Clinical Oral Investigations (2021) 25:2249–2256
https://doi.org/10.1007/s00784-020-03541-9

ORIGINAL ARTICLE

Oral submucous fibrosis transforming into squamous cell carcinoma: a prospective study over 31 years in mainland China

Xinchun Jian[1] · Yu Jian[2] · Xiaoshan Wu[1] · Feng Guo[1] · Yanjia Hu[3] · Xing Gao[1] · Canhua Jiang[1] · Ning Li[1] · Yingfang Wu[4] · Deyu Liu[5]

Received: 21 March 2020 / Accepted: 20 August 2020 / Published online: 25 August 2020

Abstract

Objective Oral submucous fibrosis (OSF) is an oral mucous disease caused by betel quid chewing. It is controversial whether OSF can transform into oral squamous cell carcinoma (OSCC).

Materials and methods In this prospective study, a group of 567 patients with OSF were enrolled from 1986 to 2017 and followed-up until 2019. The cancerous information was collected and analyzed.

Results OSF transformed into OSCC in 32 cases (32/567, 5.6%). The patient's age ranged from 20 to 69 years, and the average age was 52 years. The time taken for transformation ranged from 2 to 24 years, the average being 8.6 years. The cancerous transformation occurred in 18 patients (56%) from years 2 to 9, in 13 patients (41%) from years 10–19 and in 1 patient (3%) from 24 years. We analyzed the betel quid chewing habits and found all 32 patients with OSCC-chewed betel quid. Betel quid chewing was most prevalent in patients aged 40–69 years. Sixteen patients had chewed betel quid for 10–19 years (16/32, 50%) and 19 patients (60%) chewed 10–19 slices each day. The OSCC was located in the left or right buccal regions in 23 patients (23/32; 72%) and in the left or right lingual regions in 4 patients (4/32; 12%). Well, moderately and poorly differentiated squamous cell carcinoma was present in 23 patients (23/32; 72%), 4 patients (3/32; 9%), and 5 patients (5/32; 16%), respectively.

Conclusion Our findings supported that OSF is a real oral premalignant disorder.

Clinical relevance The long duration of the transformation from the OSF to OSCC suggests more frequent examinations and corresponding treatments are necessary for OSF patients.

Keywords Oral cancer · Oral submucous fibrosis · Carcinogenesis

Introduction

Oral submucous fibrosis (OSF) is an oral mucous disease that is mainly associated with betel quid chewing. In 1956, Paymaster described OSF as a precancerous condition and subsequently reported 650 cases with oral squamous cell carcinoma (OSCC), approximately one third of which were associated with OSF [1]. Pindborg et al. [2] then reinforced the hypothesis that oral submucous fibrosis is a precancerous condition or potentially malignant oral mucous disorder [3–8]. In 2017, Chaturvedi

Xinchun Jian and Yu Jian are Co-first authors.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00784-020-03541-9) contains supplementary material, which is available to authorized users.

✉ Xinchun Jian
drjianxinchun@163.com

[1] Department of Oral and Maxillofacial Surgery, Xiangya Hospital, Central South University, Changsha, Hunan 410008, People's Republic of China

[2] Department of Obstetrics and Gynecology, Xiangya Hospital, Central South University, Changsha, Hunan 410008, People's Republic of China

[3] Department of Oral and Maxillofacial Surgery, Xiangya Stomatological Hospital, Central South University, Changsha, Hunan 410078, People's Republic of China

[4] Center of Stomatology, Xiangya Hospital, Central South University, Changsha, Hainan 410008, People's Republic of China

[5] Department of Oral and Maxillofacial Surgery, People's Hospital of Haikou City, Central South University, Haikou, Hainan 507208, People's Republic of China

et al. reported 289 patients with OSCC, and 88 patients of them were associated with OSF. Their study had indicated that the patients with OSCC associated with OSF have better oncologic outcome than those without [9]. Similarly in 2017, Gadbail et al. compared the clinic-pathological features of OSCC in the background of OSF and OSCC. The results had showed that OSCC in the background of OSF was a distinct clinico-pathological entity with better prognosis [10].

Although the malignant potential of OSF was first identified in 1956 [1], the molecular mechanisms of malignant transformation is still under investigation. A significant number of studies have been carried out by many researchers. The major carcinogen in the malignant transformation of OSF is the arecoline [11]. It exerts effects by regulating the cell cycle-related proteins [12, 13], inhibiting the expression of suppressor genes [14] and promoting keratinocyte migration and invasion [15]. The increased fibrosis causes the hypoxia and decreased vascularity, which leads to the ischemic atrophy of the epithelium and ultimately leads to cancer [16]. Besides, the epithelial mesenchymal transition is also found to play critical role in the development of invasive carcinoma [17]. As a result, the malignant transformation in the background of OSF appears to be a complex process involving different pathways.

