Case Report

Mutational analysis of BRAFV600E in a case of sialadenoma papilliferum of oral cavity

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Abstract

This case report presents a rare benign salivary gland tumour called sialadenoma papilliferum. It comprises 3 to 5% of head and neck tumours and about 1% of all minor salivary gland tumours. The focus is on discussing the BRAFV600E mutation analysis and exploring its clinical implications, along with delving into the histopathological differentials. We documented a 73-year-old male, who had a white patch in the left retromolar trigone region for 6 months. The tumour was excised with a clinical diagnosis of verrucous lesion. Microscopic examination revealed exophytic hyperplastic parakeratotic stratified squamous epithelium and endophytic ductal papillary proliferation. The BRAFV600E mutation was present in the patient. Subsequent regular follow-ups were conducted, revealing no recurrence of the condition. This case underscores the pivotal role of BRAFV600E analysis as an invaluable diagnostic tool when confronted with the intricacies of intraoral salivary gland neoplasms.

Keywords: BRAF, male, mandible, minor salivary gland, oral cavity

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INTRODUCTION

Sialadenoma papilliferum (SP) is an uncommon benign neoplasm of salivary gland. It comprises approximately 1% of all the minor salivary gland tumours and 3 to 5% of head and neck tumours.[1,2] Sialadenoma papilliferum was initially identified by Abrams and Finck in 1969. They observed its resemblance to syringocystadenoma papilliferum. Recognizing it as an unusual tumour originating from the salivary glands, it was named as "sialadenoma papilliferum".[3] SP is typically diagnosed in individuals in their fifth, sixth, and seventh decades of life, with a slight male preponderance. The most frequent location for occurrence of SP is the hard palate, followed by the buccal mucosa, soft palate, tongue, retromolar pad area, upper lip, and occasionally in the floor of the mouth and parotid gland.[1,2] Hsieh[4] and Nakaguro[5] demonstrated the presence of genetic alterations in their respective SP cases. Hsieh conducted both immunohistochemical and molecular analyses of SP using the BRAFV600E. In the study, it was observed that all the classical SP cases tested positive for BRAF through immunohistochemistry, and the genetic alteration BRAF V600E was likewise identified in all the classical SP cases examined.[4] In Nakaguro's study involving 10 SP cases, seven of them exhibited BRAF V600E mutations, while HRAS Q61R mutations were detected in one out of eight cases, representing a frequency of 12.5% in SP.[5] In addition,

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syringocystadenoma papilliferum of the skin, sharing histological similarities with SP, has been identified to contain BRAF and HRAS mutations. This observation suggests that SP can be regarded as a salivary gland counterpart to syringocystadenoma papilliferum of the skin.[6] Considering all this information, we conducted an analysis for the BRAFV600E mutation in the current case as well.

