Tumor mimicking actinomycosis of the upper lip: report of two cases

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Abstract: Peculiar findings of orofacial actinomycosis mimicking the clinical appearance of a tumor of the upper lip were reported. A 68-year-old woman (case 1) and a 62-year-old woman (case 2) visited our hospitals towards the end of 2004 and 2007; the clinical diagnosis for each patient was upper labial tumor, and the lesions were surgically removed. Histologically, the excised specimens showed granulomas including bacterial colonies consisting of club-shaped filaments that formed a radiating rosette pattern in the submucosal layer. DNA samples were extracted from paraffin sections and examined by PCR for *Actinomyces* species. The PCR products examined by direct DNA sequencing demonstrated the presence of *Actinomyces israelii* and *Actinomyces gerencseriae* in both case 1 and case 2. Finally, pathological diagnoses of actinomycosis were made for each. Actinomycosis mimicking a tumor is very uncommon in the oral mucosa, and the use of PCR is an effective means of early and accurate diagnosis.

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Key words: actinomycosis; *Actinomyces israelii*; *Actinomyces gerencseriae*; mimicking tumor; PCR

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Introduction

Actinomyces, which are normal inhabitants of the human oral cavity, have a tendency to penetrate the submucosal tissue when there is an interruption of the mucosal barrier (1-2). Although *Actinomyces* are found in healthy persons, they can be pathogenic, with *Actinomyces israelii* being the main causative bacteria, and with *Actinomyces viscosus* running a close second (3). Actinomycosis is an infectious disease that frequently has chronic granulomatous and suppurative lesions caused by saprophytic *Actinomyces* species. In most such cases, the primary organism is combined synergistically with streptococci and staphylococci. Approximately 55% of cases of actinomycosis are diagnosed in the cervicofacial regions (4) and include intraoral and periodontal types. The remaining cases exhibit a variety of patterns, such as superficial skin or genitourinary infections. In the intraoral mucosal region, actinomycosis is

seen in rare and causative organisms which enter into tissue through preexistent ulcerative lesions.

Clinically, actinomycosis may be either an acute and rapidly progressing infection or a chronic and slowly spreading lesion that is associated with fibrosis. The suppurative reaction of the infection may discharge large yellowish flecks that represent colonies of the bacteria called sulfur granules. Additionally, actinomycosis produces a reactive inflammatory response which causes an area of necrosis and scar tissue around the abscess which is similar to indurations. Although common, sulfur granules are not invariably present. Therefore, absence of this clinical condition should not delay therapy; a high index of suspicion is of great importance for the differential diagnosis of oral mucosa masses. Lan *et al.*(2) reported a misleading lip tumor case which hinted at overdiagnosis. Diagnosis of actinomycosis is commonly made by histological examination which reveals the characteristic picture of sulfur

Fig. 1. Histopathology of tumor-mimicking actinomycosis in the upper lip. Case 1 from a 68-year-old woman; Hematoxylin and eosin (HE) stain. (a) $\times 50$; (b-c) $\times 100$. In case 1, there was a rather obscurely demarcated granulation tissue within the lamina propria to the submucosal layer. In the central part of the granulation tissue nodule, irregular-shaped bacterial colonies were surrounded by dense infiltrates of inflammatory cells and further by fibrous bundles. The covering epithelium was hyperplastic (a). The irregular-shaped bacterial colonies were characterized by their rim of eosinophilic club-like structures. The inflammatory cells were mainly neutrophils and macrophages (b). There was a surgical thread surrounded by neutrophils and further by epithelioid macrophages (c).

granules. Polymerase chain reaction (PCR) and DNA sequencing have enabled the identification of bacterial species and strains which are difficult or even impossible to grow in artificial culture. These cases have been diagnosed by histopathological findings and DNA analyses.

We present a clinical and histopathological report of an unusual presentation of orofacial actinomycosis mimicking the clinical appearance of a benign tumor of the upper lip, along with a review of the literature.

Cases report

Case 1: A 68-year-old woman was admitted to Nihon University Hospital towards the end of 2004 for tumor of the upper lip. Her dental history disclosed that she had received sutures for laceration of the upper lip caused by a traffic accident about one year prior. A tumor had gradually grown, and the patient had been sent to us from a private dental clinic for histopathological analysis. The examination showed a tumor with induration around its central area, measuring 1.0 cm in diameter and extending from the upper left incisor to canine tooth. The tumor was slightly reddish and surrounded by normal colored mucosa without ulceration. The clinical diagnosis was an upper labial tumor.

