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Confirmation of immunoglobulin heavy chain rearrangement by polymerase chain reaction using surgically obtained, paraffin-embedded samples to diagnose primary palate mucosa-associated lymphoid tissue lymphoma: A case study

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ABSTRACT

INTRODUCTION: Intraoral mucosa-associated lymphoid tissue (MALT) lymphoma is a rare lymphoma that has a good prognosis if diagnosed correctly and treated in time.
PRESENTATION OF CASE: A 64-year-old woman was referred to our department with asymptomatic swelling of the left hard palate. Computed tomography and magnetic resonance imaging revealed a mass in the left hard palate. We performed a pre-surgery biopsy; however, it was difficult to differentiate MALT lymphoma from other reactive lymphoproliferative disorders via gross or microscopic examination. Although the lesion was completely excised, histological findings did not allow a definitive diagnosis due to an absence of visible monoclonality. We then performed polymerase chain reaction (PCR) using DNA extracted from formalin-fixed, paraffin-embedded surgical samples. Capillary electrophoresis showed monoclonal peaks of immunoglobulin heavy chain gene rearrangement, thus facilitating a definitive diagnosis of MALT lymphoma.
DISCUSSION: PCR technique is rapid, accurate, and enables a definitive diagnosis without relying on traditional histological or molecular diagnostic techniques, such as Southern blotting.
CONCLUSION: We suggest that, if histological examination is ambiguous or fresh material is insufficient, PCR can be performed using paraffin-embedded materials to definitively diagnose low-grade lymphomas, such as MALT lymphoma.
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1. Introduction

Most mucosa-associated lymphoid tissue (MALT) lymphoma cases arise from gastrointestinal organs. Cases originating in the oral cavity are very rare, although a few such cases are reported [1–8,10].

MALT lymphoma is a low-grade malignancy; therefore, histological differentiation from other lymphoproliferative disorders may be difficult. Proving monoclonality with the Southern blot technique is useful for differentiating between neoplastic and lymphoproliferative disorders; however, this technique requires large amounts of DNA from fresh tissue samples or the completion of a difficult DNA extraction from formalin-fixed, paraffin-embedded (FFPE) samples [9]. To overcome these limitations, Wan et al. reported the use of polymerase chain reaction (PCR) to prove monoclonality with minimal amounts of DNA extracted from FFPE samples [9,10].

In this case, we used PCR to definitively diagnose MALT lymphoma based on FFPE materials. Based on this successful application, we suggest that PCR can be used to distinguish low-grade lymphoma from lymphoproliferative disorders when the lesion presentation is insufficient for pathological diagnosis.

2. Presentation of case

A 64-year-old woman presented to our department for evaluation of an asymptomatic mass on her left hard palate.

Intraoral examination revealed a 30 × 23-mm mass in the left hard palate's submucosal region with intact overlying mucosal tissue (Fig. 1a). Computed tomography and contrast-enhanced magnetic resonance imaging were performed. The results showed

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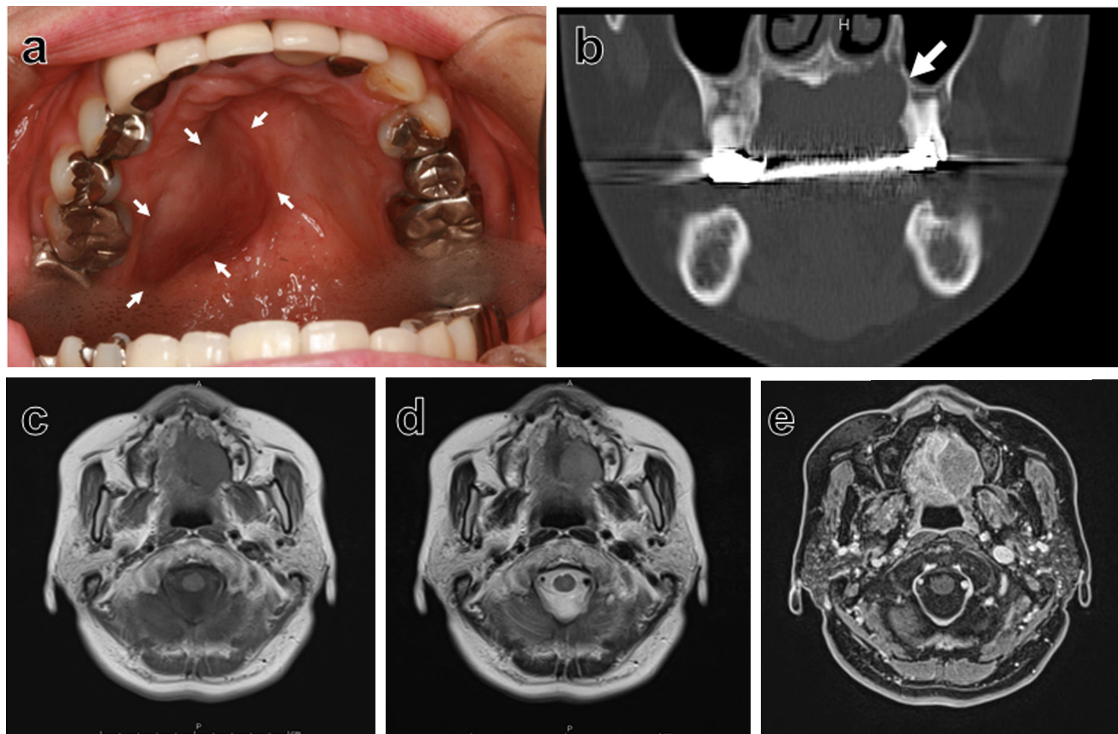