CASE HISTORY

A 73-year-old male presented with a white lesion on the left lower back tooth region of oral cavity persisting for 6 months. Clinical examination revealed a whitish nodular growth, approximately 1 × 1 cm in size, in the left retromolar trigone region. The patient had a 40-year history of cigarette smoking. Clinically, the main differentials considered were verrucous lesions. Interestingly, a prior biopsy performed at a private clinic had suggested a verrucous papillary lesion. An excisional biopsy of the lesion was conducted. Microscopic examination revealed parakeratotic stratified squamous epithelium exhibiting a sharp transition into hyperplastic exophytic projections and endophytic ductal papillary proliferation [Figure 1a]. The surface papillary epithelium was continuous with the underlying ductal proliferation component [Figure 1b]. The ductal epithelium displayed luminal cuboidal to columnar cells, featuring endophytic papillary infoldings, micropapillae and cyst-like spaces [Figure 1c and d]. The stroma exhibited a moderate lymphoplasmacytic infiltrate and clusters of mucus acini. Differential diagnoses encompassed ductal papillomas, squamous papilloma and sialadenoma papilliferum. Exclusion of ductal papillomas and squamous papillomas was based on the continuous connection between the exophytic surface epithelium and the endophytic ductal epithelium. Verrucous carcinoma and adenosquamous SCC were also considered as potential differentials, but the presence of endophytic ductal proliferation in this case eliminated verrucous carcinoma, and the absence of significant atypia ruled out adenosquamous SCC. Immunohistochemistry (IHC) examination revealed that the ductal cells displayed diffuse and intense positivity for CK7, while the surface epithelium exhibited a weaker expression of CK7 [Figure 1e]. The basal cells surrounding the ductal cells were prominently positive for p40 expression [Figure 1f]. In addition, the surface epithelium also demonstrated immunopositivity for p40. Based on histological and IHC findings, the final impression of sialadenoma papilliferum was rendered. BRAFV600E mutation was detected by real-time PCR using an allele specific PCR. DNA was extracted from formalin-fixed paraffin embedded (FFPE) tissue block using a DNA extraction kit (ReliaPrep TM FFPE gDNA Miniprep System). Purity and concentration of the DNA was quantified using nanodrop UV spectrophotometer. Real-time PCR was performed using a TB Green Premix Ex Taq II (Tli RNase H Plus, Takara RR820A) with the primers mentioned in Figure 2. The 3' ends of the inner forward and inner reverse primers are designed such that they amplify only the wild type and mutant templates, respectively. The outer primers acted as the control and would amplify both the wild type and mutant sequences and yield a 200 bp fragment (F0 and R0). Among the inner primers, Fi was against the wild type sequence: Fi and R0 would yield a 97 bp sequence only in case of the wild type allele. Ri was designed against the mutant allele. F0 and Ri would yield a 144 bp fragment only if the mutant allele was present. The current case showed amplification with all 3 primer pairs showing the presence of the BRAFV600E mutation [Figure 3].

DISCUSSION

The microscopic features of SP often resemble squamous papilloma, verrucous hyperplasia, or exophytic ductal papilloma [Table 1]. SP exhibits exophytic papillary parakeratotic stratified squamous epithelium that seamlessly transitions into an underlying endophytic ductal proliferative component. In contrast, squamous papilloma and verrucous hyperplasia only feature an exophytic surface

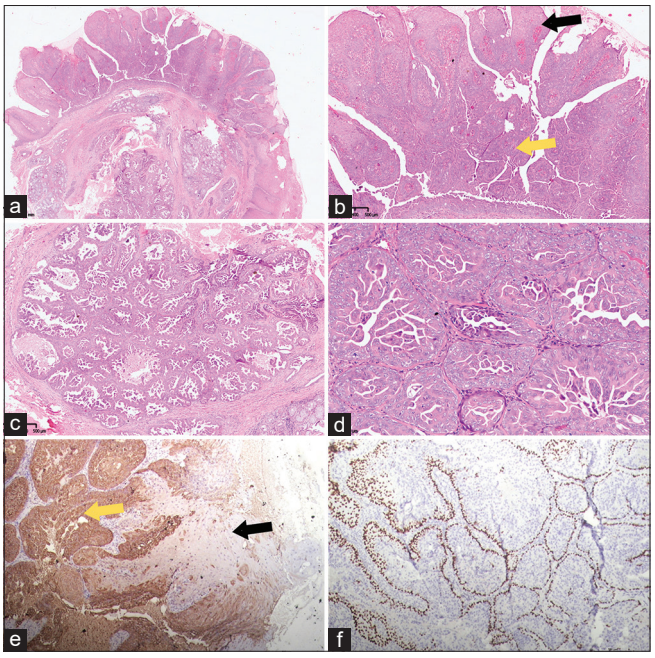

Figure 1: Microscopic features: (a) Scanner view showing classic SP with exophytic papillary surface projections. (b) Papillary squamous surface (black arrow) and a contiguously endophytic ductal proliferation (yellow arrow). (c and d) The ductal cells are composed of columnar to cuboidal cells and arranged in bilayered to multilayered structures showing central papillary projections. (e) CK7 is strongly positive in the ductal cells (yellow arrow) while the surface epithelium is immunonegative for CK7. (f) p40 shows strong immunopositivity in the basal cells

