Cavernous Lymphangioma of the Lower Lip : Clinicopathological and Immunohistochemical Study

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Abstract

A rare case of cavernous lymphangioma of the lower lip in a 9-year-old girl is reported with clinicopathological, histopathological, and immunohistochemical analyses. Of the benign soft tissue tumors (610 cases) treated at Nihon University School of Dentistry at Matsudo over the past 12 years, angiomas have accounted for about one-third (33.9%), including nine cases of lymphangioma and 198 cases of hemangioma, for a 1 : 23 ratio. Lymphangioma is composed of diffusely arranged lymph vessels lined by a single layer of endothelial cells. On immunostaining with D2-40, endothelial cells of lymph vessels showed positivity, but those of blood vessels were negative. The Ki-67 labeling index (LI) was lower for endothelial cells of lymph vessels (LI : 2.0) than for blood vessels in cavernous hemangioma (LI : 4.0). These results indicate that D2-40 is useful for discriminating between lymphatic vessels and blood vessels, and that cavernous lymphangioma is a hamartoma.

Keywords :

cavernous lymphangioma, lower lip, D2-40, Ki-67

Introduction

Lymphangiomas are relatively rare, non-epithelial lesions consisting of proliferating lymph vessels with lymphatic fluid, and they have a marked predilection for the head and neck, accounting for 50-75% of all soft tissue tumors in this location (1). About half of all lymphangiomas are evident at birth, and around 90% develop by 2 years of age (1). Oral lymphangiomas are most frequent on the anterior two-thirds of the tongue, and they often cause macroglossia (2). Histopathologically, lymphangiomas are classified into three groups (3). Lymphangioma simplex or capillary lymphangioma is composed of small thin-walled lymphatics. Cavernous lymphangioma consists of larger lymphatic channels with adventitial coats. Cystic lymphangioma, or cystic hygroma, consists of large macroscopic lymphatic spaces that have investitures of collagen and smooth muscle. Clinically, oral lymphangiomas usually occur superficially and have a pebbly and vesicle-like appear-

ance. Deeper oral lymphangiomas occur as soft and ill-defined masses. It is generally accepted that lymphangioma is not a true neoplasm but a congenital hamartomatous anomaly (4, 5), although details of the histogenesis and the nature of lymphangioma are still unclear. Furthermore, the differential diagnosis between lymphangioma and hemangioma is always difficult because the endothelial cells of both have similar morphology.

Here we describe a case of cavernous lymphangioma of the lower lip in a girl, together with details of the clinical pathology, histopathology, and immunohistochemistry of the lesion, in order to better understand the nature of the endothelial cells and the histogenesis.

Case report

A 9-year-old girl was referred with a chief complaint of repeated swelling on the left side of the lower lip. Her parents had first noticed the swelling

when she was 2 years old. She had undergone resection surgery twice at the ages of 4 and 8 years, and the pathological diagnosis of the specimens had been cavernous lymphangioma. Recently, swelling and remission had occurred. Oral examination revealed an elevation on the lower lip (Fig. 1). The results of ultrasound (Fig. 2) and MRI (Figs. 3, 4) examinations were compatible with a clinical diagnosis of recurrent lymphangioma. A debulking operation was performed under general anesthesia to avoid functional or cosmetic disorders. Macroscopically, the excised material was an ash gray, solid, soft mass, measuring $2.8 \times 1.7 \times 0.1$ cm. The patient is currently being followed.

Microscopic and immunohistochemical analyses

Light microscopy

After fixation in 10% neutral formalin solution, the specimen was cut into several pieces, and routine paraffin sections were prepared. The sections were stained with hematoxylin-eosin, periodic acid-Schiff reaction, and silver impregnation.

Immunohistochemistry

Primary antibodies against D2-40 (clone, D2-40), von Willebrand factor (clone, F8/86), and Ki-67 (clone, MIB-1) were purchased from a commercial source (DakoCytomation, Glostrup, Denmark). For detection of the antigens, the dextran polymer method (Envision kit, DakoCytomation) was used. Briefly, the deparaffinized sections were pretreated in citrate buffer (10 mM, pH 6.0) using a hot water bath at 95 °C for 40 min. The primary antibodies were used at a dilution of 1 : 50, and the incubation time was 40 min at room temperature, according to the supplier's instructions. Peroxidase activity was visualized using diaminobenzidine. Double staining of D2-40 and von Willebrand factor was visualized using diaminobenzidine (DAB) and 3-amino-9-ethyl carbazole (AEC) liquid substrate chromogen, respectively.

