Observing the bony canal structure of the human maxillary sinus in Japanese cadavers using cone beam CT

By

Iwao SATO¹, Taisuke KAWAI², Shunji YOSHIDA¹, Yoko MIWA¹, Kosuke IMURA¹,
Rieko ASAUMI², Masataka SUNOHARA¹ and Takashi YOSUE²

¹Department of Anatomy

²Department of Oral and Maxillofacial Radiology, School of Dentistry at Tokyo, Nippon Dental University,
1-9-20 Fujimi Chiyoda-ku, Tokyo, 102-8159, Japan

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Summary: We observed the location of the posterior superior alveolar artery (PSAA) and nerve at the macroscopic level between the maxillary sinus (MS) and surrounding bone of the anterior region of the maxilla. This study was completed using cone beam computed tomography (CBCT) imaging of 19 human cadavers with 38 sides of Japanese origin (ranging in age from 59–94 years, mean 77.7 ± 9.8 years) that were prepared for this study. The bony canal structure of the inner surface of the maxilla was clearly apparent in our results, and the bony canals were classified into three types according to the structure along the course of the PSAA: canal-like, ditch-shaped tunnel and fragmented, and the last sides were undefined.

Calcitonin gene-related peptide (CGRP)-positive fibers were identified along the PSAA in the bony canal of the maxilla by immunohistochemistry. The presence of the bony structure and CGRP-positive nerve fibers along the PSAA suggests that there is risk to the PSAA during surgery involving graft implant in the floor of the maxillary sinus.

Introduction

The maxillary blood supply forms a complex network on the surface of the maxilla. This vascular network is at risk during surgical treatment involving graft implant in the floor of the maxillary sinus. Many previous reports have described the distribution of the posterior superior alveolar artery (PSAA) of the maxillary sinus (MS) and its intraosseous or intrawall arteries. The maxillary vascular network of the lateral wall of the MS involves two arteries, the infraorbital artery and the PSAA, which are connected via anastomoses (Solar *et al.*, 1999). These anastomoses contribute to the sinus membrane of the anterolateral wall of the sinus, and it is clinically important to know the location of the PSAA to avoid laceration during sinus floor elevation in the region of the molars. Solar *et al.* (1999) previously described the bony canal structure surrounding the PSAA. This bony canal structure is apparent in the MS in a large percentage of CT images (53%) (Elian *et al.*, 2005); 55%, (Mardinger *et al.*, 2007), CBCT images (71.4%), (Ella *et al.*, 2008) and cadaveric dissections (62.2%) (Murakami *et al.*,

1994). The presence of the bony tunnel and nerve fibers running along the PSAA may pose a risk of vascular impairment or ischemia or of pain and inflammation during surgical treatments that involve drilling the alveolar bone. CBCT analysis of the morphological structure of the maxilla can provide useful information about the bony structure of the maxillary sinus.

The presence of CGRP-positive fibers along the PSAA indicates the capacity for vascular regulation, vasodilation and vasoconstriction (de Hoz *et al.*, 2008). Furthermore, CGRP-immunoreactive fibers have been found in the nasal mucosa of patients with obstructive sleep apnea (Ju *et al.*, 1991). The distribution of CGRP-positive fibers changes during vasodilatation and can be controlled by infusion of CGRP. The distribution of CGRP-positive fibers poses a risk during surgical treatment involving graft implant in the floor of the maxillary sinus. Thus, the distribution of CGRP-positive fibers along the PSAA in the lateral wall of the MS must be examined prior to treatment.

In the present study, we analyzed the bony structures within the maxillary vascular network of the lateral wall

of the MS and the distribution of CGRP-positive fibers using immunohistochemistry and CBCT analyses.

Materials and Methods

Maxillae were examined in 19 human cadavers aged 59–94 years (mean, 77.7 ± 9.8 years; male, 80.0 ± 10.7 years; female, 75.1 ± 8.5 years) that were donated for human dissection. For each cadaver, both sides of the maxilla were studied. We eliminated four sides that had remarkable external injury and artifact with metal materials.

Cone beam CT (CBCT) imaging

We examined both sides of the maxilla in 19 human cadavers (34 sides; right sides 16; left sides, 18) using CBCT imaging (Alioth: ASAHI Roentgen Industry, Kyoto, Japan). Cone beam scans were carried out around the maxilla with a tube potential of 80 kV, a tube current of 4 mA, and a cylindrical area of 51×51 mm at high resolution (voxel size, 0.1 mm). The Frankfurt horizontal (FH) plane of the maxilla was placed parallel to the floor, and the region of interest (ROI) was set at the lateral region of the maxilla, placing the MS at the center of the ROI. Images were reconstructed from the CBCT data using proprietary Asahi Vision image reconstruction software (Asahi Roentgen Industry Co., Ltd).