epithelium. Exophytic ductal papilloma displays exophytic papillary ductal epithelial proliferation but lacks the underlying ductal proliferation beneath the epithelium. SP consists of two distinct subtypes: classic SP and oncocytic SP. The classic SP shows strong immunopositivity for SOX10 and genetically analogous to syringocystadenoma papilliferum with consistent BRAFV600E mutations. The oncocytic SP is SOX10 negative and BRAF wild-type.[2] The current case comes under the category of classic SP. On occasion, from a histopathological perspective, SP may bear resemblance to malignant conditions like papillary squamous cell carcinoma (SCC), acantholytic SCC, and adenosquamous SCC. However, due to its benign nature, the absence of invasion and overt atypia serves as a differentiating factor, allowing SP to be distinguished from these malignant lesions.[2,7] The histogenesis of the SP remains uncertain. Numerous theories explain its origin, including those involving the excretory ducts,[8] myoepithelial cells[3] or intercalated duct.[9] Nonetheless, the present case demonstrates distinct CK7 immunopositivity for the endophytic ductal component surrounded by p40 basal/myoepithelial cells, supports the theory that SP originated from the excretory duct. Histopathologically, SP is analogus to syringocystadenoma papilliferum of the skin. The syringocystadenoma papilliferum possesses mutations related to BRAF and HRAS.

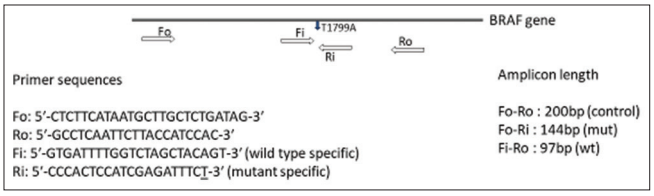

Figure 2: The 3' ends of the inner forward and inner reverse primers are designed such that they amplify only the wild type and mutant templates respectively. The outer primers are used as a control

A comprehensive review of the literature identified a total of 37 cases of SP, including our own, that have undergone BRAFV600E analysis [Table 2]. The mean age of patients was 65.13 years, with a nearly equal distribution between males and females. The most common site of the lesion was the hard palate, followed by the buccal mucosa and tongue, with average duration of 4 months. Less frequently, lesions were found on the retromolar gingiva, soft palate, lip and oral floor. The most common clinical differentials

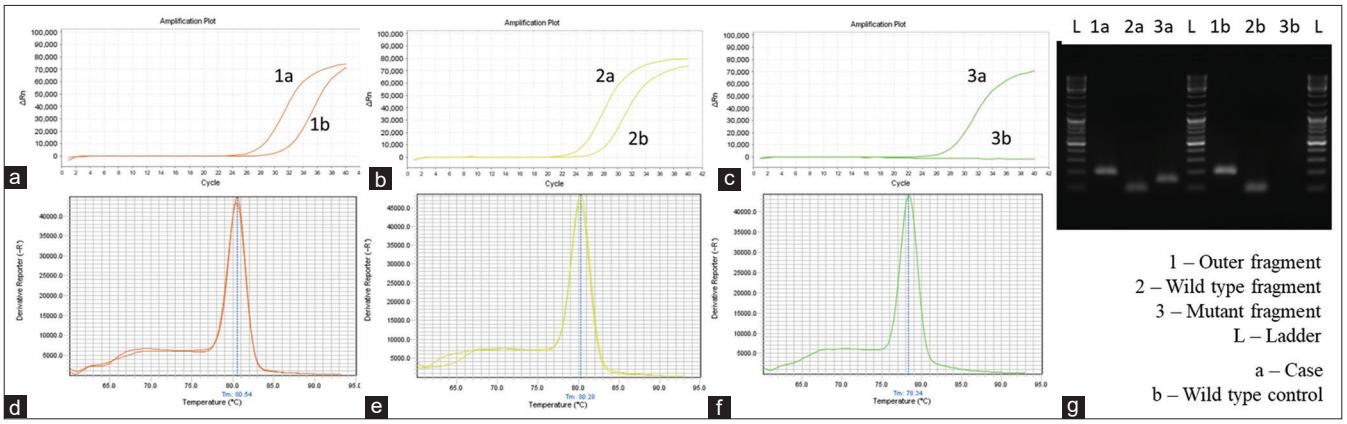

Figure 3: Molecular analysis of BRAFV600E on quantitative PCR: (a) Amplification of outer fragment. (b) Amplification of wild type fragment (c) Amplification of mutant fragment. (d) Melt curve analysis of the outer fragment. (e) Melt curve analysis of wild type fragment. (f) Melt curve analysis of mutant fragment. (g) Gel electrophoresis picture showing the amplified product of target size