However, these researches are not definitively known whether OSF can transform into squamous cell carcinoma. We, therefore, sought to determine whether OSF is a real oral mucous precancerous lesion or a potentially malignant oral disorder.

Materials and methods

Study design

The design was a multiple-center prospective study, in which 567 patients with OSF were enrolled from 1986 to 2017. This multiple-center trial was approved by the ethics committee and was monitored by the Institutional Data and Safety Monitoring Committee.

Subjects

Five hundred and sixty-seven patients with OSF over aged 18 years were enrolled from September 1986 to August 2017 and followed-up until 2019. The diagnosis and personal information and patients were recorded with the research registration system. All the subjects chewed betel quid before he/she was diagnosed as OSF. All the patients got rid of the habit of chewing betel quid after the diagnosis. There is not any active treatment provided after the diagnosis. However, the pathological fibrosis was not reversed during the follow-up period. Among these subjects, 32 patients were diagnosed with OSCC during the follow-up.

The diagnosis of OSF was indicated according to the criteria of Pindborg [2]:

(1) A history of betel quid chewing (more than five slices for every day).
(2) When eating spicy food, a burning sensation in the mouth is experienced.
(3) The oral mucosa is blanched and white and has fibrous bands.
(4) It is difficult for the patient to open their mouth.

The pathological characteristics of OSF tissues were the following:

(1) The oral epithelium in the affected areas is atrophic or hypertrophic.
(2) In the early stage, the juxta-epithelial area shows hyalinization, and collagen is seen as separate bundles. Blood vessels are dilated and congested. The inflammatory cells, mononuclear lymphocytes, eosinophils, and occasional plasma cells are present.
(3) In the moderately advanced stage, collagen is moderately hyalinized, and inflammatory cells are mainly lymphocytes and plasma cells and occasionally eosinophils. Blood vessels are either normal or constricted.
(4) In the advanced stage, collagen is completely hyalinized and is seen as a smooth sheet. Inflammatory cells are mainly lymphocytes and plasma cells. Blood vessels are obliterated or narrowed.

The indicated patients were photographed and biopsied with patient consent. Follow-up examination was performed annually to determine the malignant transformation rate and to understand the natural history of OSF.

Patients with TMJ disorders, severe hyperplasia, OSF associated with leukoplakia, or OSF associated with leukoplakia and squamous cell carcinoma in the oral cavity were excluded. Carcinoma in situ and transformation of OSF into squamous cell carcinoma were included in this study. The histopathological diagnostic criteria of dysplasia, carcinoma in situ, and squamous cell carcinoma were as follows:

(1) Dysplasia: when hyperchromatic and slightly pleomorphic nuclei are noted in the base and parabasal cell layers of the stratified squamous epithelium, mild epithelial dysplasia is diagnosed. If dysplastic changes extend to the mild point of the epithelium, characterized by nuclear hyperchromatism, pleomorphism, and cellular crowding, moderate epithelial dysplasia is diagnosed. If marked pleomorphism, hyperchromatism, scattered mitotic figures, and atypical cells are present in most of the epithelial thickness, severe epithelial dysplasia is diagnosed [18].

(2) Carcinoma in situ: when epithelial cells extend from the basal layer to the surface of the mucosa, carcinoma in situ is defined. The most important feature of carcinoma in situ is that no invasion has occurred, and a focus of invasive squamous cell carcinoma in the adjacent tissue may be present [18].
(3) Squamous cell carcinoma: histopathological criteria of squamous cell carcinoma are graded on a 3-point scale as follows.

I. Poorly differentiated squamous cell carcinoma: numerous pleomorphic cells within the lamina propria are present.
II. Moderately differentiated squamous cell carcinoma: malignant cells are easily recognizable as being of squamous epithelial origin under medium-power and high-power view.
III. Well-differentiated squamous cell carcinoma: islands of malignant squamous epithelium invaded into the lamina propria under low-power view. High-power view shows dysplastic epithelial cells with keratin pearl formation [18].

Follow-up

Patients who were diagnosed with OSF were followed-up once every 12 months. When OSF transformation into squamous cell carcinoma in the oral cavity was diagnosed and confirmed by pathological examination, treatment of squamous cell carcinoma was started. After squamous cell carcinoma treatment, patients were followed-up every month for the first year, every 2 months for the second year, and every 3 months thereafter.

Statistical analysis

The results are presented according to the diagnosis of OSF and to the diagnosis and treatment of OSCC. The data cut off point was August 31, 2017. All data were statistically analyzed using the SPSS 20.0 software (International Business Machines, Armonk, New York, USA). The descriptive data were analyzed using the methods of frequencies, percentages, medians, standard deviations, and ranges.