Fig. 1. Case images and clinical findings. (a) Clinical photograph of the left palatal tumor reflected in the glass. (b) Computed tomography image showing a homogeneous lesion and pressure bone resorption by the tumor (arrow). (c–e) Enhanced magnetic resonance image of the lesion. (c: T1 weighted image; d: T2 weighted image; and e: diffusion weighted image).

a homogeneous mass with well-delineated margins and pressure bone resorption of the hard palate (Fig. 1b–e). There were no other oral lesions or lymph node abnormalities.

We initially suspected a benign or low-grade malignant tumor, such as pleomorphic adenoma of the left palate, but an incisional biopsy specimen of the lesion did not confirm this or any other diagnosis. Histological findings revealed minimal lymphocytic infiltration in the stroma of the minor salivary glands. These findings were insufficient to distinguish between a lymphoproliferative disorder and MALT lymphoma. Therefore, we determined to excise this lesion under general anesthesia.

Pre-procedure blood tests revealed no unusual hematological or biochemical data. As the correlation of MALT lymphoma with Sjögren's syndrome is widely reported [1,2,4], we measured the amount of secreted saliva and performed appropriate immunological tests. The patient had normal salivary secretion ability, and had normal ranges of SS-A/Ro antibody and SS-B/Ro antibody.

The intraoperative rapid diagnosis of the lesion strongly suggested MALT lymphoma. The tumor was resected with an adequate safety margin of 5 mm and the palatal bone touching the tumor was shaved off along with the bone bar (Fig. 2a, b). The palatal mucosal defect was reconstructed using artificial dermis (Terudermis®). At a follow-up examination 9 months later, no recurrence or metastasis was seen (data not shown). An upper gastrointestinal endoscopy failed to detect *Helicobacter pylori* or any other abnormal endoscopic findings.

Histopathological examination showed lymphocytic infiltrate between the lymphoid follicles. Hyperplasia was seen from the germinal center to the stroma under an oral squamous epithelium surface. This hyperplastic tissue was largely composed of centrocytes and centroblasts with infiltration of small lymphocyte-like cells (Fig. 2c, d). Particularly in the infiltrated area, the lymphoid follicle was irregularly expanded with irregular polarity. Small and medium lymphocyte-like cells and plasma cell-like cells were clustered relatively densely between the follicles. Lymphoepithelial lesions or Dutcher bodies were not observed in the fibrous tissue partitions and salivary gland tissues (Fig. 2c, d). Immunohistochemistry revealed positivity for CD20 (Fig. 2e) and CD79a between the lymphoid follicles. CD10 and bcl-2 positivity were observed only in the germinal center cells and nongerminal center cells, respectively. CD3 (Fig. 2f) and CD5 positivity were observed in the T-lymphocytes between follicles. Since CD138, Igκ, and Igλ were positive in some cells, we assumed no immunoglobulin light chain restriction. A few plasma cells between lymphoid follicles were positive for immunoglobulin G (IgG) and IgG4 (<40%). In addition, the positive Ki-67 ratio in cells between follicles was about 10%. Based on these histological results, MALT lymphoma was strongly suspected; however, the diagnosis was atypical follicular and interfollicular hyperplasia because of the lack of tumor cell clonality (Fig. 2).

