Anatomic–histologic study of the floor of the mouth: the lingual lymph nodes

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Abstract

Objective: The lingual lymph nodes are inconstant nodes located within the fascial/intermuscular spaces of the floor of the mouth. Oral tongue squamous cell carcinoma has been reported to recur and metastasize in lingual lymph nodes with poor prognosis. Lingual lymph nodes are not currently included in basic tongue squamous cell carcinoma surgery.

Methods: Twenty-one cadavers (7 males, 14 females) were studied, aged from 57 to 94 years (mean age 76.3 years). The gross specimen of the floor of the mouth was divided into blocks: A (median nodes), B, B′ (parahyoid), C, C′ (paraglandular). Serial histological microslides were cut and stained with hematoxylin–eosin. Frequency of lingual lymph nodes in each block and their microscopic features were assessed.

Results: The lingual lymph nodes in overall number of 7 were detected in 5 of the 21 cadavers (23.8%). The total incidence of lingual lymph node was 33.3% (7 nodes/21 cadavers). Block A failed to demonstrate any lymph nodes (0%); blocks B, B′—2 nodes (9.5%) and 2 nodes (9.5%), respectively; blocks C, C′—1 node (4.8%) and 2 nodes (9.5%), respectively. The mean lingual lymph node length was 4.1 mm (from 1.4 to 8.7 mm), the mean thickness was 2.8 mm (from 0.8 to 7.5 mm). Five cadavers (23.8%) revealed MALT. Atrophic changes appeared in 4 (57.1%) lingual lymph nodes.

Conclusion: The presence of lymph node-bearing tissue in the floor of the mouth is demonstrated. In account of resection radicalism and better local control the fat tissue of the floor of the mouth should be removed in conjunction to glossectomy. Further anatomic and clinical research is required to establish the role of lingual lymph node in oral squamous cell carcinoma recurrence and metastasis.

Key words: oral tongue, squamous cell carcinoma, lingual lymph node
Introduction

Understanding the lymphatic system’s anatomy is of paramount significance in surgical oncology. Cancers frequently spread through the network of lymphatic channels that transport tumor cells to the regional lymph nodes. The presence of regional lymph node metastasis is an important predictive factor that influences both, treatment and prognosis. In the head and neck region the patterns of lymphatic spread are well recognized and on their basis several modifications of neck dissection have been proposed (1). In oral tongue cancer the submandibular and jugulodigastric nodes are considered to be the first-echelon nodes of lymph flow, though unusual cases of skip-metastases to lower neck levels have been reported (2).

In 1985, Ozeki et al. (3) described secondary tumor burden from tongue squamous cell carcinoma (SCC) located in the lingual lymph nodes (LLN), occurring in three patients. Until then not much attention has been drawn to this group of inconstant lymph nodes, situated in the floor of the mouth, in terms of oral tongue SCC spread. Several observations were chronicled as regional metastasis or primary site recurrence (Table 1–3). The LLNs are not commonly removed during neck dissection neither at the time of glossectomy. The anatomical data on these nodes as well as their microscopic appearance may be of some importance in developing more effective surgical treatment for oral tongue SCC.

Patients and methods

The anatomical properties of the floor of the mouth were studied on 21 cadavers (7 males, 14 females), aged from 57 to 94 years (mean age 76.3 years). The gross specimen contained the hyoid bone caudally and the tissues of the floor of the mouth cranially, the tongue mass and the epiglottis were dissected off the specimen. The material was gained during routine autopsies in the department of pathologic anatomy and fixed in 10% buffered formalin. After fixation it was separated into blocks resembling the fascial/intermuscular spaces of the floor of the mouth, one median compartment (Block A) and two paired lateral compartments [Blocks B, B’ and C, C’] were identified for each gross specimen (Fig. 1). Every block was then cut into serial stepwise cross-sections, step distance—50 µm, microslide thickness—7 µm. The cross-sections were stained with H&E. Pathologist investigated the cross-sections, the anatomical contents for every tissue block were judged and the presence of each group of LLN as well as their histological features evaluated. With digital camera in scanning function (AxioCam, Carl Zeiss, Jena, Germany) microphotographs of the slide were obtained, from these a reconstruction of the whole cross-section was performed. The size of the nodes was measured in histomorphometric computer program (AxioVision, Carl Zeiss).

Histomorphometry was performed on those microslides that contained the largest portion of the node (e.g. the closest to the structures’ equator). The LLNs were divided into three groups, according to their topography: Block A—the median nodes (located between the genioglossus and geniohyoid muscles); Blocks B, B’—the paraglandular nodes (situated along the course of the lingual artery at the cornu of the hyoid bone) and blocks C, C’—the paraglandular nodes (lying in the proximity of the sublingual salivary gland).

