Histologic evaluation of inferior alveolar lymphatics: an anatomic study

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The purpose of this study was to investigate the presence of a lymphatic system within the inferior alveolar canal, using specific immunohistochemical staining. Lymphatic capillaries are characterized by a thin wall with an irregular profile. They are superficial to organs and collect the lymph. Currently, the lymphatic system of the mandible and the floor of the mouth are attributed to the periosteum. The inferior alveolar canal within the mandible houses the inferior alveolar nerve, artery, and vein. Owing to the surgical vulnerability of the canal, several recent studies have focused on defining the exact contents and arrangements within the canal as well as precise measurements of the diameter of its components. No study has been performed on the existence of a lymphatic system within the inferior alveolar canal, the presence of which has significant importance for surgical management of malignant lesions. Presence of lymph channels would make the inferior alveolar nerve a potential route for spreading malignant cells. In this study, podoplanin-positive thin-walled vessels, considered to be lymphatic vessels, were traced within the inferior alveolar canal. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:564-567)

The lymphatic structure of the facial skeleton is not fully understood. The oldest definition of the lymphatic drainage was found in the literature as depicted by Sappey in 1874.1 It is suggested that the lymphatic drainage of the intraoral structures and the floor of the mouth communicates with the periosteal lymphatic drainage of the mandible2. Lymphatics of the anterior neck lay above the platysma muscle and course in horizontal, oblique, and upward patterns toward the mandible. It is suggested that these pathways also join the periosteal lymphatic drain of the mandible.3 Periosteal lymphatic drainage has great importance when defining the surgical margins of a lesion. Perineural invasion has been reported to be responsible for 76% of local recurrences.4 The inferior alveolar canal (IAC) is considered to be the most important anatomic feature within the body of the mandible, containing the inferior alveolar nerve (IAN), artery, and vein. Several studies have investigated the pattern of the IAN, its correlation to dental roots, and its distance from the outer cortex of the mandible.5-12 The anatomic configuration of the IAC has been clearly described in recent years: The artery is usually a solitary compartment, positioned lingual to the nerve, and the vein appears to be mostly a multiple structure and lies superior to the nerve.13 However, the presence of the inferior alveolar lymphatic (IAL) has never been investigated before. Recently, podoplanin, a specific lymphatic microvessel marker, has enabled selective immunostaining of the lymphatic microvessels in paraffin-embedded sections.14-16 The purpose of the present study was first, to assess the presence of a lymphatic draining system within the IAC and second, to define its correlation with the IAN.

MATERIALS AND METHODS

Ten right hemimandibles were selected from the forensic anatomy hall, after being ethically approved by the Iranian forensic medicine ethical committee. Harvested hemimandibles were either dentate or partially

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edentulous. Samples were frozen at −18°C. Using a fissure bur, the body of each hemimandible was cut into 3 parts: central to canine, canine to second premolar, and second premolar to the most posterior tooth, named, respectively, anterior, middle, and posterior sections. Sections were then fixed in a 10% formalin solution for 10 days, rinsed, decalcified in a 10% nitric acid for 30 days, dehydrated using a graded concentration of ethanol, and embedded in paraffin wax. An ultrathin microtome was used to prepare 5-μm-thick cross-sections along the mandibular canal and mental foramen.

One-half of the cross-sectioned specimens were stained with hematoxylin-eosin (H&E) and examined under the light microscope (400E-Eclipse; Nikon, Tokyo, Japan). The remaining sections were prepared for podoplanin (11-003; Angio Bio, Del Mar, CA, USA) antibody staining (NovoCastra System, Newcastle, UK) according to the process described by Liang et al.16 A lymphatic was defined as a podoplanin-positive endothelial-lined structure. For this purpose, samples were heated for 16 hours in 37°C and then for 1 hour in 60°C in an oven. To reaktivate the antigen, samples were heated with nitrate buffer solution in microwaves for 5 minutes (700 W) and 15 minutes (450 W), which was followed by a double wash in Tris-buffered saline solution (TBS), each for 5 minutes. Then samples were incubated at room temperature for 20 minutes with peroxidase and rinsed for another 5 minutes in TBS. Next, samples were incubated with the antibody against podoplanin as a primary antibody with 1:50 dilution for 60 minutes at room temperature. The samples were then incubated with the secondary antibody for 30 minutes and washed twice for 5 minutes in TBS, after which the samples were incubated with Novolink polymer (NovoCastra) for 30 minutes. It was followed by another double wash with TBS, each for 5 minutes. Finally, samples were incubated with 3,3’-diaminobenzidine tetrahydrochloride solution for 10 minutes, washed in tap water for 5 minutes, and then counterstained with Mayer hematoxylin. Negative control sections were stained by omitting the primary antibody. External positive control samples were lymphangiomas and lingual varicosities. The internal positive control was the presence of lymphatic vessel. Slides were evaluated by light microscope. Data collection was carried out through observations of a single operator, and all the findings were recorded manually.