Finally, we ordered a molecular analysis of the immunoglobulin heavy chains using the FFPE sections of a postoperative sample. We performed PCR using extracted DNA from the materials and observed a monoclonal peak of VH(FR1)/JH, VH(FR2)/JH, and VH(FR3)/JH immunoglobulin heavy chain (*IgH*) gene rearrangement on capillary electrophoresis (Fig. 3). The final diagnosis was MALT lymphoma of the hard palate.

3. Discussion

Most malignant lymphomas in the oral and maxillofacial region are non-Hodgkin's lymphoma (NHL), of which MALT lymphoma is a subtype [2]. Head and neck NHL most commonly arises in Waldeyer's ring (comprising the pharyngeal, tubal, palatine, and lingual tonsils) followed by the major salivary glands. Intraoral NHL is relatively rare; malignant lymphomas comprise 3.5% of all oral malignancies [1]. Most intraoral NHL cases are of diffuse large B-cell

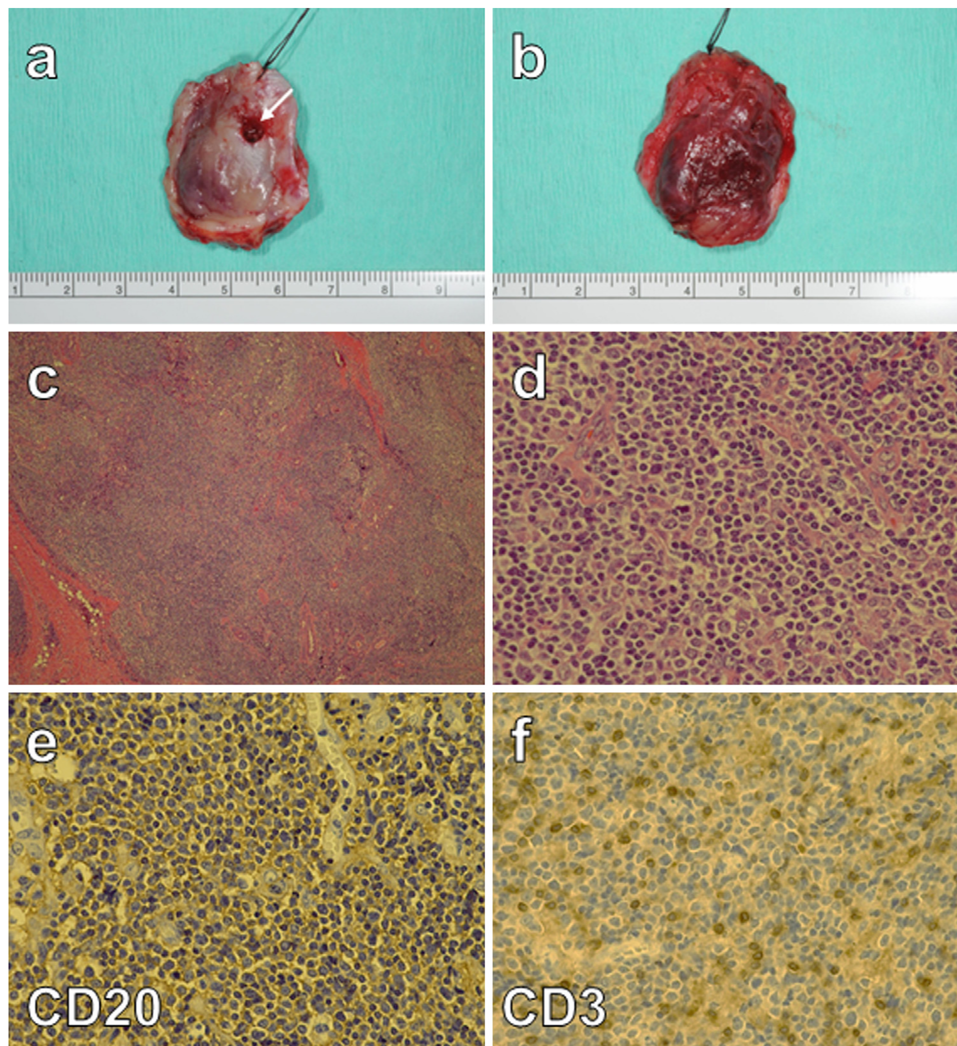

Fig. 2. Case histopathology. (a and b) Post-excision clinical photograph of the tumor. The arrow indicates an intraoperative biopsy on the oral side (a) and deep side (b). (c and d) Hematoxylin-eosin staining. The lymphocytic infiltrate is observed between the lymphoid follicles, showing hyperplasia from the germinal center to stroma deep to an oral squamous epithelium (c). High-power magnification shows a tumor composed of centrocyte-like cells with small lymphocyte-like cell infiltrates (d). (e and f) Tumor immunohistochemistry: CD20 (positive) (e) and CD3 (negative; positive at normal T-lymphocytes) (f). Original magnification (c) ×40, (d–f) ×400.

lymphoma [3]. Intraoral MALT lymphoma is a particularly uncommon type of lymphoma, with very few cases being reported till now [1–8,10].