Results

Block A in all 21 specimens contained a discrete fibro-fatty lamina accounting to the lingual septa and various amounts of skeletal muscle fibers, the genioglossus and geniohyoid muscles. Blocks B and B’ in 95.2% (20/21 specimens) showed skeletal muscle fibers (mm. genioglossus and/or geniohyoid on one side and m. hyoglossus laterally. Block B demonstrated the lingual artery in 17 specimens (80.9%) in these 12 (57.1%) were accompanied by the lingual vein. The corresponding numbers for Block B’ were 20 specimens (95.2%) with the lingual artery, and 14 specimens (66.7%) with the sublingual vein. Blocks C and C’ contained moderate amounts of fibro-fatty tissue which surrounded different anatomic elements all inferiorly bordered by the mylohyoid muscle fibers. The sublingual salivary gland was included in 21 (100%) and 20 (95.2%) blocks C and C’, respectively. The sublingual artery was included in 20 (95.2%) of both C and C’ blocks. The sublingual vein was identified in 14 (66.7%) of Blocks C and 15 (71.4%) of Block C’. The salivary duct got involved in 16 (76.2%) of Blocks C and 15 (71.4%) of Blocks C’. An additional arterial lumen, a branch of submental artery, was seen in 4 (19.1%) of Blocks C and 7 (33.3%) of Block C’.

The observed lymphatic structures were true regional lymph nodes and mucosa-associated lymphoid tissue (MALT). The LLNs in overall number of 7 were detected in 5 of the 21 studied cadavers (23.8%). The total incidence of these regional nodes was 33.3% (7 nodes/21 cadaver). The frequency of their presence for each tissue block: Block A—0/21 cadavers (0%); Block B—2 nodes/21 cadavers (9.5%); Block B’—2 nodes/21 cadavers (9.5%); Block C—1 node/21 cadavers (4.8%); Block C’—2 nodes/21 cadavers (9.5%). All of the tissue blocks exhibited at least one LLN presence except Block A (Fig. 1). The cadaver No. 13 had multiple unilateral LLNs, two nodes in Block C’ and one node in Block B’. No other cadavers had evidence of multiple lingual nodes uni- or bilaterally. On histomorphometry the mean LLN length was 4.1 mm (from 1.4 to 8.7 mm), the mean thickness was 2.8 mm (from 0.8 to 7.5 mm) (Table 4). The lingual nodes were similar in morphologic structure to normal regional lymphatic nodes (Fig. 2). The mean age of the cadavers revealing the LLN was

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Table 1. Anatomic descriptions of LLNs

| Year | Author     | Anatomic data                                                                 |
|------|------------|-------------------------------------------------------------------------------|
| 1898 | Kuttner (34)| Depicted two-three nodes at the lower mandibular margin situated posterior and anterior to the sublingual salivary gland. |
| 1938 | Rouviere (35) | First grouped the LLNs into rare median and more prominent lateral.             |
| 1943 | Katayama (9)  | Stated the incidence of medial and lateral LLNs as 15.1 and 30.2%, respectively. |
| 1967 | Mashkov (36)  | Found LLNs within tongue musculature in 8.6% of 104 cadavers investigated. Grouped LLNs as regional draining lymph nodes of the oral tongue. |
| 1972 | Feind (37)   | Described LLNs as a group of interrupting nodules located along the collecting trunks of the tongue and the sublingual salivary gland. |