Case 2: In 2007, a 62-year-old woman noticed a swelling in her upper lip and consulted Niigata University

Hospital two days later. The examination showed an elastic hard and movable mass in the left side of upper lip measuring 1.0 cm in diameter and surrounded by normal mucosa with ulceration. The clinical diagnosis was an upper labial tumor.

In both cases, the wound healed well after surgery and there was no evidence of local recurrence. Informed consent was obtained from the patient before performing surgical treatment.

Histopathological findings

Case 1: There was a granuloma including bacterial colonies in the submucosal layer of the lip. The covering mucosa was covered by hyperplastic squamous epithelium (Fig. 1a). These colonies were surrounded by abundant inflammatory cells and an outer fibrous wall. An island of ectopic squamous cell epithelium was observed adjacent to the bacterial colonies. The colonies consisted of club-shaped filaments that formed a radiating rosette pattern. With HE stain, the central core stained basophilic and the peripheral portion was eosinophilic. These colonies were surrounded by neutrophils with an outer zone of mononuclear cells (Fig. 1b). The serial section demonstrated a surgical thread in the center of the granuloma surrounded by numerous neutrophils, agminated epithelioid histiocytes and neovascularization (Fig. 1c). At this point, pathological diagnoses were

Fig. 2. Histopathology of tumor-mimicking actinomycosis in the upper lip. Case 2 from a 62-year-old woman; HE stain. (a) $\times 50$; (b-c) $\times 100$. In case 2, there was granulation tissue, including bacterial colonies in the submucosal layer of the lip adjacent to the minor salivary gland. In the central part of the granulation tissue nodule, irregular-shaped bacterial colonies and salivary concretions were surrounded by inflammatory cells and by fibrous bundles (a). The irregular-shaped bacterial colonies consisted of club-shaped filaments that formed a radiating rosette pattern. These colonies were surrounded by abundant inflammatory cells (b). Salivary concretion, surrounded by lots of neutrophils, existed as a core of the granulation tissue (c).

Table 1. PCR primers and amplicon size in this study

Primer name	DNA sequences of PCR primer (5'→3')	Amplicon size
ACM-F-1	AAGTCGAACGGGTCTGCCTTG	237bp
ACM-R-1	TCAAAGCCTTGGCAGGCCATC	
ACM-F-2	TAACCTGCCCTCACTTCTGG	170bp

compatible with actinomycosis.

Case 2: There was an agranulomatous lesion including bacterial colonies adjacent to the minor salivary gland in the submucosal layer of the lip. The surface mucosa was covered by hyperplastic squamous epithelium with partial ulcerative changes (Fig. 2a). Salivary concretion with periodic acid-Schiff (PAS), diastase-digested PAS, and mucicarmine-positive staining existed as a core of the granulation tissue (data not shown). The bacterial colonies were surrounded by abundant inflammatory cells and further by fibrous granulation tissue. These colonies consisted of club-shaped filaments that formed a radiating rosette pattern. With HE stain, the central core stained basophilic and the peripheral portion was eosinophilic. These colonies were surrounded by neutrophils with an outer zone of mononuclear cells (Fig. 2b). The salivary concretion adjacent to the bacterial colonies was surrounded by lots of neutrophils (Fig. 2c). At this point, pathological diagnoses were compatible with actinomycosis.

DNA analysis

Serial paraffin sections were cut at 10 µm thickness from each of the formalin-fixed and paraffin-embedded tissues of