Extranodal complications arising from MALT occur most often in the gastrointestinal tract, thyroid, and major salivary glands. *H. pylori*-induced chronic gastritis may actually trigger gastric MALT lymphoma [5]. However intraoral MALT lymphoma concurrent with gastrointestinal MALT lymphoma has not been reported, so we considered it unlikely that oral *H. pylori* had caused the patient's oral MALT lymphoma. Sjögren's syndrome is often considered a major contributor to MALT lymphoma of the oral and maxillofacial region; in our case, it was considered as an underlying cause. Although we determined that the patient did not have Sjögren's syndrome, MALT lymphoma of the palate with Sjögren's syndrome was observed in one-third of the patients described in Table 1, which presents the clinicopathological features of characteristic cases of primary MALT lymphoma of the palate. Thus, considering the syndrome's presence and ruling it out was appropriate.

MALT lymphoma is histologically heterogeneous because it is composed of monocytoid B cells (both small and medium lymphocytes) and plasma cells. Therefore, as in this case, it can be difficult to distinguish MALT lymphoma from reactive lymphoproliferative disorders.

Molecular diagnostic techniques for categorizing MALT lymphoma are fairly advanced and include several characteristic chromosomal and genetic abnormalities. The most common marker is t [11; 18] (q21; q21) *API2-MALT1*, a chimeric gene. Despite its accuracy and capacity for definitive diagnosis, actually detecting the presence of the *API2-MALT* chimeric gene is difficult (Table 1) [1,6]. Thus, detecting monoclonality remains the most ideal diagnostic technique for confirming MALT lymphoma.

In cases of lymphoma, the B cells are reconstituted monoclonal rearrangements of the *IgH* genes; however, the B cells in non-neoplastic lesions are reconstituted polyclonal rearrangements, providing a clear differentiation between cell types.

To capitalize on the characteristic monoclonality of proliferative lymphoma, several types of molecular diagnostic techniques are currently employed. Southern blotting is ideal for revealing monoclonal immunoglobulins, but it requires a large amount (1–5 μg) of fresh DNA (as opposed to FFPE DNA) [9,10]. To overcome this limitation, Wan et al. reported a PCR technique for detecting monoclonality using DNA obtained from FFPE materials [9]. The authors reported a 92% positive monoclonal detection rate for cases of