Immunohistochemistry

After dissection, the MS was apparent in the maxilla. Samples underwent whole-mount immunohistochemical analysis of the MS and its blood vessels and nerves. The mucosa surrounding the MS was immersed in a 10 mg/L solution of alizarin red S in ethanol to differentiate between peripheral nerves and vessels in 30 sides using a modification of previously reported methods (Yi *et al.*, 2007). The solution was changed three times. Additional four sides were examined by whole-mount immunohistochemistry.

Whole-mount specimens were washed with distilled water for 24 hrs, incubated with 3% H_2O_2 for 20 min to eliminate endogenous peroxidase activity and digested with 0.02% proteinase K (Wako, Tokyo, Japan) for 1 hr at 38°C. After overnight fixation in 4% paraformaldehyde, the samples were washed with distilled water for 50 min. They were then washed with phosphate-buffered saline (PBS) for 30 min; sequentially incubated in 2.5%, 5% and 10% sucrose in PBS; and subjected to three freeze/thaw cycles. After overnight incubation with 2% Triton X-100 in PBS at 4°C, the samples were washed three times with PBS for 1 hr and incubated for 1 hr at room temperature with 2% normal goat serum in PBS (pH 7.2) containing 0.05% Tween 20 to prevent non-specific antibody binding. The samples were then incubated with rabbit polyclonal antibodies against

CGRP (1:1000; Biogenesis, NH, USA) or normal goat serum as a negative control. Subsequently, samples were washed three times with PBS for 1 hr and incubated with HRP-conjugated goat anti-rabbit IgG according to the manufacturer's instructions (Santa Cruz Biotechnology, USA). The samples were then washed three times with PBS for 1 hr. Staining was visualized using 0.02% H_2O_2 and 0.1% (1 mg/ml) diaminobenzidine tetrahydrochloride in 0.1 M Tris-HCl, pH 7.2. Images were acquired using a stereomicroscope (Leica MZ 16FA; Leica Microsystems, USA) with Leica Application Suite software (Leica Microsystems).

Ethics

Cadavers were obtained by consensual donation, according to the guidelines from the Law Concerning Body Donation for the Law Concerning Cadaver Dissection and Preservation (LCCDP).

Results

Microscopic observations

Two branches of the infraorbital artery, the middle superior alveolar artery and the anterior superior alveolar artery, were clearly identified on the lateral wall of the MS. The PSAA was identified parallel to the posterior superior alveolar nerve and branched several times around the lateral region of the MS. Numerous vessels and nerves forming complex fibers were observed near the floor region and the upper wall of the MS, and the PSAA and infraorbital artery formed anastomoses on the lateral wall of the MS (Figs. 1a–b). CGRP-positive nerves were clearly visible around the PSAA in the floor region of the MS. In addition, numerous anti-CGRP nerve fibers were scattered along the large vessels located on the lateral wall of the MS (Figs. 1c–d).

CBCT analysis

The bony canal structure of the inner surface of the maxilla was found and classified into three types: canal-like, ditch-shaped tunnel, fragmented (Figs. 2a–c and 3a–c). In 5 images (2 males and 3 females, representing 14.7% of the 34 images), the inner surface of the maxilla was canal-like structure along the course of the PSAA; in 23 images (11 males and 12 females, representing 67.6% of the 34 images), there was a ditch-shaped tunnel structure; and in 6 images (5 males and 1 female, representing 17.6% of the 34 images), there was a fragmented structure (Figs. 4a–c).

Discussion

Conventional dental imaging techniques such as dental and panoramic radiology provide limited informa-

Fig. 1. (a) Lateral view of the maxillary sinus (MS) in the maxilla. The posterior superior alveolar artery (PSAA) (arrows) and the infraorbital artery (IOA) are shown in the lateral region of the MS by alizarin red S staining (black arrow heads) (bar = 1 mm). (b) Fine nervous fibers (black arrow heads) were identified along the PSAA (bar = 1 mm). (c) Distribution of CGRP-positive nerve fibers (black arrow heads) identified by immunohistochemical staining at the macroscopic level (bar = 0.2 mm). (d) Large magnification of Fig. 1c. CGRP expression (black arrow heads) was observed in small fibers along the PSAA (bar = 0.02 mm).