Table 1: Histopathological differentials of sialadenoma papilliferum (SA)

Entities with overlapping histopathological features with SA	Histopathological features	Differentiating features from SA
Squamous papilloma	Exophytic papillary proliferation of hyperplastic stratified squamous epithelium with fibrovascular stromal cores.	Absence of endophytic glandular component.
Inverted ductal papilloma	An unencapsulated, endophytic epithelial tumour with well-defined borders. Presence of both squamous and glandular features with circumscribed lower border.	Generally don't have significant exophytic component.
Verrucous hyperplasia	Verruco-papillary hyper ortho-/parakeratosis with/without dysplasia	Absence of endophytic glandular component.
Verrucous carcinoma	Broad-based well differentiated exophytic and endophytic squamous epithelial proliferation with marked surface keratinization and keratin plugging.	Absence of endophytic glandular component.
Mucoepidermoid carcinoma (MEC)	Characterized by mucous, intermediate and squamoid cells. Architectural configurations include cystic and solid areas where proportions of tumour cell types vary widely.	MEC does not usually have a papillary component even when it emerges at the surface from a salivary duct.

were squamous papilloma and verrucous lesions. For BRAFV600E analysis, both immunohistochemical (IHC) methods and molecular techniques (PCR followed by Sanger sequencing) were used. Among the 37 cases, IHC was

Table 2: BRAFV600E analysis in sialadenoma papilliferum

Author and year	No. of cases	Age	Gender	Location	Duration	Size (mm)	Clinical differentials	Histopathological impression	BRAF analysis (IHC)	BRAF analysis (Molecular)	
										Mutation	Wild type
Owosho AA *et al*. 2023[10]	1	58	F	Hard palate	N/A	4.0	Verrucous lesion	CSP	Positive	Not done	Not done
	2	56	M	Hard palate	N/A	3.0	Papilloma	CSP	Positive	Not done	Not done
	3	65	M	Buccal mucosa	N/A	34.0	Papilloma	CSP	Positive	Not done	Not done
	4	80	F	Buccal mucosa	N/A	2.0	Verrucoid lesion	CSP	Positive	Not done	Not done
	5	28	M	Buccal mucosa	N/A	15.0	Papilloma versus verruca vulgaris	CSP	Positive	Not done	Not done
	6	81	M	Labial vestibule	N/A	3.0	Epulis	CSP	Positive	Not done	Not done
	7	66	F	Lip	N/A	3.0	Not provided	CSP	Positive	Not done	Not done
	8	79	F	Hard palate	N/A	-	Papilloma	OSP	negative	Not done	Not done
Chen *et al*. 2020[1]	9	78	M	Hard palate	1 month	5.0	Squamous papilloma	CSP	Positive	Present	N/A
	10	56	F	Hard palate	5 month	10.0	Squamous papilloma	CSP	Positive	Present	N/A
	11	72	M	Nasal cavity	2 month	10.0	Squamous papilloma	CSP	Positive	Present	N/A
	12	50	F	Esophagus	9 month	6.0	Squamous papilloma	CSP	Positive	Negative	N/A
	13	53	M	esophagus	1 month	8.0	Squamous papilloma	CSP	Positive	Not done	Not done
Nakaguro M *et al*. 2020[5]	14	71	F	Soft palate	N/A	4.0	N/A	CSP	Not done	Present	N/A
	15	80	F	Retromolar gingiva	N/A	8.0	N/A	CSP	Not done	Present	N/A
	16	45	M	Hard palate	N/A	7.0	N/A	CSP	Not done	Present	N/A
	17	75	M	Buccal mucosa	N/A	6.0	N/A	CSP	Not done	Present	N/A
	18	67	M	Retromolar gingiva	N/A	23.0	N/A	CSP	Not done	Negative	N/A
	19	79	M	Hard palate	N/A	6.0	N/A	CSP	Not done	Negative	N/A
	20	75	F	Tongue	N/A	6.0	N/A	CSP	Not done	Present	N/A
	21	63	F	Tongue	N/A	9.0	N/A	CSP	Not done	Present	N/A
	22	78	F	Oral floor	N/A	13.0	N/A	CSP	Not done	Present	N/A
	23	66	F	Tongue	N/A	8.0	N/A	CSP	Not done	Negative	N/A
Hsieh *et al*. 2020[4]	24	65	M	Hard palate	N/A	3.0	N/A	CSP	Positive	Present	N/A
	25	77	M	Hard palate	N/A	1.5	N/A	CSP	Positive	Present	N/A
	26	83	F	Soft palate	N/A	3.0	N/A	CSP	Positive	Present	N/A
	27	52	M	Hard palate	N/A	5.2	N/A	CSP	Positive	Present	N/A
	28	2	M	Buccal mucosa	N/A	4.0	N/A	CSP	Positive	Present	N/A
	29	91	F	Hard palate	N/A	4.5	N/A	CSP	Positive	Present	N/A
	30	58	F	Tongue	N/A	6.0	N/A	CSP	Positive	Present	N/A
	31	77	F	Hard palate	N/A	2.0	N/A	CSP	Positive	Not done	Not done
	32	36	F	Hard palate	N/A	2.0	N/A	CSP	Positive	Not done	Not done
	33	61	M	Buccal mucosa	N/A	2.0	N/A	OSP	Negative	Negative	positive
	34	73	F	Hard palate	N/A	3.0	N/A	OSP	Negative	Negative	positive
	35	77	F	Hard palate	N/A	4.0	N/A	OSP	Negative	Negative	positive
	36	64	M	Hard palate	N/A	2.5	N/A	OSP	Negative	Negative	positive
Current case	37	73	M	Retromolar trigone region	6 months	10	Verrucous lesion	CSP	Not done	Present	Negative
Total	37	65.13	F=19 M=18	Buccal mucosa=6; Hard palate=16; Retromolar gingiva=3; Soft palate=2; Lip=1; Tongue=4; Esophagus=2; Labial vestibule=1; Nasal cavity=1; Oral floor=1	Mean of 6 cases=4 months	Mean 6.64	Squamous papilloma/ verrucous lesion	CSP=32 OSP=5	positive=21 Negative=5 Not done=11	Present=18; Negative=8; Not done=11	positive=4 Negative=1 Not done=11 N/A=21