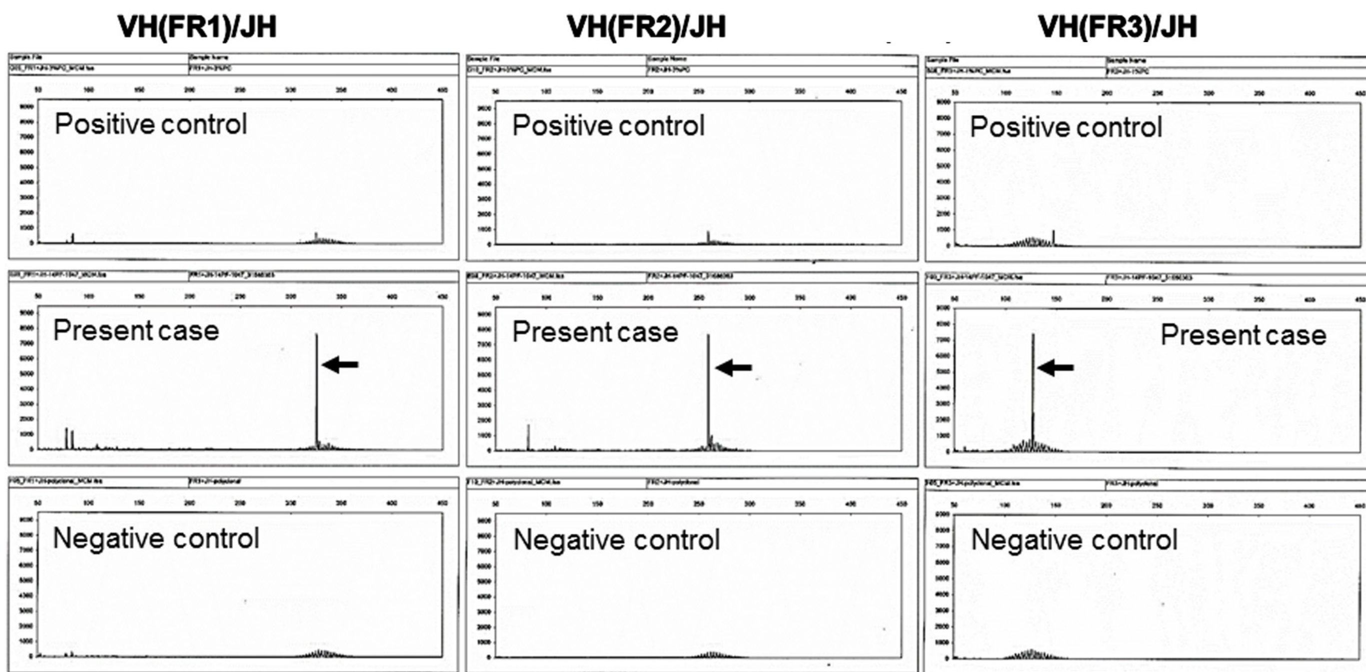

Fig. 3. PCR analysis of IgH rearrangements. The PCR product showed monoclonal peaks of VH(FR1)/JH, VH(FR2)/JH, and VH(FR3)/JH IgH gene rearrangement on capillary electrophoresis.

Table 1
Clinicopathological features of palatal MALT lymphoma.

Case	Reference	Age/sex	Autoimmune disease	Size (mm)	Location	Regional node metastasis	Treatment	Response	Outocome	Follow up	API2-MALT1	Monoclonality
1	Odell et al. [2]	52/F	SjS	NR	Palate + parotid	No	RT	CR	A(−)	18 m	NT	NT
2	Kolokotronis et al. [3]	73/F	No	NR	Palate	No	Excision	RD	DOD	15y	NT	NT
3	Pijpe et al. [4]	42/F	SjS	20	Palate + parotid	No	Chemo (rituximab)	CR	A(−)	6 m	NT	PCR(+)
4	Sakuma et al. [1]	70F	SjS	17	Palate	No	Spontanous regression	CR	A(−)	38 m	ND	PCR(+)
5	Tauber et al. [5]	71/F	NR	20	Palate	No	Excision	CR	A(−)	4y	NT	NT
6	Kojima et al. [6]	51/M	No	15	Palate	No	Excision	CR	A(−)	10y	NT	NT
7	Kojima et al. [6]	64/F	No	20	Palate	No	Excision	RD	A(+)	180 m	ND	NT
8	Kojima et al. [6]	77/F	No	15	Palate + parotid	No	RT	CR	A(−)	14 m	ND	NT
9	Manveen et al. [7]	40/M	No	45	Palate	No	Excision	CR	A(−)	6 m	NT	NT
10	Shah et al. [8]	55/F	NR	19	Palate	No	Excision	CR	A(−)	2y	NT	IHC(+)
11	Present case	64/F	No	30	Palate	No	Excision	–	–	–	NT	IHC(ND) PCR(+)

A: alive, CR: complete remission, DOD: died of disease, IHC: immunohistochemistry, ND: not detected, NR: not recorded, NT: not tested, PCR: polymerase chain reaction, RD: relapsed disease, RT: radiation therapy, SjS: Sjögren's syndrome, (−); without disease, (+); with disease.

known B-cell NHL with this technique [9]. The PCR technique uses a small amount of DNA, which can be taken from paraffin-embedded samples. The technique is rapid, simple, and sensitive. Table 1 shows the results of this technique in 3 cases of reported palatal MALT lymphoma, including the present case.

4. Conclusion

We report a diagnosis of MALT lymphoma of the hard palate. We used FFPE materials obtained during surgery to perform PCR and confirm monoclonality.

This successful diagnosis is promising, but additional, larger studies are needed. As diagnostic methods develop, intraoral MALT lymphoma will be treated earlier and with greater accuracy. Further optimization of surgery, chemotherapy, radiotherapy, and combination therapy will improve patient prognosis.

Conflict of Interests

None declared.

Source of funding

None.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Author's contribution

Shighiro Abe, Naoko Yokomizo, and Yutaka Kobayashi were the surgeons, Kouhei Yamamoto was the Pathologist for the study. Shigehiro Abe was responsible for writing the article and responsible for the manuscript preparation.

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