Fig. 2. (a) CBCT image showing a lateral view of the maxilla. A bone canal structure is shown with the PSAA (black arrowhead). (b) CBCT (frontal) section image showing a bone canal structure (white arrowhead) on the buccal surface of the MS. The indicated location of white arrowhead is same region of black arrowhead in Fig. 2a. (c) A view of the inner surface of the MS. The PSAA with nerve fibers were observed in the buccal surface region after the bony parts of the maxilla were removed.

Fig. 3. Three patterns of CBCT image of the bony canals. Right side, 3D image; Left side, frontal section of CBCT image.
 (a) CBCT image of a canal-like structure (black arrow) in a floor view of the MS in the maxilla.
 (b) CBCT image of a ditch-shaped tunnel structure (black arrows) in a floor view of the MS in the maxilla.
 (c) CBCT image of a fragmented structure (black arrows) in a floor view of the MS in the maxilla.

tion for visual examination because they produce only two-dimensional images of the mesiodistal and occlusal-apical dimensions of the maxilla. CBCT systems have the ability to reconstruct full three-dimensional models of the maxilla, allowing precise definition of anatomical structures (Garg, 2007); (Monsour and Dudhia, 2008). Many reports have indicated that complex and anastomotic branches of the PSAA and the infraorbital artery are present in the lateral wall of the MS as determined by cadaver dissection (Murakami *et al.*, 1994; Solar *et al.*, 1999; Traxler *et al.*, 1999; Rosano *et al.*, 2009) and CT analysis (Mardinger *et al.*, 2007; Ella *et al.*, 2008). There are no available data, however, regard-

ing visualization of the branches of the PSAA and the infraorbital artery using a combination of CBCT imaging and macroscopic analysis. Our macroscopic observations show that branches of the PSAA with CGRP-positive fibers were present in the maxillary sinuses of the Japanese cadavers. The varied appearance of the bony canal structure along the course of the PSAA has been reported in both CT (53%, Elian *et al.*, 2005; 55%, Mardinger *et al.*, 2007) and CBCT (71.4%, Ella *et al.*, 2008) studies. The CT system was used to generate overlapping 1.2-mm axial cuts, and axial images were reconstructed into cross sections. In contrast, our CBCT system generated high-resolution images (voxel size, 0.1 mm). The smallest

Fig. 4. Percentage of CBCT images showing the bony structure. Three types of structures were identified: canal-like, ditch-shaped tunnel and fragmented. a, male; b, female; c, total.

diameter of the PSAA has been reported to be less than 1 mm (Mardinger *et al.*, 2007), and the largest diameter of the infraorbital artery has been reported to be 1.6 mm (Solar *et al.*, 1999). Thus, most of the surrounding bony canal structure is not large enough to be detected by CT imaging. In the present study, this bony canal structure with canal-like and ditch-shaped tunnel were evident in 82.3% of the images, greater than the value of 71.4% observed in a previous CBCT analysis (Ella *et al.*, 2008). Furthermore, we inspected the bony canal structure by maxillary dissection of our specimens following CBCT analysis. We also defined this structure in detail as one of three types: canal-like, ditch-shaped tunnel or fragmented. The ditch-shaped tunnel structure was the most common (67.6% of the 34 images), and the thin wall of this structure may pose a risk during implant surgery. The bony canal structure may be formed by the inflammation of maxillary membrane with paranasal sinuses, which may be due to infection, allergy or autoimmune issues. Especially most of our examined specimens were mainly elderly subjects, and therefore formed canal structure may be concerned with aging.

CGRP is released by nociceptive sensory nerves. In addition, CGRP-positive fibers are associated with the regulation of blood vessels supplying local tendons and contribute to vasodilation (de Hoz *et al.*, 2008; Danielson *et al.*, 2006). Our results demonstrate that CGRP-positive fibers are located along the PSAA in the bony structure of the maxilla. It has been suggested that CGRP-positive fibers mediate blood vessel regulation. Dental surgeons must be mindful of this point when performing maxillary sinus graft procedures for dental implants. Post-operative pain and discomfort can occur in the maxillary sinus floor following surgical treatment with sinus floor elevation (Trombelli *et al.*, 2010). Post-operative pain along with posterior superior alveolar nerve damage is related to anatomical variations in the paranasal sinuses (Whittet, 1992; Solar *et al.*, 1999). In the present study, numerous nerve branches with CGRP-positive fibers were identified along the vessels in the human maxillary sinuses that we examined. This region is susceptible to inflammation following implant placement (Penarrocha-Diago *et al.*, 2009). In conclusion, identification of the arterial supply and of the innervation of the maxillary sinus area is essential prior to dental surgical treatment, and CBCT imaging is required to observe the intraosseous vessels in this region.

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