Results

Patients with OSCC

Of the 567 patients with OSF, 32 (5.6%) presented transformation of submucous fibrosis into OSCC (Table 1). Among the 32 patients, 31 were male and 1 was female. Their age distribution was 20–69 years, the average being 52 years (Table 1 and Supplementary Table 1). All 32 patients chewed betel quid. Six percent were aged between 20 and 39 years, 44% were aged between 40 and 49 years, and 50% were aged between 50 and 69 years. Betel quid chewing was most prevalent in patients aged 40–69 years. (Fig. 1a, Table 1).

The amount and duration of betel quid chewing in OSCC patients

The OSCC patients had chewed betel quid for different amounts of time: six patients (19%) for 1–9 years, 16 patients (50%) for 10–19 years, 7 patients (22%) for 20–29 years, and 3 patients (9%) for 30–39 years (Fig.1b and Supplementary Table 2).

The amount of betel quid chewing (slices/day) also varied: Five patients (16%) chewed 5–9 slices per day, 19 patients (59%) chewed 10–19 slices per day, 5 patients (16%) chewed 20–29 slices per day, 2 patients (6%) chewed 30–39 slices per day, and 1 patient (3%) chewed ≥ 40 slices per day (Fig.1c and Supplementary Table 3).

When we separated the patients into 4 groups according to the amount of betel quid chewing per day (≤ 5, 6–10, 11–15, and ≥ 16) and compared the duration of suffering from OSF among the groups, the results showed that the more betel quid chewing per day, the less of the duration length required for transformation from OSF to OSCC ($P < 0.05$) (Fig.2).

The transformation latency

The transformation rate from OSF to OSCC was 32/567(5.6%), and transformation occurred after 2–24 years with a median time of 8.6 years: transformation occurred in 18 patients (57%) after 1–9 years, in 13 patients (41%) after 10–19 years, and in 1 patient (3%) after 20–29 years (Fig. 3; Fig. 1d).

Stages and sites of OSCC transformed from OSF

Among the 32 patients, squamous cell carcinoma was located in left or right buccal regions in 23 patients (72%), in left or right lingual regions in 4 patients (12%), in the right gingival region in 2 patients (6%), and in the right floor of the mouth in 2 patients (6%) (Fig. 1e and Supplementary Table 4).

Well-differentiated squamous cell carcinoma accounted for 23 patients (72%), and moderately differentiated squamous cell carcinoma accounted for 8 patients (25%) (Fig.1f, Fig.4–5, and Supplementary Table 5).

Discussion

OSF is a premalignant and crippling lesion of the oral mucosa that is associated with various chewing habits [19]. In 2006, Ariyawardana et al. showed that betel chewing was the only significantly associated factor in the etiology of OSF. Other

Table 1 Characteristics of patients with OSF transforming into oral cancer

Case No.	Sex	Age (y)	Duration of betel quid chewing (y)	The number of betel quid chewing (slice/day)	Duration of suffering from OSF (y)	Duration of suffering from OSCC (m)	Sites	Diagnostic results of biopsy
1	M	65	20	20	13	7	Right buccal	Well
2	M	40	15	10	7	2	Left buccal	Well
3	M	45	16	10	3	3	Right oral corner	Well
4	M	55	5	15	3	2	Left buccal	Well
5	M	43	5	5	2.5	15	Right buccal	Well
6	M	52	15	10	9	5	Right buccal	Well
7	M	44	20	5	10	1	Left oral floor	Well
8	M	48	15	10	5	1	Right buccal	Carcinoma in situ
9	M	42	15	30	10	2	Right buccal	Well
10	M	59	4	10	2	2	Left buccal	Poorly
11	M	59	4	10	2	2	Left buccal	Well
12	M	41	10	5	2	2	Left root of tongue	Well
13	M	67	30	20	18	1	Right anterior part of tongue	Well
14	M	51	30	10	16	3	Right buccal	Well
15	M	60	20	20	14	3	Right gingival of the mandible	Moderated
16	M	30	3	5	2	5	Right gingival of the mandible	Well
17	F	64	10	10	7	3	Left buccal	Well
18	M	29	14	100	11	2	Right buccal	Well
19	M	65	20	20	14	10	Right buccal	Well
20	M	40	15	10	9	2	Left buccal	Well
21	M	45	16	10	11	3	Right buccal	Poorly
22	M	55	5	15	3	2	Left buccal	Hyperplasma
23	M	54	13	10	9	5	Right buccal	Well
24	M	45	20	5	14	1	Left oral floor	Well
25	M	49	16	10	10	2	Right buccal	Moderated
26	M	44	15	30	11	6	Right soft palate	Well
27	M	62	4	10	2	2	Left buccal	Poorly
28	M	46	10	5	8	2	Right lingual	Poorly
29	M	69	21	15	12	2	Left lingual	Well
30	M	53	30	20	24	3	Left buccal	Well
31	M	46	15	14	5	14	Left buccal	Moderated
32	M	52	15	10	9	8	Right buccal	Well