LLN, lingual lymph nodes; ND, neck dissection; RND, radical neck dissection; MRND, modified radical neck dissection; FND, functional neck dissection.
| Year  | Author          | Diagnosis, details                                                                 | Treatment                                                                 | Results                                                                 |
|-------|-----------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------|
| 1985  | Ozeki et al. (3) | Reported three cases of LLNs metastasis: (1) a 49-year-old male with SCC of the lateral lingual margin with root of the tongue invasion, T3N3M0. Intraoperatively an enlarged median LLN was encountered and resected. (2) A 54-year-old male with SCC of lingual margin, T1N0M0. A lateral LLN with extracapsular spread noted in the surgical specimen. (3) A 63-year-old male with SCC of lingual margin, T2N1M0. A lateral LLN identified in the specimen. | Neoadjuvant 240 mg bleomycin, hemi-glossectomy en-bloc with bilateral RND. | The patient died 9 months post-operatively due to regional recurrence. |
| 2002  | Dutton et al. (4) | A 73-year-old male with SCC of the lateral tongue, T2N2bMX. In the surgical specimen two positive LLN and 27 negative cervical lymph nodes were identified. | Partial glossectomy in-continuity with MRND, post-operative radiation.   | No loco-regional recurrence in more than 2 years post-operatively.       |
| 2008  | Han et al. (38)  | A 46-year-old male with SCC of the lateral tongue, cT2N0M0. Two LLN spotted intraoperatively exhibited metastasis on frozen section. | Partial glossectomy en-bloc with lateral mouth floor and sublingual gland excision. | No loco-regional recurrence in >2 years post-operatively.               |
| 2009  | Umeda et al. (39)| Reported two cases of LLNs lesions: (1) a 62-year-old male with SCC of the anterior mouth floor, T3N2cM0. CT and MRI showed an enlarged LLN. (2) A 62-year-old male with SCC of the median mouth floor, T2N2cM0. CT and MRI showed an enlarged LLN. | Resection of the tumor with mylohyoid and anterior digastrics sacrifice, bilateral FND, free flap reconstruction. | No loco-regional recurrence, the patient died of pneumonia 10 months post-operatively. |
| 2010  | Ando et al. (40) | A 63-year-old male with mouth floor and tongue base invasive SCC, three enlarged nodes shown on MRI. The specimen had six ipsilateral positive nodes, three LLNs in close contact with sublingual gland and one at the root of lingual artery. | Subtotal glossectomy in-continuity with bilateral ND (ipsilateral level I-IV, contralateral level I), no radiotherapy. | No loco-regional recurrence in 10 months post-operatively.               |
| 2011  | Zhang et al. (41)| Reported two cases of LLNs lesions: (1) 47-year-old female with ventral tongue SCC initially cT1N0M0 received three surgery. Seven months after first operation (a) PET-CT suspected LLN lesion, second surgery (b) and 60 Gy radiotherapy were performed. In 6 months, the third operation (c) was carried out for extensive loco-regional recurrence. (2) A 42-year-old man with ventral tongue SCC, T2N0M0. Surgical specimen showed only LLN metastasis with extracapsular spread. | (a) partial glossectomy and discontinuous supraomohyoid ND; (b) wide local resection with contralateral RND; (c) wide local resection, subtotal thyroidectomy internal carotid artery reconstruction. | Patient died 3 months after the third surgery because of multiple organ failure. |
| 2012  | Satio et al. (42)| An 81-year-old female with lateral tongue SCC, T2N0. CT lymphography showed positive LLN and no neck disease. | Partial glossectomy en-bloc with mouth floor and in-continuity supraomohyoid ND and free flap reconstruction, post-operative 60 Gy radiotherapy. | No loco-regional recurrence in 2 years post-operatively.                 |
2011 Calabrese et al. (44) Designed a modification of in-continuous resection. One hundred forty-three patients with previously untreated tongue SCC, cT2–4a, cN0, cN+, M0 treated with the approach. In 5 years, local control achieved in 88.4% (16.8% improvement on standard surgery); loco-regional control—83.5% (24.4% improvement); overall survival—70.7% (27.3% improvement).

Discussion

In present study groups of LLNs, their prevalence and histologic appearance are outlined. These lymphatic structures are located in the floor of the mouth and upper neck. Limited anatomical depictions are available in the literature (Table 1). According to the lymphatic location of these nodes on the pathway from the primary site on the tongue into the targeted Levels I and II they were named: intervening (4), in-transit (5) and intercalated (6).

In our study, MALT structures of the floor of the mouth mucosa were present in five subjects. They had similar appearance to regional lymph nodes proper (Fig. 3). Two characteristics were helpful in differentiating MALT from the LLNs: immediate subepithelial localization with lymphocytes, situated in the overlying epithelium; and the absence of afferent lymph vessels (7,8).

In the dissected cadavers we observed median LLN in 0%, paraglandular together with parahyoid LLNs in 23.8% of the subjects studied, which is incompatible with published data as regarding the frequency of median nodes. Katayama (9) studied oral lymphatics on embryos and newborn cadavers, and stated that incidence of median and lateral LLNs was 15.1 and 30.2%, respectively. The difference may be explained by a natural hallmark of lymph nodes to atrophy with age. Zyb and Nestajko (1983) pointed out two non-neoplastic conditions, which cause incomplete filling of the node by the contrast material during direct lymphography. Fibrosis usually results from chronic inflammatory processes in the draining area lipomatosis is a common aging feature (10). Hadamitzky et al. (11) observed different degeneration stages of human inguinal nodes and found that lipomatosis of the medulla and lymphocyte reduction are characteristic to senile changes. Our findings of degenerative changes in LLN support these observations. Pan et al. (12) introduced the transparent lymph node that reached such a stage of degeneration in which both cortex and medulla are completely replaced by connective tissue. This transparent node hence cannot be identified by basic histologic methods and requires special lymph vessel tracing (13). According to this information, further anatomic studies, implicating more sophisticated