For analysis of Ki-67 proliferative activity, five areas showing high positivity were chosen, and the average cell positivity rate (LI, labeling index) was calculated after analysis. Five cases of cavernous

hemangioma were used as controls.

Results

Histopathological findings

The tumor was composed of lymph vessels lined by a single layer of endothelial cells. The lymph vessels were diffusely arranged and dilated, lying mainly under the mucosal epithelium (Figs. 5, 6). Acidophilic lymph fluid was recognized in most of the lumina. In the stromal connective tissue, mild to moderate lymphocyte infiltration, proliferation of blood capillaries and fibroblasts, and collagen fibers were observed in some areas. In the surrounding and lower area, lymph follicles, peripheral nerves, muscle, fatty tissue, and minor salivary glands were also recognized. The surface was covered by parakeratotic, stratified squamous epithelium with irregular extension of the rete pegs in some areas. The histopathological diagnosis was recurrence of cavernous lymphangioma.

Immunohistochemical findings

Most of the endothelial cells of the lymph vessels showed immunoreactivity for D2-40, but they were negative for von Willebrand factor. In contrast, most endothelial cells of the blood capillaries were negative for D2-40, but positive for von Willebrand factor. Double immunostaining revealed no overlapping positivity in the endothelial cells of either the lymph vessels or the blood capillaries (Fig. 7).

Some endothelial cells of the lymph vessels in the lymphangioma (Fig. 8) and the blood vessels of cavernous hemangiomas showed positivity for Ki-67. The Ki-67 LIs of the lymphangioma and the hemangiomas were 2.0 and 4.0 (range : 3.2-4.5), respectively.

Discussion

Lymphangiomas can affect almost any part of the body served by the lymphatic system, but they show a predilection for the head and neck (3). It is estimated that 50-65% of these tumors are present at birth, and as many as 90% become manifest by the end of the second year of life, the gender incidence

Fig. 1. Macroscopic view of the lesion on the left side of the lower lip.
 Fig. 2. Ultrasonography reveals a mass lesion (arrows) with less Doppler blood flow.
 Figs. 3, 4. The lesion has uniform low-signal intensity (arrows) on T1-weighted (3) and non-uniform high-signal intensity (arrows) on T2-weighted (4) axial MR images.
 Figs. 5, 6. Histopathologically, the tumor is composed of diffusely arranged and dilated lymph vessels, which are lined by a single layer of endothelial cells (HE, 5, $\times 40$; 6, $\times 200$).
 Fig. 7. Immunohistochemically, the endothelial cells of the lymph vessels show positivity to D2-40 (arrows), but those of the blood capillaries react to von Willebrand factor (arrowheads) (Double-stain, $\times 200$).
 Fig. 8. Positive reactions (arrows) for Ki-67 are recognized in some endothelial cells of the lymph vessels ($\times 200$).

being roughly equal (3). Anderson (6) reported that lymphangioma accounts for only 6% of all benign tumors in children. Sato et al (7). suggested that lymphangioma accounted for 11% of all tumors and 19% of all soft tissue tumors of the oromaxillofacial region in children. It has been reported that the tongue, especially the anterior dorsal surface, is the most common site of oral lymphangioma (5, 7).

