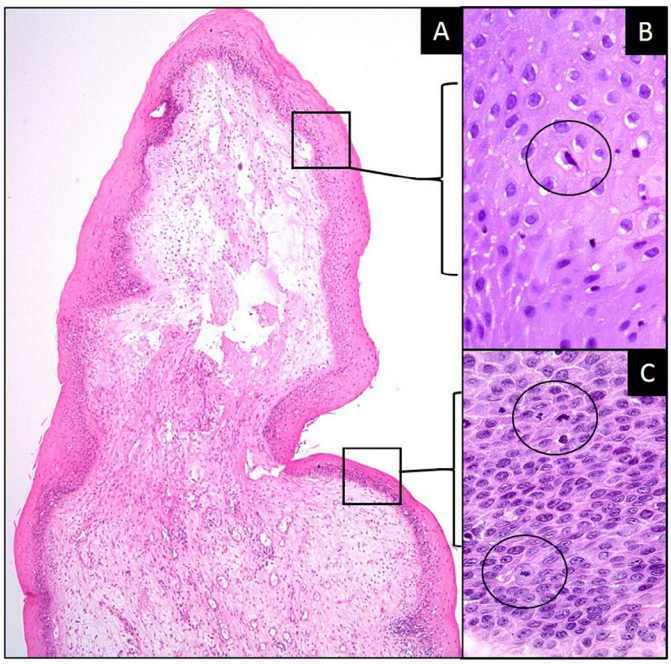

FIGURE 1. **A.** A vocal fold polyp with hyperplastic lining epithelium and a fibrous core with intervening vascular proliferation. **B.** Apoptotic cell in the epithelium with deeply shrunken eosinophilic cytoplasm detached from the surrounding cells and pyknotic-degenerated nuclei. **C.** Mitotic figures in the lining epithelium of the vocal fold polyp.

microscopically intact vocal fold from laryngectomy specimens was collected. Only patients with full clinical history and available pathology material were included in the study.

Clinical data included age, gender, and history of smoking. Diagnosis of polyp was established microscopically by the presence of polypoid protrusion in the vocal fold with intense fibrous core and intervening vascular proliferation with acute and chronic hemorrhage[11,12] (Figure 1A). Microscopically, the parameters studied were as follows: apoptosis, mitosis, inflammation, and exocytosis. Apoptotic index (number of apoptotic cells) was determined by the number of apoptotic cells per millimeter square in the epithelium.[13] Apoptotic cells were readily identified by deeply shrunken eosinophilic cells detached from the surrounding environment with pyknotic-degenerated nuclei, which is a well-established criterion for identification of apoptotic cells as an alternative to the Terminal deoxynucleotidyl transferase-mediated d-UTP Nick End Labeling (TUNEL) method of quantification[14] (Figure 1B). Mitotic index (number of mitotic cells) was determined by the number of mitotic cells per millimeter square in the epithelium (Figure 1C). Inflammation was quantified in the subepithelium and divided into nonbrisk and brisk. Nonbrisk describes absent to patchy presence of inflammatory cells. Brisk is defined as inflammatory cells completely cuffing the epithelium (Figure 2A). Exocytosis is established by inflammatory cells migrating into the overlying epithelium and was divided into nonbrisk and brisk. Nonbrisk describes absent to focal presence of inflammatory cells in the overlying epithelium. Brisk is defined as numerous and diffuse inflammatory cells in the epithelium (Figure 2B). The microscopic features were evaluated and graded on multiple hematoxylin and eosin stained slides by two of the authors in a blinded fashion (IK and GT). The same variables (apoptosis, mitosis, inflammation, and exocytosis) were entertained in the control specimens.

Statistical analysis

Means and standard deviations were reported for continuous variables, whereas frequencies and percentages were reported for

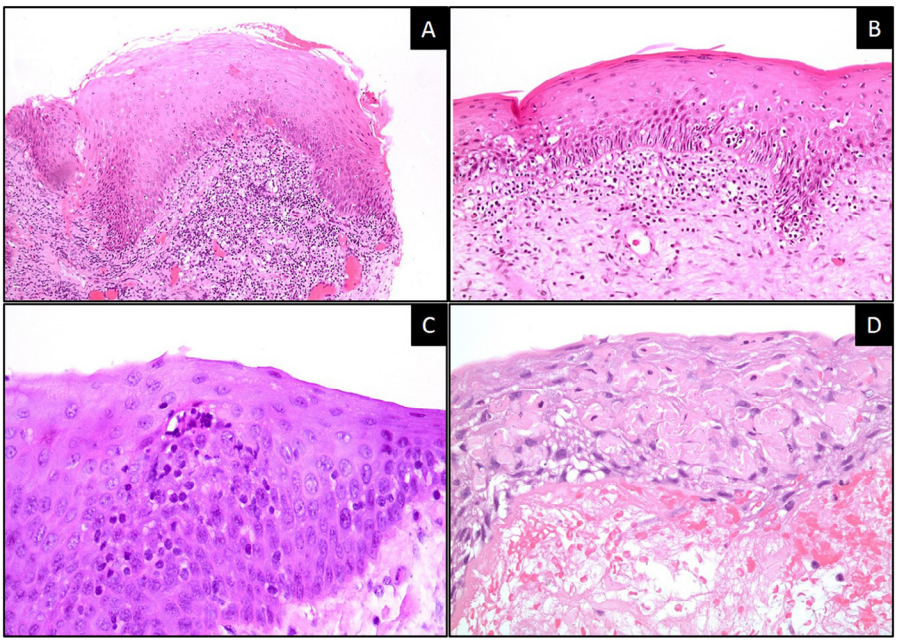

FIGURE 2. **A.** A vocal fold polyp with brisk inflammation cuffing the overlying epithelium. **B.** A vocal fold polyp with brisk exocytosis showing inflammatory cells migrating to the squamous epithelium. **C.** Apoptotic cell cuffed by inflammatory cells. **D.** Clustering of apoptotic cells in vocal fold polyp.