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Osteogenetic changes in elongated styloid processes of Eagle syndrome patients

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ABSTRACT

Abnormal elongation of the styloid process, or Eagle syndrome, can be painful, and is associated with differential diagnoses including cranio-facial malformations and vasculo-neurological disturbances. The precise molecular mechanism leading to styloid process elongation is unknown. In this study, elongated styloid processes with periosteal fibrous ligament tissue were obtained from three patients with Eagle syndrome and examined by immunohistochemical methods using different antisera. In all cases, marked bony deposition was found at the apex of the styloid process. The osteogenetic proteins, such as osteonectin, osteocalcin, BMP-2, BMP-4, and RANKL were strongly positive by immunohistochemistry in both the ligament fibers and the periosteal membrane attached to the styloid process apex. Staining for protective proteins, HO-1, HSP-70, and HSP-90 was also positive. These results suggest that styloid process elongation is related to increased expression of osteogenetic and protective proteins. Therefore, we propose that Eagle syndrome results from a protective response to increased tensile stress in the ligament attached to the styloid process, which could also signal osteogenetic protein expression in the periosteal fibrous tissue.

1. Introduction

The stylohyoid complex is composed of the styloid process, the stylohyoid ligament and the lesser horn of the hyoid bone. The styloid process is a long cylindrical, cartilaginous extension of the temporal bone. Embryologically, the anatomical structures of the stylohyoid complex originate from Reichert's cartilage, or the second branchial arch. The second branchial arch gives rise to the stylohyoid chain and contains potential ossification centers which eventually mineralize to varying degrees (Camarda et al., 1989a, 1989b). The normal styloid process length is approximately 20–30 mm (Gokce et al., 2008), with a mean length of 21.6 mm on the right side and 21.2 mm on the left side. There are three groups of styloid processes: short (under 21 mm), normal (21–30 mm) and elongated (more than 30 mm). This classification is both biologically based and clinically relevant (Sokler and Sandev, 2001; Fini et al., 2000).

In the general population, the frequency of an elongated styloid process is estimated to be 4%, of which only 4% show clinical manifestations. This suggests that the incidence of styloid syndrome is 0.16% (about 16,000 persons in Serbia). Deviation of the styloid process causes external or internal carotid impingement and pain that radiates along the arterial trunk (Petrovic et al., 2008a, 2008b). Styloid process deviation is considered important, because it is clinically similar to other painful cranio-facial syndromes, making diagnosis for treatment, difficult (Petrovic et al., 2008b). The American otolaryngologist Eagle was the first to describe this styloid syndrome in 1937. Eagle syndrome is a rare condition in which an elongated styloid process (more than 30 mm) impinges on adjacent anatomical structures (Mortellaro et al., 2002; Karam and Koussa, 2007). Ossification of the stylohyoid chain leads to a progressive decrease in elasticity and the potential for associated clinical symptoms. Theories about the stimulus for ossification include aging and reactive healing following surgical or

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nonsurgical trauma (Gokce et al., 2008; Keur et al., 1986). The aetiologic triggering factor for Eagle syndrome has not yet been discovered.

Two forms of Eagle syndrome exist: classic and vascular form. Patients with classic "Eagle syndrome" present with unilateral sore throat, dysphagia, tinnitus, unilateral facial and neck pain, and otalgia (Murtagh et al., 2001; Thot et al., 2000). In patients with vascular "Eagle syndrome", the elongated styloid process is in contact with the extracranial internal carotid artery; this can cause compression while turning the head or dissection of the carotid artery, causing a transient ischemic accident or a stroke (Gokce et al., 2008). Classical stylohyoid syndrome is found after tonsillectomy and is characterized by pharyngeal, cervical, and facial pain and headache (Leong et al., 2007; Lorman and Biggs, 1983; Kim et al., 2008). Stylo-carotid syndrome is the consequence of irritation of the pericarotid sympathetic fibers and compression of the carotid artery (Bafaqeeh, 2000; Farhat et al., 2009; Chuang et al., 2007). Clinical manifestations are most frequent after head-turning and neck compression. Eagle syndrome is defined as clinical symptoms of the neck with cervicofacial pain caused by an elongated styloid process that compresses neural and vascular structures including the glossopharyngeal nerve. Eagle syndrome can also cause stroke from carotid artery compression (Gokce et al., 2008). Eagle syndrome can be classified as an entrapment syndrome requiring neurosurgical attention (Karam and Koussa, 2007; Slavin, 2002).

This study aimed to elucidate the molecular mechanism of styloid process elongation in Eagle syndrome through immunohistochemical detection of osteogenetic and protective proteins in the apical areas of surgically removed styloid processes.