RESULTS

H&E staining confirmed the presence of the IAN, inferior alveolar artery, and inferior alveolar vein within the mandibular canal (Fig. 1). Podoplanin-positive thin-walled channels, considered to be lymphatic vessels, were also seen within the IAC along with the veins. The lymphatic system was seen in interfascicular area as well as within the proximity of the arterioles and venules (Figs. 2 and 3). There was no significant difference in the distribution of the lymphatic vessels on either side of the canal. The lymphatic system around the main trunk of the IAN was small, and there was no evidence of large lymph-
phatic trunks. The distribution of the lymphatic walls in the anterior, middle, and posterior sections was following the same pattern. Five of the middle sections were edentulous. No qualitative histologic differences were found regarding the presence or distribution of the lymphatic channels between the dentate and edentulous parts of the mandible.

DISCUSSION
The IAN is the largest branch of the mandibular division of the trigeminal nerve. It enters the lower jaw from the mandibular foramen, travels through the body of the mandible, and divides into 2 terminal branches (incisive and mental nerves) at the mental foramen. Recent studies have focused on the arrangements and exact diameters of the contents of the canal. The mean diameters of the IAN, inferior alveolar artery, and inferior alveolar vein have been measured to be 2.52 mm, 1.84 mm, and 0.42-0.58 mm, respectively. These studies have mostly dealt with the canal position and its proximities, space relationship between the IAN and the lower dental roots, or the surgical vulnerability of the canal. Nothing, however, could be found in the literature regarding the presence of a lymphatic system within the mandible.

Until now, the periosteum of the mandible has been known to be the main source of the lymphatic drainage and has been overemphasized when treating carcinomas of the floor of the mouth. Internal structures within the mandible have never been recognized as potential routes to carry exotoxins or malignant cells. Nerve invasion by malignant tumors, especially salivary gland tumors, was thought to be the etiologic factor for local metastasis.4

The pathogenesis of perineural invasion is not fully elucidated. In 1905, it was theorized that tumor cells spread along nerve trunks via perineural lymphatics. However, the literature did not provide histologic evidence, and suggestions were based on this theory that all vascular bundles contain lymphatic vessels. Larson et al.20 demonstrated that the lymphatic channels have no role in the local recurrences of the adenoid cystic carcinoma. They believed that tumor cells spread through nerve sheaths. Feasel et al.21 reviewed the clinical pattern of perineural invasion. They studied the presence of tumor cells adjacent to epineural bundles and around venules and noted the perineural lymphatics and perineural sheath spaces as important routes for the spread of cutaneous malignancies. Presence of tumor cells within and around the IAC at the so-called safe margins of resection would be crucial in changing the current concept of locoregional spread, which would then warrant a more radical resection to reduce the incidence of recurrences. Considering the IAL as a potential route to spread malignant cells may change the surgical treatment protocols in preserving or resecting the IAN. Several specific lymphatic endothelial cell markers have become available, such as podoplanin, lymphatic vessel endothelial hyaluronan receptor 1, vascular endothelium growth factor receptor 3, D2-40, and Prox-1.22-26

The significance of the present findings regarding the presence of a lymphatic system in the body of the mandible is not yet clear, and further studies are required to verify the exact role of the lymphatic vessels in the inflammatory responses of the pulp, local metastasis of the malignant tumors, distribution of odontogenic tumors, and resistance to surgical trauma.

The lymphatic pathway is attributed to collecting veins. The mammalian lymphatic vascular system originates solely from venous endothelial cells. After arterial and venous endothelial cells differentiate, a subpopulation of venous endothelial cells is thought to become competent to acquire lymphatic endothelial cell characteristics.27 The maxilla and mandible are components of the facial skeleton which contain the teeth. Having a vital pulp, teeth are considered to be vital organs. Each dental root has its own feeding and collecting vessels that drain into the IAC. A lymphatic system is also found within the pulp.28 Recent studies have shown that lymphatic drainage in dental pulp has a major role in the equilibrium of interstitial fluid.29 Lymphatic networks have also been identified in human periodontal tissues with a higher density in the radicular section.30
CONCLUSIONS
Evidence for the existence of a lymphatic system within the IAC may contribute in postulating new anatomic, physiologic, and functional properties for the IAC of the mandible.

REFERENCES

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