investigations then provided further evidence for the role of betel quid chewing in the etiology of OSF [4–8, 20]. Our follow-up study also indicated that betel quid chewing is a significant factor in the etiology of OSF and that there is a strong dose response relationship between the frequency and duration of betel quid chewing and the risk of developing OSF and oral cancer.

OSF was defined as an oral precancerous condition by Paymaster in 1957. In his series, one third of 650 cases with buccal mucous cancer were associated with OSF. In 1984, Pindborg suggested OSF to be a precancerous condition [3] according to the following five criteria: (1) a higher incidence of oral cancer in patients with submucous fibrosis, (2) high occurrence of submucous fibrosis in oral cancer patients, (3) histological diagnosis of oral cancer without any clinical suspicion among submucous fibrosis cases, (4) higher prevalence of leukoplakia among submucous fibrosis cases, and (5) high frequency of epithelial dysplasia. In 2016, Jayasinghe et al. [7] reported five cases of clinically malignant exophytic lesions in a background of OSF. These five patients were all

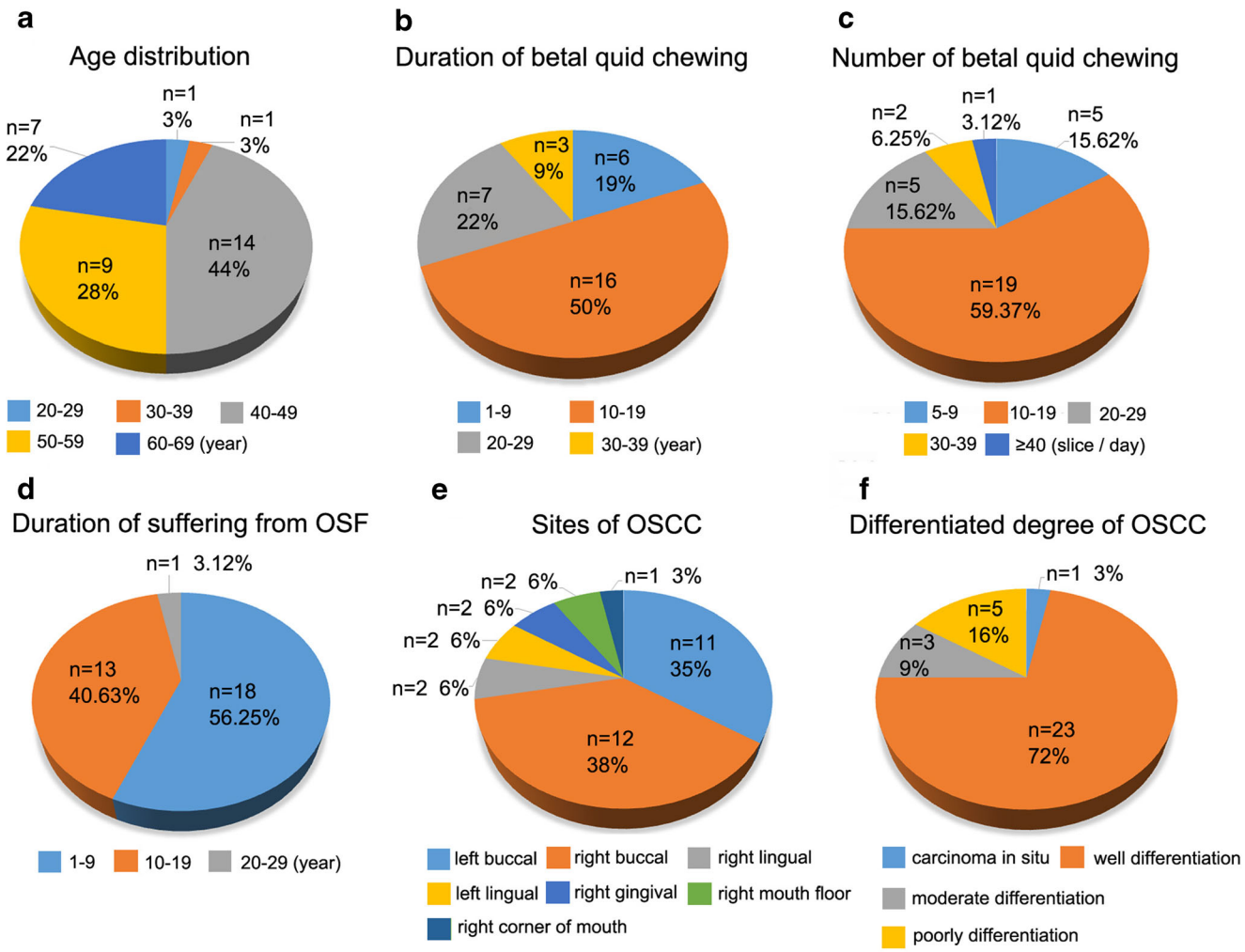

Fig. 1 Characteristics and betel quid chewing habits of OSF and OSCC patients. (**a**) Age distribution. (**b**) Duration of betel quid chewing. (**c**) Quantity of betel quid chewed. (**d**) Duration of suffering from OSF. (**e**) Sites of OSCC transformed from OSF. (**f**) Degree of OSCC differentiation