For the present study, the authors selected 610 cases of benign soft tissue tumors of the oromaxillofacial region, excluding recurrent cases, from the files of the Department of Oral Pathology, Nihon University School of Dentistry at Matsudo, covering the last 12 years. Classification was based on comprehensive criteria (8). Fibrous tumors (304 cases, 49.8%) were the most common, followed by tumors of blood and lymph vessels (207 cases, 33.9%; hemangioma (198 cases), lymphangioma (9 cases), lipomatous tumors (43 cases, 7.1%), peripheral nerve sheath tumors (27 cases, 4.5%), fibrohistiocytic tumors (14 cases, 2.3%), other tumors (11 cases, 1.8%), angiomyoma (2 cases, 0.3%), and myxofibroma (2 cases, 0.3%) (Table 1). Thus, there were nine cases of lymphangioma, accounting for only 1.5% of all benign soft tissue tumors and 4.3% of all angiomas in the oromaxillofacial region. Clinicopathologically, the incidence of lymphangioma is lower than that of hemangioma (3). The reason for this is unclear, but it might be due to the fact that there are fewer lymph vessels than blood vessels in the body, and that the proliferative activity of lymphangioma (LI: 2.0) is lower than that of hemangioma (LI: 4.0). Six (66.7%)

Table 1. Benign soft tissue tumors in the present study

Tumor	Number of cases (%)
Fibrous tumors	304 (49.8)
Tumors of blood and lymph vessels	207 (33.9)
Lipomatous tumors	43 (7.1)
Peripheral nerve sheath tumors	27 (4.5)
Fibrohistiocytic tumors	14 (2.3)
Angiomyoma	2 (0.3)
Myxofibroma	2 (0.3)
Others	11 (1.8)
Total	610

Table 2. Clinical data of nine lymphangiomas in the present study (cases)

Sex :	
Male	6
Female	3
Age range :	1 year to 63 years
Average age :	13.6 years
Location :	
Tongue	5
Lower lip	2
Gingiva	2

of the nine affected patients were males. The patients ranged in age from 1 to 63 years (average, 13.6 years), and five (55.6%) and three (33.3%) cases arose in the first and second decade, respectively. Five cases (55.6%) occurred in the tongue, and two cases each (22.2%) were located in the gingiva and the lower lip (Table 2). The present data are in agreement with the site predilection of oral lymphangioma reported previously (3, 5-7), but they nevertheless indicate that lymphangiomas account for a very small proportion of all benign soft tissue tumors, and that the age of affected patients is relatively high. In the present case, it was presumed that the lower lip had been affected by lymphangioma when the patient was 2 years old. Hence, this is a rare lip lymphangioma from the viewpoint of site predilection.

With regard to the pathogenesis of lymphangiomas, most researchers regard them as malformations that fail to communicate normally with the lymphatic system, as most cases become manifest clinically during childhood and develop in areas where the primitive lymph sacs occur (3). Some consider lymphangiomas to be true neoplasms that are capable of locally aggressive behavior, while others have suggested that they arise when inflammation causes fibrosis and obstruction of lymphatic channels (3). Recently, D2-40, a MW 40,000 O-linked sialoglycoprotein, has been found to be expressed specifically on the endothelium of normal lymphatic vessels and lymphangiomas (9, 10). In the present study, most endothelial cells of lymph capillaries showed positivity for D2-40, while blood capillaries

were negative. The present study demonstrated that D2-40 was a useful marker for distinguishing the endothelial cells of lymphatics from those of blood capillaries.

Proliferative activity is a useful indicator for estimating whether or not a lesion has a neoplastic character. Only one previous study has demonstrated proliferative activity of endothelial cells in lymphangioma using anti-PCNA antibody (11). That study reported that PCNA immunostaining was rarely positive in the endothelial cells. In the present case, Ki-67-positive endothelial cells were scattered, and the Ki-67 LI was only 2.0, suggesting that the lesion was not a neoplasm but a malformation/ hamartoma.

Various treatments, such as surgical excision, radiation therapy, cryotherapy, electrocautery, sclerotherapy, steroid administration, embolization, ligation, laser surgery, and radiofrequency ablation, have been proposed for lymphangioma. It has been reported that sclerotherapy using OK-432 was an effective initial treatment for head and neck lymphatic malformations, especially for cystic hemangiomas and pediatric patients (12). Surgical excision is considered to be the best and most commonly used approach (13, 14), because the recurrence rate is reported to be 10-20% (4, 15). However, lymphangiomas frequently show aggressive spread to surrounding tissues and organs. When complete excision is likely to lead to severe dysfunction, a debulking operation is recommended. In the present case, as cosmetic and functional disturbances were considered likely, debulking was chosen.

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