the two biopsy specimens. Three sections each were treated by using DEXPAT® (Takara Bio Inc., Kyoto, Japan) for DNA extraction. The oligonucleotide primers used in this study are summarized in Table 1. The PCR mixture (20 µl) contained 1×PCR buffer (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA), 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems) and 5 pM each of the primers. The primer pair of nested PCR for the first round was ACM-F-1 and ACM-R-1, and for second round was ACM-F-2 and ACM-R-1 for 16S ribosomal RNA gene of *Actinomyces israelii* (Table 1). The size of the first round PCR product was 237 bp, and the second round PCR product was 170 bp (5). Following preheating at 94°C for 10 min, 30 cycles of consecutive incubation at 94°C for 30 sec, 52°C for 30 sec and 72°C for 1 min was carried out, followed by post-incubation at 72°C for 5 min. Two microliters of the PCR products were resolved in 3% agarose gel and photographed after staining in ethidium bromide. DNA direct sequencing was performed by using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Fig. 3. PCR products for *Actinomyces* separated by 3% ethidiumbromide stained agarose gel electrophoresis (a) case 1; (b) case 2. In case 1, *Actinomyces*-specific DNA was amplified by nested PCR. PCR products 170 bp in size were observed in agarose gel. Lane 1, 100 bp ladder DNA marker; lane 2, sample; lane 3, negative control (TE solution was used as template DNA)(a). In case 2, *Actinomyces*-specific DNA was amplified with only a second round PCR primer set, ACM-F-2 and ACM-R-1. PCR products 170bp in size were observed in agarose gel. Lane 1, 100 bp ladder DNA marker; lane 2, sample; lane 3, negative control (TE solution was used as template DNA)(b).

Fig. 4. Target sequences of 16S ribosomal RNA gene of *Actinomyces israelii* (upper line) and *Actinomyces gerencseriae* (lower line). Asterisks indicate homologous nucleotides, and symbol (-) denotes the deletion of bases. Arrows show the primers. In case 1, DNA sequence of nested PCR product was corresponding to *Actinomyces israelii*. In case 2, DNA sequence of PCR product with only second round PCR primer set (ACM-F-2 and ACM-R-1) was corresponding to *Actinomyces gerencseriae*. First round forward PCR primer, ACM-F-1, was not specific for *Actinomyces gerencseriae* but was specific for *Actinomyces israelii*. It was difficult to amplify the target DNA of *Actinomyces gerencseriae*.

Table 2. Clinical findings of Actinomycosis in the oral mucosa as reported in the English-language literature

case #	age & sex	site	size (cm)	duration (months)	gross features	induration	clinical diagnoses	dental history	medical history	reference #	yr
1	55 M	tongue	2-3	6	swelling	+	malignant tumor	self-inflicted bite	unremarkable	12	1989
2	32 F	upper lip	2	2	swelling	+	tumor of minor salivary gland	NS**	unremarkable	1	1995
3	58 M	hard palate	3-4	—	ulcer	-	—	—	hypertension/ arthritis	8	1995
4	65 F	hard palate	1.5	1 week	ulcer	-	mucormycosis/ tumor	oral candidosis	diet-controlled hypertension	9	1998
5	74 F	anterior floor	3	5 weeks	ulcer	+	ulcer	extraction	diabetes	6	2000
6	5 M	hard palate	3*	10 days	swelling	+	inflammation	peculiar habit***	unremarkable	13	2003
7	60 M	lower lip	1	3	mass	+	tumor of minor salivary gland	NS	unremarkable	2	2003
8	60 F	gingiva	—	—	abscess	-	fungal infection	poor oral hygiene	unremarkable	14	2003
9	52 F	tongue	2	2	mass	-	tumor	NS	unremarkable	10	2006
10	68 F	upper lip	1	10	mass	+	tumor	suture of laceration	unremarkable	present case	2011
11	62 F	upper lip	1	2 days	mass	+	tumor	mucocoele	unremarkable	present case	2011

*Author described that the lesion extended from the rugae area to the junction of the hard and soft palate.

**Not specified

***The patient had a peculiar but rare habit of poking his anterior hard palate with his mother's safety pin.

DNA sequence was compared with the 16S ribosomal RNA gene of *Actinomyces* in Genbank.

Case 1: *Actinomyces israelii* specific DNA was amplified by using nested PCR and was observed in 3 % agarose gel (Fig. 3a). The DNA sequence of the PCR product was determined and searched with the NCBI BLAST program. The sequence corresponded to *Actinomyces israelii* (GenBank accession number: X82450.1) (Fig. 4). This molecular biological evidence indicated that tumor-like granulation tissue was infected with *Actinomyces israelii*.

Case 2: PCR amplified band was observed in 3% agarose gel without nested PCR procedure but using PCR with a second round primer set (ACM-F-2 and ACM-R-1) (Fig. 3b). The DNA sequence of the PCR product was determined and searched with NCBI BLAST program. The sequence was corresponding to *Actinomyces gerencseriae* (GenBank accession number: X80414.1) (Fig. 4). This molecular biological evidence indicated that tumor-like granulation tissue was infected with *Actinomyces gerencseriae*.