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Table 3. Clinical series related to LLNs

| Year | Author | Clinical data | Recommendations |
|------|--------|---------------|----------------|
| 2009 | Ando et al. (43) | Evidenced parahyoid nodes disease in 6.3% of their 248 patients with T1–2 oral tongue SCC. | Careful intraoperative inspection of loose fibro-fatty tissue along the course of lingual artery for possible firm nodes in any type of ND. |
| 2010 | Hoshina et al. (5) | Positive ‘parasubmandibular’ nodes observed in 16.3% of their 43 patients. | Routine pre-operative imaging and meticulous data interpretation in light of possible occult LLN disease. |
| 2011 | Calabrese et al. (44) | Designed a modification of in-continuous resection. | Advocated a wide en-bloc resection of a hemi-tongue and required the underlying mouth floor tissues, the stylohyoid and mylohyoid muscles. |

Supported the extrinsic muscles to be included in the resection.

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Table 4. Histomorphometric parameters of the LLNs

| Size, mm | Specimen | Length | Thickness |
|----------|----------|--------|----------|
|          | 5 B      | 5 B    | 2.7      |
|          | 8 A      | 8 A    | 0.8      |
|          | 12 C     | 12 C   | 3.9      |
|          | 13 C     | 13 C   | 7.5      |
|          | 13 C′    | 13 C′  | 2.1      |
|          | 18 B     | 18 B   | 1.0      |
|          | Mean, mm | 1.4    | 2.8      |

Histomorphometry was performed on those microslides that contained the largest portion of the node (e.g. the closest to the structures’ equator).

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Figure 1. Illustration of the floor of the mouth fascial spaces. From every dissected cadaver five tissue blocks of the floor of the mouth were obtained, Blocks: A, B, B′, C and C′. The numbers represent the frequency of the lingual lymph nodes (LLNs) observed in each tissue block in 21 cadavers studied.

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techniques, may be needed to establish actual frequency of the LLNs, especially the median ones, which may normally obtain this transparent status. A fact worth to be mentioned is that among the first three cases of LLN metastasis, Ozeki et al. (3) described a patient with a median lesion between the inner surfaces of the two genioglossus muscles.

Explorations of lingual and mouth floor lymphatics demonstrated pathways penetrating the mylohyoid muscle to drain into the submandibular nodes. These mainly start from the lateral border of the mobile tongue (14,15). Werner et al. (16) illustrated that the draining pathways of the lateral lingual border and sublingual mucosa are directed toward the floor of the mouth before reaching the regional nodes.

In most documented cases of LLN metastasis an in-continuity approach was utilized. Resection radicalism is established but functional damage usually takes place. Fistula formation, delayed healing and radiation therapy postponement are common in patients undergoing in-continuity resections (4). Shelomentsev and Sushentsov (1975) extensively studied lymphatic vasculature of the tongue. They postulated that one crucial step to elude local recurrences after tongue and mouth floor resection is the choice of surgical approach. Close connections of the lingual lymphatic vessels with the sublingual gland passing through its parenchyma and draining the gland itself were documented. Main extraorganic efferent lymphatic trunks were found to locate along the branches of lingual artery and vein. To
achieve better local control the authors advocated wide dissection along the lingual artery system with sublingual gland removal in oral tongue SCC surgery (17). Dutton et al. suggested a local excision of floor of the mouth tissues in conjunction with partial glossectomy for oral tongue SCC as an alternative for in-continuity neck dissection. The lymph node-bearing tissue of the floor of the mouth is accessed orally in conjunction to glossectomy. The sublingual gland should be removed with the floor of the mouth fatty tissue a refined operating technique can preserve the lingual and the hypoglossal nerves (4). The extrinsic tongue musculature would be salvaged, which is of benefit for the patient’s quality of life. Removing first-echelon lymph structures in sake of loco-regional tumor control improvement while maintaining swallowing ability and discriminative speech, seems especially attractive in respect of functional surgery principles.