verrucopapillary lesions or verrucous leukoplakia, which were all histologically diagnosed as oral verrucous hyperplasia (OVH) on a background of OSF. Additionally, many other

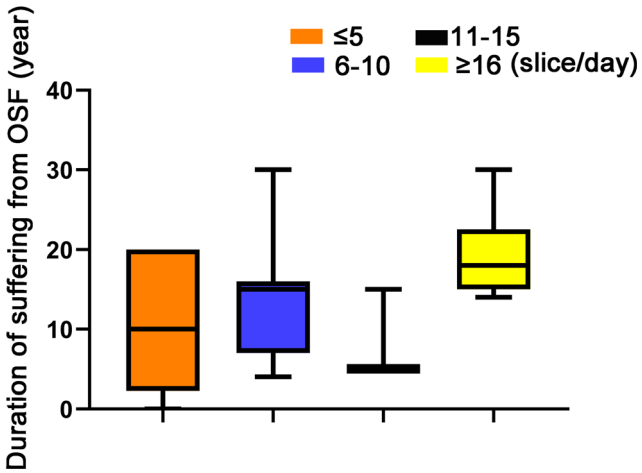

Fig. 2 Comparison of the duration of suffering from OSF among the groups with different amounts of betel quid chewing per day

similar studies have been published in the English literature [1–6, 8, 20, 21]. However, whether OSF can transform into squamous cell carcinoma remains unclear.

In the present study, among the 567 patients with OSF followed-up over long period, 32 cases had transformed into OSCC. Our study revealed that the average duration of malignant transformation was 8.7 years, longer than that in other studies [5, 6]. Most of the 32 patients with a malignant transformation were presented in the buccal mucosa (72%) and the tongue (12%). Moreover, we found that OSF in the buccal mucosa had a higher malignant transformation potential than that in other regions of the oral cavity. Excepted for the use of betel quid, cigarette and alcohol were identified as risk factors for malignant transformation of OSF in the Mainland China previously. Synergistic effects between betel quid chewing and cigarette or alcohol consumption were revealed [22].

The limitations of the study are that we only observed how OSF in the oral cavity transformed into oral cancer and the patients with both OSF and oral leukoplakia had been