Discussion

Actinomycosis is a chronic granulomatous infective disease caused by microaerophilic Gram-positive bacteria of the genus *Actinomyces*. It occurs most frequently in the cervicofacial, abdominal, and pulmonothoracic regions. Actinomycosis usually involves the cervicofacial region as a form of actinomycotic osteomyelitis; infection is endogenous, and a tooth socket, most commonly a lower third molar or an infected root canal, is thought to be the portal of entry, and localization in the oral mucosa is extremely rare (6). As for actinomycotic osteomyelitis, typical clinical appearance is a slowly enlarging, painless,

firm or fluctuant swelling at the angle of the mandible. Multiple draining cutaneous fistulas are the characteristic sign of this infection (7). In contrast, a tumor-mimicking, slow-growing, indurated, nontender submucosal mass was observed in actinomycosis in the oral mucosa. The present case report was a limited to the upper lip and showed tumor-like findings in which the diagnosis was based on histopathological examination and DNA analyses.

Actinomycosis of the oral mucosa is very uncommon, and a review of the English-language medical literature revealed only 9 reported cases of oral mucosa involvement over the last 30 years. Table 2 showed clinical characteristics of actinomycosis in the oral mucosa by a review of literature, including these present cases. As for actinomycosis in the oral mucosa, females are predominantly affected (1:1.5), with an age range of 5-74 years, with a male predominance of 1.5-3:1 and an age range of 40-70 years without any racial predilection in the head and neck region (4). Clinically, actinomycosis may be either an acute, rapidly progressing infection or a chronic, slowly spreading lesion that is associated with fibrosis. It is a reactive inflammatory response, resulting in scar tissues around abscesses or necrotic foci. This scar or fibrous granulation tissue, which is palpable as an induration, might lead to a diagnosis of neoplasm. Out of the nine cases of oral actinomycosis documented in the literature, seven cases were clinically diagnosed as tumors, of which one was malignant. Macroscopically, six cases showed induration with the duration of swelling ranging from 2 to 10 months. The seven cases with tumor mimicking appearances were found in the lip (4 cases) (1-2), hard palate (2 cases) (8-9), and tongue (1 case) (10).

Actinomycosis does not appear to be an opportunistic

disease (7). Poor oral hygiene and dental problems have been suggested as triggers of foractinomycotic infection. Dental problems, namely loss of integrity of the mucosa by injury, tooth extraction, root canal treatment, and periodontal or periapical lesions, might be opportunities for infection (7, 11). Bacterial culture tests from actinomycosis lesions have also shown the presence of other bacteria. It has been suggested that these bacteria diminished the oxygen in the tissues and led to the growth and more easy expansion of saprophytic *Actinomyces* (7, 11). In case 1 of the present series, suture of a laceration of the upper lip caused by a traffic accident might have provided a pathway of infection and aberration of epithelial cells. In case 2, granulomatous and ulcerative changes associated with a mucocele might have been a trigger of infection. Our review of the literature demonstrated that two cases had dental problems (6, 12), while one case each had oral candidosis (9), a peculiar dental habit (13), and poor oral hygiene (14), respectively.

A diagnosis of actinomycosis has typically been made based on bacterial culture tests. However, this method is not reliable because bacteria cannot always be efficiently isolated. Therefore, final diagnosis is usually dependent on histopathological confirmation of actinomycotic sulfur granules by biopsy (4, 7, 11). Although sulfur granules have also been found in nocardiosis, their detection in the presence of gram-positive rods is considered to be pathognomonic for actinomycosis (15). Nocardia is weakly or partially positive for Ziehl-Neelsen staining, and this should be an important discriminative point from *Actinomyces* (16). Although the present cases demonstrated Gram-positive (+) and Ziehl-Neelsen-negative (-) filaments with peripheral Gram (-) clubbing, PCR was able to demonstrate the presence of *Actinomyces*. Another infection that produces sulfur granules and mimics actinomycosis is botryomycosis, an unusual host reaction to *S. aureus* and other bacteria. For the differential diagnosis from botryomycosis, the molecular genetic method may be helpful. Thus, the PCR method is very advantageous when microorganisms are few (17-18). In the present study, the *Actinomyces* species were identified not only by PCR, but also by DNA sequencing using DNA samples extracted from formalin-fixed and paraffin-embedded tissues. DNA analysis is effective for early and accurate diagnosis of oral lesions infected with microorganisms in order to prevent delay in appropriate treatment.

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