2. Material and Methods

Four elongated styloid processes including the periosteal fibrous ligament from three Eagle syndrome patients were studied with approval of the Institutional Review Board of Gangneung-Wonju National University (Table 1). One patient had surgery on both sides via an extraoral submandibular approach. After incision of the skin and identification and division of the posterior extension of the platysma muscle, blunt dissection was performed to divide the posterior border of the mandible and identify a portion of the external carotid artery. Immediately below the investing fascia of the external carotid or internal maxillary artery, the styloid process was identified and easily palpated. Once the fascia was removed from the surface of the styloid process, an incision was made in the periosteum, facilitating reflection of the periosteum and muscle attachments. The styloid process was removed near its base with dissection of the stylohyoid ligament at a point distal to the calcified portion. The wound was closed with a traditional layer suture.

The other two patients had surgery on each side via an intraoral trans-tonsillar approach. With the patient in a hyperextended open-mouth position, a trans-tonsillar incision was made in the pharyngeal mucosa, and the fascial plane was identified by blunt dissection of the involved muscle with a non-sharpened dissection scissors, palpating the hard tip of the calcified styloid process in the lateral pharyngeal space.

Removed specimens were fixed in 10% neutral formalin and decalcified with 0.5-M EDTA solution (pH 8.0), embedded in paraffin, and cut into 4 m sections. Microsections were stained with hematoxylin and eosin, followed by immunohistochemical staining using antibodies against osteonectin, osteocalcin, BMP-2, BMP-4, RANKL, HO-1, HSP-70, or HSP-90. All immunostaining was performed with an indirect triple sandwich method as previously described (Lee et al., 2005; Kim et al., 2009). Background cross-reaction was minimized by negative control staining without primary antibodies using the same procedures. Histological images of representative samples were captured with a digital camera (DP-70®, Olympus Co., Japan). Each slide was evaluated for intensity of positive immunostaining, graded as ++++, +++, ++, +, +/−, −, corresponding to extremely strong, strong, moderate, weak, rare and negative, respectively. A rare grade of +/− was defined as a focal or questionable weakly positive signal. Images were analyzed and compared by ANOVA using SPSS for Windows® (Version 12.0, SPSS Inc., USA) and mean values with 95% confidence intervals. Correlations were analyzed by Pearson's correlation test ($p < 0.05$).

Table 1
Eagle syndrome patients in this study.

	Age/ gender	Symptoms	Site	Operation	F/U period	Complications
Case 1 (S2001-72[a])	37/M	Neck pain, otalgia	Both	Extraoral	2 years	None
Case 2 (S2002-148)	23/M	Neck pain	Left	Intraoral	2.5 years	None
Case 3 (S2008-452)	41/M	Neck pain, dysphagia	Right	Intraoral	2 years	None

[a] Biopsy number registered in the Department of Oral Pathology, Gangneung-Wonju National University Dental Hospital.

3. Results

Removed styloid processes were mainly composed of compact bone with sparse marrow space. Processes were covered with a thin fibrous periosteal membrane that was thickened in the apical area (Figs. 1b1 and 3a1–b5). All four cases of elongated styloid processes showed active osteophytic bone deposition at the apical end with rare bony deposition on the lateral side (Figs. 1b1, 2a and 3c1–d5). Immunohistochemical localization of osteogenetic proteins and protective proteins was characteristic of the periosteal membrane, osteoid matrix, ligament fibers, and muscle bundles of each styloid process apex (Table 2). Immunoreaction for the osteogenetic protein BMP-2 was strongly positive in the apical periosteal membrane, and accentuated in the ligament fibers attached to the apical end of the styloid processes. Immunoreaction for RANKL was relatively weak in the periosteum but consistently positive in the ligament fibers attached to the apical end of the styloid processes (Figs. 1b2, c and 2b, i). Immunoreaction for BMP-4, a marker of chondroid differentiation, was rare in the apical area of the styloid processes (Fig. 2g). Staining for the bone matrix proteins osteonectin and osteocalcin was positive at the bone deposition site of the styloid process apex, with osteonectin staining strongly in the periosteum and osteoid matrix (Fig. 2d), and osteocalcin staining relatively weak in the periosteum but consistently positive in the bone deposition site of the styloid process apex (Fig. 2e).

Staining for the protective proteins HSP-70 and HO-1 was clearly positive in the apical areas of the styloid processes. HSP-70 was strongly positive in the muscle fibers attached to the apical end and consistently positive in the periosteum near apical bone deposition (Fig. 2f). HO-1 was localized in the apical periosteum of the styloid processes (Fig. 2h).

Both elongated styloid processes from one of the patients showed increased axial growth with multiple callus formation in the proximal areas of the styloid processes (Fig. 3). The callus of hyalinized chondroid tissue was clearly distinguishable by Masson trichrome stain (Fig. 3b2–b4), and contained hypertrophic chondrocytes (Fig. 3c2, c4, d2, d4). Multiple calluses appeared in the marrow space, growth plate-like thickening occurred in the proximal area of the styloid process (Fig. 3c, d). The growth plate-like callus consequently produced fibrous immature bone that stained