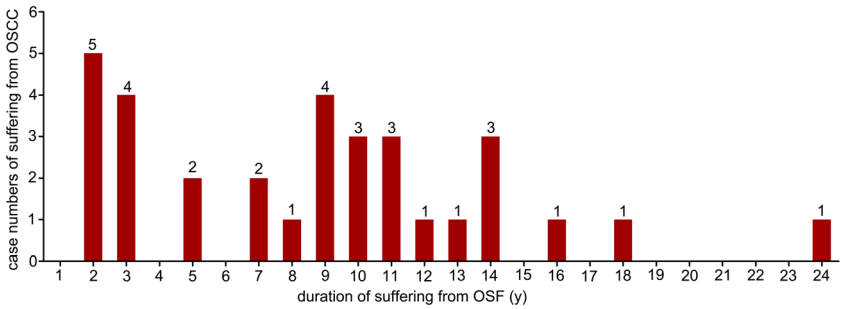

Fig. 3 Duration of follow-up before transformation of OSF to OSCC among patients

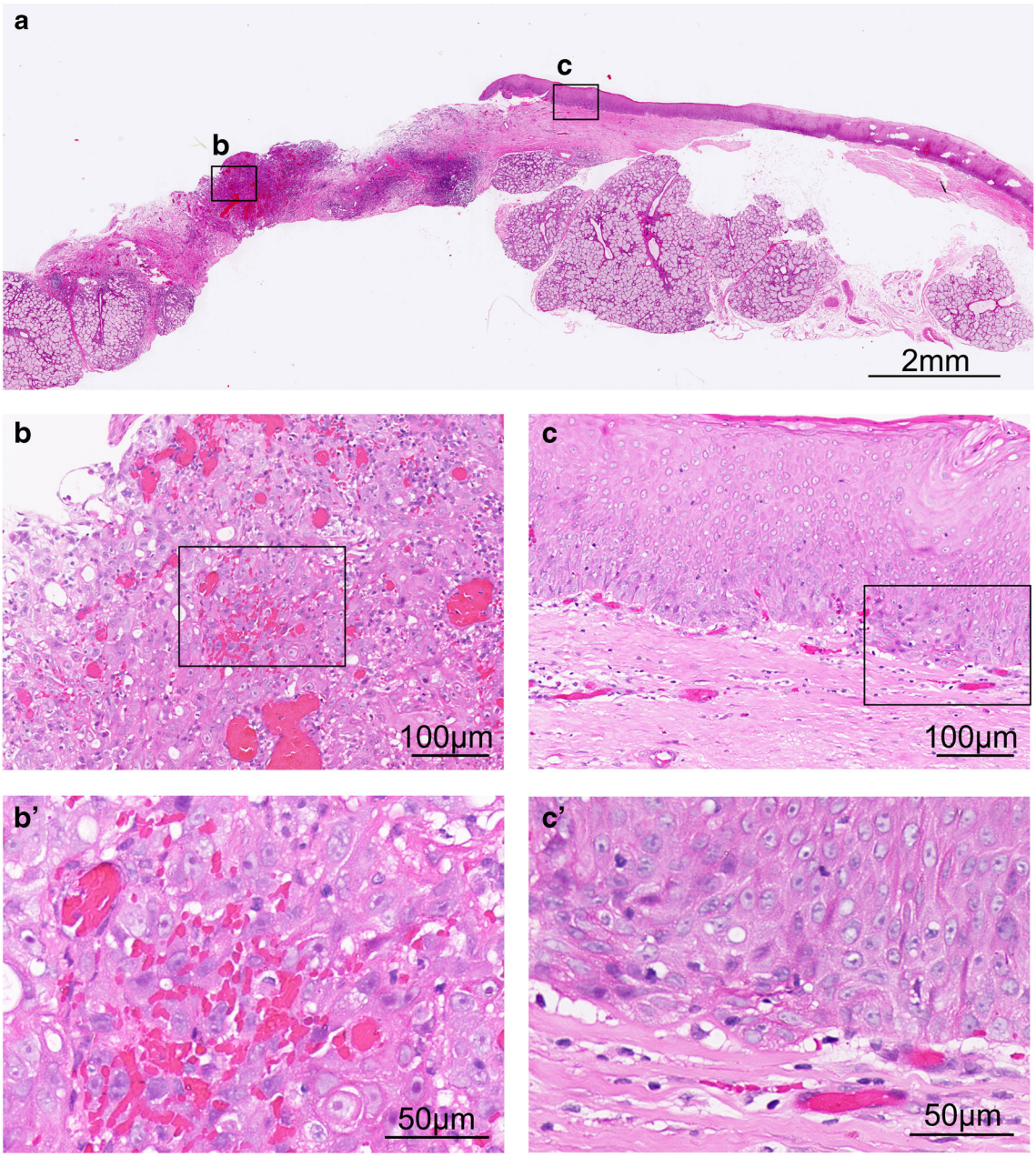

Fig. 4 Histopathology of OSF transformation into OSCC. **A** Histopathological overview of OSF transformation into OSCC. **B-B′,** Area of OSF transformed into moderately differentiated OSCC; the boxed region is the higher magnification area shown in B′. **C-C′,** Area of OSF; the boxed region is the higher magnification area shown in C′. **C′,** Moderate epithelial dysplastic changes