*F: female; M: male; CSP: classic sialadenoma papilliferum; OSP: oncocytic sialadenoma papilliferum; N/A: not available

performed on 26, with 80.76% showing positivity for BRAF. Molecular analysis was also conducted on 26 cases, revealing a BRAF mutation in 69.23% of them. The BRAFV600E mutation was reported by Hsieh and Nakaguro in the minor salivary gland SP patients.[4,5] Owosho AA *et al*.[10] examined BRAFV600E using immunohistochemistry in eight SP cases (seven classical SP cases and one oncocytic SP case). They found that BRAFV600E immunopositive status was present in all classical SP patients. The current case's molecular investigation reveals a positive result for the BRAFV600E mutation. All of these results point to the possibility that SP is the salivary equivalent of skin's syringocystadenoma papilliferum. The BRAFV600E mutation in SP indicates that the endophytic ductal epithelium and the exophytic squamous epithelium are both neoplastic. Conservative surgical excision is the treatment of choice, but follow-up at regular intervals is required. Literature review showed a recurrence rate of 7.4% with up periods of 2 to 96 months.[2] This case highlights the significance of BRAFV600E analysis as a potentially valuable diagnostic tool when faced with a complex intraoral salivary gland neoplasm.

Key Messages

Sialadenoma papilliferum (SP) shares histopathological similarities with syringocystadenoma papilliferum of the skin, often exhibiting BRAF and HRAS mutations. Molecular analysis in our current case confirms the presence of a BRAFV600E mutation. Histopathologically, SP presents a spectrum of potential diagnoses, spanning from benign to malignant lesions, including ductal papillomas, squamous papilloma, verrucous carcinoma, acantholytic squamous cell carcinoma (SCC), and adenosquamous SCC. Therefore, accurate diagnosis of the case is crucial.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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