The potential location of the LLNs can be easily determined intraoperatively. The grouping of the nodes which was used at the stage of gross specimen division is based on the topographic arrangement of the floor of the mouth region. It consists of five fascial or intermuscular spaces (gaps), one unpaired sagittal median and two paired lateral, the base for all of them is comprised by the mylohyoid muscle (Fig. 1). The median space (Block A) is situated between genioglossus and geniohyoid muscles on both sides of the lingual septum and fat are contained here, in this space the median LLN node was not encountered in our study. Next the intermediate gap (Blocks B, B’) lies between the genioglossus and geniohyoid medially and hyoglossus laterally, the lingual artery, traveling along the cornu of the hyoid bone is contained within this gap, the artery in most cadavers was accompanied by vena sublingualis (Fig. 5). This space harbored three nodes. The third space (Blocks C, C’) is limited by the hyoglossus medially and the internal surface of the mandibular body laterally, this space harbors the deep lingual vein, three nerves, lingual, hypoglossal and mylohyoid, the deep portion of the submandibular salivary gland with its duct as well as the sublingual salivary gland, our results show the existence of paraglandular LLNs in three cadavers. At the level of the hyoid the first intermediate lateral space (Block B, B’) is limited laterally by the hyoglossus muscle which covers the lingual artery, more anteriorly it is connected with the outmost lateral space (Blocks C, C’) where the sublingual salivary gland is located. Posteriorly the fatty tissue contained in the intermediate space extents into a cleft, formed by the bucco-pharyngeal fascia and the hyoglossus muscle and further to the parapharyngeal space. The outmost lateral space is continuous with the submandibular space via the interval between the mylohyoid and

![Figure 4. Specimen 13 C demonstrates two paraglandular lymph nodes. The submandibular salivary gland parenchyma (star) is located in the proximity of LLNs. The medulla of the smaller node (1) is partially substituted by fatty tissue (arrows). The larger node (2) exhibits several foci of fibrosis (asterik). H&E, x15.](image1)

![Figure 5. Specimen 5 B (male, 57 years). The anatomical contents of the intermediate lateral space are exposed. The parahyoid LLN (1) is surrounded by fat and lies next to lingual vessels. The base of the space is comprised by the mylohyoid muscle (2), the anterior belly of digastic muscle (3) inferiorly got included in the cross-section. The geniohyoid muscle makes the medial wall of the space (4). H&E, x5.](image2)

![Figure 6. Specimen 8 A (female, 75 years). The LLN (1) is located beyond the median space, specifically it was counted for Block B’, because of its location lateral to the muscles. The median space is limited by the genioglossus (2) and geniohyoid (3) muscles bilaterally. H&E, x10.](image3)
Rehabilitation.

The mouth has been demonstrated. Topographic and histologic metastasis and loco-regional recurrence.

It is required to establish the evident role played by LLNs in oral SCC.

Reviewing tongue cancer treatment strategies Harrold C.C. stated in 1969: ‘In selecting the surgical approach for excision of a tongue cancer, it is good to remember that whereas the prognosis for survival is largely dependent upon the status of the lymph nodes, the prognosis concerning disability, dysfunction and deformity is dependent upon the management of the primary lesion’ (21). Since then regional pedicled flaps and free tissue transfer have become widely accepted in reconstruction of head and neck defects after ablative surgery. Still functional deterioration is a usual sequence of such interventions. Post-glossectomy swallowing and speech are believed to be determined by variables as the extent and the site of resection (22,23); the reconstruction type (24,25); tongue mobility and residual volume (26,27) and adjuvant radiotherapy (28,29). The in-continuity or composite approach consists of severance of the majority of suprahyoid musculature ipsilaterally, as a result a so-called ‘pull-through’ defect is developed (30,31). Regional or free flaps used for repair are potent in filling the defect, but unfortunately the active mobile structures involved in the resection are replaced by functionally passive, static tissue (32). Partial and hemi-glossectomy defects are usually closed primarily or with skin auto-grafts which gives higher functional results (25,32,33). Reasonably, a composite resection is not suitable for early oral tongue tumors nevertheless strong anatomic evidence tends to suspect some lack of radicalism in traditional partial and hemi-glossectomy operation. As for locally advanced disease of the oral tongue the in-continuity approach may be regarded as the treatment of choice according to the anatomical details and certain clinical observations (Table 1–3). The value of detailed pre-operative imaging of floor of the mouth in tongue SCC is unquestionable. Further research is required to establish the evident role played by LLNs in oral SCC metastasis and loco-regional recurrence.

The presence of lymph node-bearing tissue in the floor of the mouth has been demonstrated. Topographic and histologic characteristics of LLNs are presented. The authors hope this information may be of some value in achieving better tumor control and functional outcomes in a competitive field of oral cancer treatment and rehabilitation.

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Conflict of interest

None declared.

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