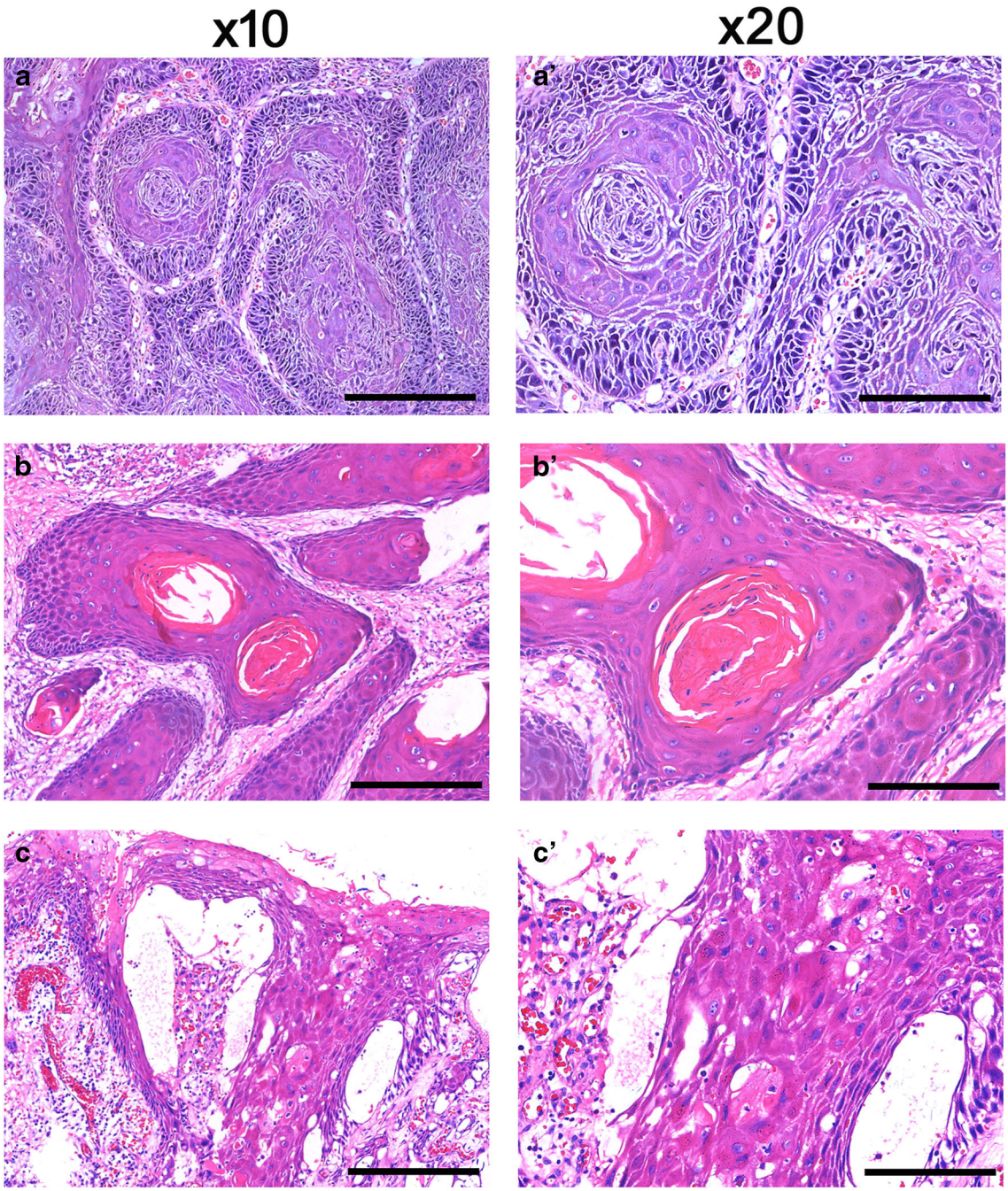

Fig. 5 Differentiation degrees of OSCC transformed from OSF **A, A'**, Carcinoma in situ. **B, B′**, Well-differentiated squamous cell carcinoma. **C, C′**, Moderately differentiated squamous cell carcinoma. Scale bar represents 200 μm in A, B, and C; 100 μm in A', B′, and C′

excluded in our study. However, the patients who have two or more kind of oral precancerous lesions are gradually increased nowadays. As a result, we need to study the malignant transformation of the patients with both OSF and other precancerous lesions in the future.

Our study indicated that OSF is a really malignant oral disorder. It is, therefore, of great importance to prevent malignant change in patients diagnosed with OSF.

Acknowledgments The authors thank the staff of the Oral and Maxillofacial Surgery of Xiangya Hospital, Central South University.

Funding This project was supported by the National Natural Science Foundation of P. R. China (No. 81260166, No. 30572044).

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the Ethical Committee of Xiangya Hospital, Central South University.

Informed consent Informed consent was obtained from all individual participants enrolled in this study.

References

1. Parmaster JC (1956) Cancer of the buccal mucosa; a clinical study of 650 cases in Indian patients. Cancer 9:431–435
2. Pindborg JJ, Sirsat SM (1966) Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 22:764–779
3. Pindborg JJ (1972) Is submucous fibrosis a precancerous condition in the oral cavity? Int Dent J 22:474–480
4. International Agency for Research on Cancer. Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines (1985) Lyon: IARC:141–200
5. Hsue SS, Wang WC, Chen CH, Lin CC, Chen YK, Lin LM (2007) Malignant transformation in 1458 patients with potentially malignant oral mucosal disorders: a follow-up study based in a Taiwanese hospital. J Oral Pathol Med 36:25–29
6. Wang YY, Tail YH, Wang WC, Chen CY, Kao YH, Chen YK, Chen CH (2014) Malignant transformation in 5071 southern Taiwanese patients with potentially malignant oral mucosal disorders. BMC Oral Health 99
7. Jayasinghe LA, Peiris PM, Tilakaratne WM, Attygalla AM, Jayasinghe RD, Sitheeque MA, Siriwardena BS (2016) Clinically malignant exophytic lesions in the background of oral submucous fibrosis: report of five cases. Oral Surg Oral Med Oral Pathol Oral Radiol 122:210–215
8. Jian X, Huang X, Liu D, Xu P, Liu X (2013) Analysis of clinical symptoms and signs in cases of oral submucous fibrosis which have transformed into squamous cell carcinoma. Open J Stomatology 3: 235–240
9. Chaturvedi P, Malik A, Nair D, Nair S, Mishra A, Garg A (2017) Oral squamous cell carcinoma associated with oral submucous fibrosis have better oncologic outcome than those without. Oral Surg Oral Med Oral Pathol Oral Radiol 124:225–230
10. Gadbail AR, Chaudhary M, Gawande M, Hande A, Sarode S, Tekade SA, Korde S, Zade P, Bhowate R, Borle R, Patil S (2017) Oral squamous cell carcinoma in the background of oral submucous fibrosis is a distinct clinicopathological entity with better prognosis. J Oral Pathol Med 46:448–453
11. Lin KH, Lin CY, Liu CC, Chou MY, Lin JK (2011) Arecoline N-oxide: its mutagenicity and possible role as ultimate carcinogen in areca oral carcinogenesis. J Agric Food Chem 59:3420–3428
12. Ji WT, Yang SR, Chen JY, Cheng YP, Lee YR, Chiang MK, Chen HR (2012) Arecoline downregulates levels of p21 and p27 through the reactive oxygen species/mTOR complex 1 pathway and may contribute to oral squamous cell carcinoma. Cancer Sci 103:1221–1229
13. Lee PH, Chang MC, Chang WH, Wang TM, Wang YJ, Hahn LJ, Ho YS, Lin CY, Jeng JH (2006) Prolonged exposure to arecoline arrested human KB epithelial cell growth: regulatory mechanisms of cell cycle and apoptosis. Toxicology 220:81–89
14. Choudhury Y, Sharan RN (2011) Altered BRCA1 and BRCA2 responses and mutation of BRCA1 gene in mice exposed chronically and transgenerationally to aqueous extract of betel nut (AEBN). Environ Toxicol Pharmacol 31:57–69
15. Moutasim KA, Jenei V, Sapienza K, Marsh D, Weinreb PH, Violette SM, Lewis MP, Marshall JF, Fortune F, Tilakaratne WM, Hart IR, Thomas GJ (2011) Betel-derived alkaloid up-regulates keratinocyte alphavbeta6 integrin expression and promotes oral submucous fibrosis. J Pathol 223:366–377
16. Jayasooriya PR, Nadeeka Jayasinghe KA, Tilakaratne WM (2011) Relationship between thickness of fibrosis and epithelial dysplasia in oral submucous fibrosis. J Investig Clin Dent 2:171–175
17. Das RK, Anura A, Pal M, Bag S, Majumdar S, Barui A, Chakraborty C, Ray AK, Sengupta S, Paul RR, Chatterjee J (2013) Epithelio-mesenchymal transitional attributes in oral submucous fibrosis. Exp Mol Pathol 95:259–269
18. Neville BW, Damm DD, Allen CM et al (2009) Oral and maxillofacial pathology (ed 3). Saunders Elsevier, St Louis, MO
19. Canniff JP, Harvey W (1981) The etiology of oral submucous fibrosis. The stimulation of collagen synthesis by extracts of areca nut. Int J Oral Surg 10:153–157
20. Ariyawardana A, Athukorala ADS, Arulamandam A (2006) Effect of betel chewing, tobacco smoking and alcohol consumption on oral submucous fibrosis: a case-control study in Sri Lanka. J Oral Pathol Med 53:197–201
21. Ray JG, Ranganathan K, Chattopadhyay A (2016) Malignant transformation of oral submucous fibrosis: overview of histopathological aspects. Oral Surg Oral Med Oral Pathol Oral Radiol 122:200–209
22. Liu B, Shen M, Xiong J, Yuan Y, Wu X, Gao X (2015) Synergistic effects of betel quid chewing, tobacco use (in the form of cigarette smoking), and alcohol consumption on the risk of malignant transformation of oral submucous fibrosis (OSF): a case-control study in Hunan Province, China. Oral Surg Oral Med Oral Pathol Oral Radiol 120:337–345